Editorial
Creative Commons, Open Access, and Living Cultures (37)

News
Major new research initiative to tackle life threatening fungal infections – Fungal Conservation and IUCN Red Listing (39)
– Volkswagen Foundation supports the African Mycological Association (AMA) to build mycological capacities in West Africa – FUNGEN – A national fungal genetic resource for India – Czech Culture Collection of Fungi (CCF)
– State Key Laboratory of Mycology (SKLM) in China celebrates 30 years – Fungi and Global Challenges
Advice to mycologists concerning Article 57.2 – Fungal Biodiversity Calendar 2017

Reports
Asian Mycological Congress (AMC) 2015 – 2nd Iranian Mycological Congress (IMyC2) – Essential Skills for Young Mycologists

Awards and Personalia
Awards: The Gordon and Tina Wason Award (51)
Birthdays: Katharina Bickerich-Stoll centenarian – Andreas Bresinsky’s 80th – Renowned lichenologist Irwin Brodo turns Eighty – Huub van der Aa at 80 years

Research News
Host-jumps drove rust evolution – Sensing host plant signals: a new role for pheromone-sensing machinery? – Fifty key events in fungal systematics

Mycolens
“The need to engage with citizen scientists to study the rich fungal biodiversity in South Africa” by Marieka Gryzenhout

Correspondence

Book News

Forthcoming Meetings

Erratum

Notices

Articles
“Phylogenetic placement of Itajahya: An unusual Jacaranda fungal associate” by Seonju Marincowitz, Martin P.A. Cserze, P. Markus Wilken, Brenda D. Wingfield, and Michael J. Wingfield 257
“Accepted Trichoderma names in the year 2015” by John Bissett, Walter Gams, Walter Jaklitsch, and Gary J. Samuels 263
“New sequestrate fungi from Guyana: Jimtrappea guyanensis gen. sp. nov., Castellanea pakaraimophila gen. sp. nov., and Cantasporus cyanescens gen. sp. nov. (Boletales, Boletaceae)” by Matthew E. Smith, Kevin R. Amses, Todd F. Elliott, Keiisuke Obuse, M. Catherine Aime, and Terry W. Henkel
“Physallana boodjera sp. nov., a damping-off pathogen in production nurseries and from urban and natural landscapes, with an update on the status of P. alticola” by Agnes V. Simamora, Mike J. C. Stukely, Giles E. StJ. Hardy, and Treena I. Burgess 297
“Matsushimamyces, a new genus of keratinophilic fungi from soil in central India” by Rahul Sharma, Rohit Sharma, and Pedro W. Crous 319
“Phylogeny of Hirsutella species (Ophiocordycipitaceae) from the USA: remedying the paucity of Hirsutella sequence data” by D. Rabern Simmons, Ryan M. Kepler, Stephen A. Rehner, and Eleanor Groden 345
“New 1F1N Species Combinations in Ophiocordycipitaceae (Hypocreales)” by Joseph W. Spatafora, C. Alisha Quandt, Ryan M. Kepler, Gi-Ho Sung, Bhushan Shrestha, Nigel L. Hywel-Jones, and J. Jennifer Luangsa-ard 357

TABLE OF CONTENTS CONTINUED INSIDE BACK COVER
EDITORIAL BOARD

Editor-in-Chief
Prof. Dr. D.J. Hawksworth CBE, Departamento de Biología Vegetal II, Facultad de Farmacia, Universidad Complutense de Madrid, Plaza Ramón y Cajal, 28040 Madrid, Spain; and Department of Life Sciences, The Natural History Museum, Cromwell Road, London SW7 5BD, UK; Comparative Plant and Fungal Biology, Royal Botanic Gardens, Kew, Surrey TW9 3DS, UK; E-mail: d.hawksworth@nhm.ac.uk

Managing Editor
Prof. Dr. P.W. Croes, CBS-KNAW Fungal Biodiversity Centre, P.O. Box 85167, 3508 AD Utrecht, The Netherlands; E-mail: pcroes@ch.uk.nl

Layout Editors
M.J. van den Hoven-Ververij & M. Vermaas, CBS-KNAW Fungal Biodiversity Centre, P.O. Box 85167, 3508 AD Utrecht, The Netherlands; E-mail: mervervij@ch.uk.nl

Associate Editors
Dr E.V. Andersen, M.G. Kloosndy Institute of Botany, Terseshchikovska Street 2, Kiev, 01601, Ukraine; E-mail: tand@duan.microsoft.com
Prof. Dr. D. Beguina, Lehrstuhl für Evolution und Biodiversität der Pflanzen, Ruhr-Universität Bochum, Universitätstrasse 150, Gebäudef-N3-0174, 44780, Bochum, Germany; E-mail: dominik.beguin@rub.de
Prof. Dr. Mary Berbee, Department of Botany, University of British Columbia, 6352-b 4th Street, Vancouver, BC V6T 2Z4, Canada; E-mail: maryberbee@gmail.com
Dr B. Carpinteri, Department of Plant Pathology and Crop Physiology, Louisiana State University, Agricultural Centre, 455 Life Sciences Building, Baton Rouge, LA-70803, USA; E-mail: bscarpinte@louisiana.edu
Dr P.S. Dyne, School of Biology, Institute of Genetics, University of Turin, Newtown Park, University of Northumbria N7G 2RD, UK; E-mail: paul.dyne@nottingham.ac.uk
Dr Ana Esperanza Franco Molina, Instituto de Botánica, a Universidad de Antioquia, A.A. 1226, Medellin, Colombia; E-mail: anesper@uantioquia.edu.co
Dr K. Hansen, Kryptogamobuktika Naturhistoriska Riksmuseet, Box 50007, 104 05 Stockholm, Sweden; E-mail: karen.hansen@hkr.kth.se
Prof. Dr. David Hibben, Biology Department, Clark University, Lancy Biological Science Center, 950 Main St., Worcester, MA 01610, USA; E-mail: dhibben@clark.edu
Prof. Dr. Xingzhong Lin, State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences, No. 1, 31st Beichen West Road, Chaoyang District, Beijing 100101, P. R. China; E-mail: lianxin@im.ac.cn
Dr Janet Jennifer Divinagracia Luangsa-ard, National Center for Genetic Engineering and Biotechnology (BIOTEC), 373 Khlong Luang, Pathum Thani 12120, Thailand; E-mail: jajen@biotec.or.th
Prof. Dr. W. Meyer, Molecular Mycology Research Laboratory, SDIM-ICPM, Level 3, Room 314,1/4, Westmead Hospital, Dacey Road, Westmead, NSW, 2145, Australia; E-mail: w.meyer@syd.edu.au
Dr Chiharu Nakashima, Graduate School of Bioresources, Mie University, Kamisaka-Michiyu 1577, 574-8507, Tei, Mie, Japan; E-mail: chiharu@biosci-mie.ac.jp
Dr Marc-Olivier Riquelme, Department of Microbiology, Centro de Investigación Científica y de Educación Superior de Ensenada CICESE, Carretera Ensenada-Tijuana N. 3918, 22860 Ensenada Baja California, Mexico; E-mail: riquelmar@icm.csic.es
Prof. Dr. K.A. Sifon, Research Scientist / Biodiversity (Mycoology and Botany), Agriculture & Agri-Food Canada, K.W. Nearby Building, 960 Carling Avenue, Ottawa, ON, K1A 0C6, Canada; E-mail: sifonk@cfs.gc.ca
Dr. J.W. Taylor, Department of Plant and Microbial Biology, University of California, 111 Kohlheid Hall, Berkeley, CA 94720, USA; E-mail: j.w.taylor@berkeley.edu
Prof. Dr. M.J. Wingfield, Forestry and Agricultural Research Institute (FABI), University of Pretoria, Pretoria 0002, South Africa; E-mail: m.j.wingfield@fabi.up.ac.za

"Bringing Laboulbeniales into the 21st century: enhanced techniques for extraction and PCR amplification of DNA from minute ectoparasitic fungi" by Danny Hadelwouters, Michel Gorcezak, Walter P. Pfeiffer, András Tarrally, Marta Tusich, Marta Wrzosek, Donald H. Pfister

“Carcepsorid fungus (Myxogastriidae) & Species on dican (Anastomus to Anastomus)" by Uwe Braun, Pedro W. Croes, and Chiharu Nakashima

"Knudsonia proteus is not the only Knudsonia-symbiont of Protea repena" by Janneeke Aylward, Léanne L. Dreyer, Emma T. Steenkamp, Michael J. Wingfield, and Francois Roets

"Bacterial rusts of hollyhock (Aria rusta)" by Jill E. Demers, Megan K. Romberg, and Lisa A. Castlebury

"Raskolhapi, a new genus of grammicosilicous downy mildews from tropical Australia, with an updated key to the genera of downy mildews” by Marlon Thines, Sabine Telle, Young-Joo Choi, Yu Pei Tan, and Roger G. Shivas


INSTRUCTIONS TO AUTHORS

Instructions to authors can be found on the IMA Fungus website http://www.imafungus.org/instruction.aspx
CREATIVE COMMONS, OPEN ACCESS, AND LIVING CULTURES

It has now been just over a year since the Nagoya Protocol went into force, and many of you have heard concerns expressed about its potential effect on research. Many countries protect their biological resources by restricting or preventing export of living material; Nagoya strengthens this, applies to all groups of organisms, establishes procedures for "fair and equitable sharing of benefits", and defines enforcement protocols. As of this writing, 62 countries have ratified, accepted or approved the Protocol, about 50 have signed the original declaration but have not proceeded further, and about 80 countries, including several who actively enforce the 1992 Convention on Biological Diversity (CBD), have done neither. Both the CBD and Nagoya benefit the developing world by recognizing that biological resources are sovereign property of countries of origin, and providing legal and economic frameworks for sustainable exploitation of these biological resources. What now needs to be developed is a mechanism that allows public good international scientific research to progress, while continuing to protect intellectual property and economic development rights. Long-standing scientific standards in microbiology include the deposition of cultures, especially taxonomic types, in public collections as a requirement to support publication. This also needs attention.

The situation is analogous to commercial publishing, where copyrighted works are the commodities rather than biological resources. Copyright is governed by an intricate mixture of national and international laws and is often subject to a similar conflict between commercial and academic interests. Although formal exemptions are often made for fair use in teaching, many academics quietly bypass copyright and distribute copies of publications to colleagues; “pirated” copies of books are a reality. The development of the related concepts of Creative Commons, free Open Access (rather than OA for a fee), and Open Data were inspired by historical ideas of Public Domain copyright, and attempt to create an arena where the free sharing of ideas, data and publications for non-commercial use is legal and encouraged.

Biologists have long treated cultures in ways that parallel publishing. Some cultures have great commercial value and are carefully preserved and protected under regulations under the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure, which came into effect in 1981. But to most scientists, cultures are research subjects, reference vouchers (e.g. ex-types), teaching material, and more profoundly, doorways to exploring the microscopic world that enable the development of new ideas, new research and sometimes dramatic discoveries.

Could the same Creative Commons (CC) concepts and processes be applied to living cultures in some circumstances? In publishing, permission to engage in either commercial or non-commercial (NC) activity is explicitly stated by the owner of the resource. Whether that resource can be modified or included as a component in other commercial or non-commercial activities is also defined. The credit expected by the originator of the resource (BY=Attribution) and the requirement that anyone receiving the resource second hand is bound by the same conditions (SA=Share Alike) is defined up-front (summarized in Fig. 1). For cultures originating in countries that either do not enforce the CBD or explicitly choose not to enforce Nagoya for non-commercial...
EDITORIAL

It could be as simple as putting check boxes on culture deposit forms, allowing the original owner to designate their cultures as CC-BY-NC-SA (Fig. 1). If this could be done legally, researchers would declare their intentions in writing and ensure that they comply with their employer’s requirements, funder’s policies, and national laws, each time they submit a culture to a public culture collection. For biological resources originating in countries that have signed the CBD or Nagoya, and choose to enforce it, individual researchers who isolate cultures presently lack the legal authority to deposit them in international collections in support of publication requirements, or to make them available to collaborators in other countries. As advocates of Open Research, we need to find a way for public good researchers in all countries to participate in the development of mechanisms so that their cultures can be designated as Open Access for public good research or teaching, while respecting the requirements of the Treaty and Protocol.

There are many complexities. How can we avoid unintentional interference with intellectual property rights for unanticipated applications of cultures discovered after the publication of academic research? Can quick, precise strain typing mechanisms be developed to enforce the intended creative commons application of open access strains? The best public culture collections have staff that isolate new strains, repeatedly observe strains, and develop new data to enhance the value of the strains. They already suffer from an increasing burden of legal paperwork as they cope with increased national and international regulations; perhaps a system parallel to the Creative Commons would simplify this.

In the meantime, however, all who work with living biological materials must conform to the legislation enacted by international governments (e.g. Verkley 2015). As I learned during my recent travels on behalf of the IMA in India (Fig. 2), a country that enforces CBD and Nagoya rights rigorously, rewarding collaboration is possible and can stimulate the installation of modern scientific capacity, such as DNA sequencing or state of the art analytical chemistry, to further that collaboration. Scientists in the developing world are eager to work with international colleagues. In India, mechanisms exist to negotiate the legal transfer of biological material across borders, but this is not true for all countries. Perhaps the application of some of the Creative Commons concepts discussed here can further facilitate this to the great benefit of international mycology and microbiology.

Over the next few months, the IMA will be reaching out to other national and international bodies representing scientists who use microbial cultures, and to the representatives of the Creative Commons movement, to campaign for mechanisms to be developed that will enable the possibility for legal designation of selected cultures as Open Access. This will be an onerous country-by-country task because implementation of the protocol is at the national level and may require amendments to the Nagoya protocol, but we should not shirk this task. We hope that you will support the IMA in this, with the aim of stimulating the safe, legal exchange of microbial cultures among researchers for use in public good research and teaching, while protecting the sovereign rights of all countries.

I am grateful to Pedro W. Crous, David L. Hawksworth, Lene Lange and Oded Yarden for discussions during preparation of this editorial.


Keith Seifert
President, IMA
(keith.seifert@agr.gc.ca)

Fig. 2. Keith Seifert (left) with his colleague and former post doc Damodar Shenoy (right) of the National Institute of Oceanography, Visakhapatnam, India, in the Western Ghat mountains, October 2015 (photo: Ashish Prabhugaonkar).
The impact of life-threatening human diseases caused by fungi has been severely under appreciated. Estimates of the number of people dying from fungal diseases are greater than 1.5 million, which is more than the numbers that die from malaria or tuberculosis. Unfortunately there are few antifungal drugs to treat these deadly fungal diseases and resistance against antifungal drugs, particularly azoles, is increasing. As a result of a major investment by the University of Manchester, the Manchester Fungal Infection Group (MFIG) was established in 2013 to address these extremely important problems. The MFIG joins the National Aspergillosis Centre, headed by David Denning, and the Mycology Reference Centre Manchester, led by Malcolm Richardson, which are both based at the University Hospital of South Manchester. There are currently over 75 clinical and non-clinical researchers working on human fungal diseases in Manchester, and it is now the main global centre for research on aspergillosis and *Aspergillus fumigatus*.

The MFIG is focused on performing basic and translational research, which is being integrated with clinical research on fungal diseases in Manchester. A significant component of their work is also on antifungal drug discovery and target validation through collaborations with industry. The MFIG's research programme is primarily concentrated on *A. fumigatus* as an experimental system and the research complements the outstanding work being performed in other UK centres of excellence focused on studying human fungal diseases, particularly the Aberdeen Fungus Group that is mainly working on *Candida*. The research at the MFIG is covered in four main themes: (1) Cell and molecular biology of the fungal-host pathogen interaction; (2) *Aspergillus* genetics and genomics; (3) antifungal drug discovery, mode of action and resistance; and (4) the genetic basis of human susceptibility to fungal disease. The MFIG group is led by its Director, Nick Read, supported by the Deputy Director Elaine Bignell and Principal Investigators Paul Bowyer and Mike Bromley. Currently the MFIG is home to over 40 postdoctoral researchers, PhD students, and technical and administrative support staff. Recently, Jorge Amich has joined the MFIG after being awarded a prestigious 5-year Medical Research Council (MRC) Fellowship. The MFIG’s research is well supported financially by grants from the MRC, Wellcome Trust, the European Union, Blackberry Therapeutics, Du Pont, Genon Laboratories, the Global Action Fund for Fungal Infections (GAFFI), and the Fungal Research Trust.

The MFIG was officially launched on 9 September 2015 and the launch event was attended by over 100 academics and representatives from industry, including many collaborators. The scientific programme covered the significance of fungal diseases, recent advances in our understanding of their mechanistic basis, and how this understanding is being applied to develop novel antifungal therapies. Beside the MFIG Principal Investigators, the invited speakers were Jack Edwards (University of California at Los Angeles) who described the trials and tribulations of developing a fungal vaccine, and Gerald Bills (University of Houston) who spoke on the latest class of antifungals, the echinocandins, now selling nearly $1 billion annually. The Keynote Lecture was given by Keith Gull (University of Oxford) who highlighted our current lack of knowledge about infectious diseases in general. Overall, it was an extremely successful, high profile event that exemplified the range of interdisciplinary skills, specialised resources and critical mass of researchers necessary to bring about major breakthroughs in tackling life-threatening fungal diseases. It also illustrated how much exciting interdisciplinary science is now being undertaken in the field of medical mycology and how critical it will be to communicate this important area of research to other scientists, the general public and policy makers.

Nick Read
(nick.read@manchester.ac.uk)
Fungal conservation and IUCN Red Listing

Awareness of the importance of fungal conservation has steadily increased following establishment of the International Society for Fungal Conservation (ISFC) at IMC9 in Edinburgh in August 2010. Today, the ISFC has over 400 members from more than 70 countries, and acts as a coordinator and point of focus for fungal conservation initiatives worldwide. Membership is currently free; please submit details to "Membership" at: www.fungal-conservation.org.

While ISFC members have been active at national level, two initiatives are highlighted here of global and regional scope.

Anders Dahlberg and Michael Krikorev (Sweden), and Greg Mueller (USA) have developed a mechanism to promote Red Listing of fungi. The Global Fungal Red List (GFRL) Initiative comprises a website (www.iucn.ecko.se) where mycologists of all nations are invited to nominate threatened fungi that might qualify for IUCN Red Listing and to collate all information necessary to support a Red List application to IUCN. Anders and Greg also personally assist mycologists with their proposals for Red Listing to ensure that all criteria and support arguments are of a standard acceptable to IUCN.

Why should we consider Red Listing fungi? IUCN Red Listing is a globally recognised alert for governments to value and protect designated taxa, knowing that their threat status has been formally recognised internationally according to agreed criteria. While fungi constitute the second largest kingdom of multicellular life, they barely rank in most conservation efforts, in part because few have been considered for Red List status. In fact, IUCN (2015) includes just 5 Red Listed species of fungi (1 agaric and 4 lichens), compared with 11,877 species of animals and 10,896 plants. Through the GFRL Initiative and practical guidance in the application of IUCN assessment criteria to fungi (e.g. Dalhberg & Mueller 2011), mycologists now have a supportive mechanism by which to engage with fungal Red Listing. Anders and Greg have collaborated with mycologists globally to develop over 25 fungal species Red List proposals for submission to IUCN by December 2015.

Meanwhile, regional mycological congresses provide the opportunity to bring mycologists together to discuss and raise awareness about fungal conservation needs. Membership of ISFC is highest among Indian mycologists, and the Asian Mycological Congress in Goa, October 2015, provided a venue to engage this topic. A symposium on fungal conservation was co-organised with KV Sankaran (India), comprising nine speakers and attracting the largest audience of five concurrent sessions. Papers included case studies of threatened Asian species, molecular detection, culture collection preservation of fungi, information resources for fungal conservation in India, red listing, and an introduction to the ISFC – the latter presented remotely by ISFC President Dave Minter (UK) via a downloaded video file.

All mycologists with concern about the threat status of fungi are encouraged to join ISFC, to support inclusion of fungi in national Red Lists (www.nationalredlist.org), to participate in the GFRL Initiative for global IUCN Red Listing, and to submit articles about in-country or regional initiatives in fungal conservation to Paul Cannon (p.cannon@kew.org) for the ISFC society newsletter.

Participants at the Fungal Conservation symposium held during the Asian Mycological Congress in Goa.
VOLKSWAGEN FOUNDATION SUPPORTS THE AFRICAN MYCOLOGICAL ASSOCIATION (AMA) TO BUILD MYCOLOGICAL CAPACITIES IN WEST AFRICA

Mycology is presently regarded as a megascience that includes various aspects such as ethnomycoogy, food mycology, medical mycology, mycorrhizal symbiosis, taxonomy, systematics, phylogenetics, and ecology of fungi. Although modern mycology becomes a routine in temperate and boreal countries, very little is known about mycodiversity in the tropics. It is expected that tropical areas host a high diversity of fungi of nutritional, pharmaceutical, ecological, and agricultural significance. Tropical African mycodiversity can efficiently be used to face numerous food and health problems, and environmental degradation when human capacities have been generated, and when mycological teaching and research are integrated into native academic systems. In the present project, the Volkswagen Foundation (Germany) financially supports the University of Frankfurt (Germany), the University of Parakou (Benin), and the African Mycological Association (AMA), to reinforce mycological capacity in West Africa. From 2015 to 2017, a series of three summer schools of two weeks each will be organised at the University of Parakou. Target groups are composed of West African and German students and junior scientists working on different aspects of mycology. The coaching staff is composed of expert mycologists and botanists from Europe and West Africa. Through this project, we expect to sustainably promote a north-south transfer, but also a south-south exchange of mycological know-how among West African and European junior scientists. The overall goal is that the trained native West African and German students, but also the trainers, will establish a network of mycologists, and will thereby perpetuate tropical mycological teaching and research in order to sustainably launch West Africa in the mycological sphere. The Volkswagen Foundation also supports the improvement of mycological infrastructure at the University of Parakou.

For more information contact Nourou S. Youou (AMA Vice-President; n.s.yorou@gmail.com), Meike Piepenbring (piepenbring@bio.uni-frankfurt.de) or Karen Hahn (karen.hahn@bio.uni-frankfurt.de).

FUNGEN – A NATIONAL FUNGAL GENETIC RESOURCE FOR INDIA?

Genetic resource collections of fungi play a key role in conserving fungal biodiversity and making it available for research and exploration. Now a road map as to how the rich fungal resources of India might be made available on a national scale has been put forward (Suryanarayanan et al. 2015). This is envisaged as a complement to the existing collections in India, by focussing on securing fungi from little-explored habitats. FUNGEN (Fungal Genetic Resource of India) would be engaged in training graduate student mycologists in colleges and universities in isolation techniques, and operate on a crowdsourcing model with "mini-collections" developed at participating centres. Initial screening would then be undertaken at a national centre in one of the major cities. The development of a major collection is reminiscent of Subramanian’s (1982) vision and call for action, and now needs the financial commitment of government and state agencies to make FUNGEN a reality.
With the increased global concern over the sustainable use of natural resources, the need to find novel bioactive compounds of pharmaceutical interest, and the possibilities afforded by modern molecular technologies, it would be an opportune time to see such an initiative implemented.


CZECH CULTURE COLLECTION OF FUNGI (CCF)

CCF Culture Collection of Fungi

The Czech Culture Collection of Fungi (CCF) celebrated its 50th anniversary in 2015. The collection was started at Charles University in Prague in 1965 by Olga Fassatiová and now includes about 3400 isolates, mostly of ascomycetes and zygomycetes. Congratulations to Alena Kubítová and her staff, who continue the long tradition of microbial culture collections in Prague. The first ever collection of bacterial and fungal cultures selling isolates is reputed to be that started by František Král (1846–1911) at the Czech Technical University in Prague in 1885. Král’s collection was moved to Vienna after his death; some cultures were later taken to the USA and a few can be found in different collections today, but those left in Vienna were sadly lost in World War II (Ainsworth 1976). Today the CCF has a diverse and active taxonomic research programme, including Karel Prášil, Miroslav Kolářík, Ondřej Koukol, and Vít Hubka as well as Alena.


STATE KEY LABORATORY OF MYCOLOGY (SKLM) IN CHINA CELEBRATES 30 YEARS

The State Key Laboratory of Mycology (SKLM) of the Chinese Academy of Sciences (CAS) was first granted Key Laboratory status in 1985, then as the State Key Laboratory of Systematic Mycology and Lichenology. Located in Beijing, the Laboratory grew out of the former CAS Department of Mycology and Plant Mycology which dates back to 1953. In order to celebrate the occasion, the September 2015 issue of Mycosistema, 34(05), show-cases its work though 13 review and seven original research papers contributed by research groups from the Laboratory. The range of topics illustrates the impressive diversity of the Laboratory’s current research: taxonomic revisions, biodeterioration due to lichens, insect mutualisms, photoreceptors, fungal products, antifungal resistance, autophagy, quarantine lists, endophytes, heat tolerance of lichen bionts, and batch fermentation of Ophiocordyceps sinensis. Almost all papers are in Chinese, but are made more widely accessible by being accompanied by English abstracts and English legends to figures and tables. It has been a great pleasure for me to see the Laboratory develop since my first visit there in 1987, how the journal has gone from strength to strength, and for this to become one of the foremost world centres for mycology today.

FUNGUS AND GLOBAL CHALLENGES

Previous important and successful CBS Spring Symposia, One Fungus = One Name (2011), One Fungus = Which Name (2012) and One Fungus = Which Genes (2013), Genera and Genomes (2014), and the Second International Workshop on Ascomycete Systematics (2015) had a great impact on the mycological community. The CBS-KNAW Fungal Biodiversity Centre has now planned the 2016 Spring Symposium, “Fungi and Global Challenges” which will take place on 14–15 April 2016. One of the main topics of the symposium will be on how global and climate change impact on fungi, specifically those involved
Fungi and Global Challenges

This meeting will also see the launch of the International Fusarium Research Centre (IFURC), and several talks will focus on Fusarium systematics, impact, and relevance. In addition new developments on fungal taxonomy, as well as novel and emerging fungal applications, will also be considered. Contributed papers are welcome, and they will be selected for poster presentations. For more information visit: http://www.cbs.knaw.nl/index.php/meetings/659-fungi-and-global-challenges-2016

The symposium will include sessions on: Fungi, global and climate change; Fungi and human health; Taxonomic challenges: Fusarium; Fungi and food production; Fungi, novel and emerging applications; and New developments in fungal nomenclature and taxonomy. Some of the speakers who have already agreed to speak are shown in Box 1.

Takayuki Aoki (Japan)  
Balazs Brankovics (The Netherlands)  
Amanda Chen (The Netherlands)  
David Denning (UK)  
Geoff Gadd (UK)  
David Geiser (USA)  
Francine Govers (The Netherlands)  
Sarah Gurr (UK)  
David Hawksworth (Spain and UK)  
Fahimeh Jami (South Africa)  
Yanping Jiang (China)  
André Lévesque (Canada)  
Lorenzo Lombard (The Netherlands)  
David McMullin (Canada)  
Naresh Magan (UK)  
David Miller (Canada)  
Kerry O’Donnell (USA)  
Corné Pieterse (The Netherlands)  
Jack Pronk (The Netherlands)  
Martijn Rep (The Netherlands)  
Margarita Hernandez-Restrepo (The Netherlands)  
Keith Seifert (Canada)  
Anton Sonneberg (The Netherlands)  
Marc Stadler (Germany)  
Emma Steenkamp (South Africa)  
Joey Tanney (Canada)  
Paul Verwey (The Netherlands)  
Cees Wäldwijk (The Netherlands)  
Xuewei Wang (China)  
Patricia Wiltshire (UK)  
Mike Wingfield (South Africa)

ADVICE TO MYCOLOGISTS CONCERNING ARTICLE 57.2

Article 57.2 was a new article in the Melbourne International Code of Nomenclature for algae, fungi and plants (McNeill et al. 2012), introduced as part of the package of changes in the move from dual nomenclature to ‘one fungus : one name’. The Article requires a specific but rather confusing action by the Nomenclature Committee for Fungi (NCF) prior to taking up an earlier anamorph-typified name over a later synonymous teleomorph-typified name. That action is rejection by the NCF of a proposal to: (1) conserve the later name over the earlier name; or (2) reject the earlier name outright.

In the short time that it has been in operation, there have been a number of issues around the need for and application of Art. 57.2. It now seems quite clear that it should be removed from the Code and Hawksworth (2015) has formally published a proposal to delete the Article, on the basis of almost unanimous support for this action in the questionnaire distributed as part of the Nomenclature Section at the 10th International Mycological Congress in August 2014. Abolishing the Article also has unanimous support from the International Commission for the Taxonomy of Fungi (ICTF) and near unanimous support from the NCF.

The proposal to delete Art. 57.2 will be dealt with at the Nomenclature Section of the International Botanical Congress in 2017. In the interim, the Nomenclature Committee for Fungi will bundle up all situations covered by Article 57.2 and deal with them en masse (whether or not formal proposals have been submitted). The intent of author/s in regard to choices among competing names that fall under Art. 57.2 will be followed. This process will be carried out several times between now and the 2017 Congress, and reported on in IMA Fungus.

Therefore, mycologists are instructed not to submit formal proposals under Art. 57.2. However, it remains useful for the morph state to be indicated when making a choice among anamorph- and teleomorph-typified names, so that situations falling under Art. 57.2 can be identified by the NCF and dealt with en masse.

By sidelining Art. 57.2 during the period of its operation (and eventually removing it), the choice of names for fungi since dual nomenclature was removed on 30 July 2011 can be based strictly on priority, whatever the morph state of types of names. However, it should be remembered that if a later name is deemed preferable then conservation or rejection (individually) or ‘protection’ or ‘suppression’ (through a list) is, of course, still available as a means of taking up a later name, whatever the state present in its type or in those of competing names (anamorph or teleomorph; or indeed if the state of the type is uncertain).
Choice among competing anamorph- and teleomorph-typed names can be made by an individual author, but is most commonly being dealt with by international working groups. These working groups have been set up in consultation between the NCF and the ICTF to deal with the transition to one name: one fungus in important groups of fungi such as Dactidiomycetes, Leotiomycetes, Diaporthales etc. There are also working groups covering Phytopathogenic Fungi and Medical Mycology, that can deal with names that fall outside of the taxonomic scope of the other working groups. The working groups ensure that choice of competing names is dealt with by a community of experts in the particular group, taking into account both the usage of competing names and the views of user groups about the optimum choice of name.

In the case of any confusion about the application of Art. 57.2, authors are encouraged to contact the NCF Secretary.


---

Fungal Biodiversity Calendar 2017

In April 2013 the CBS-KNAW Fungal Biodiversity Centre launched its fungal calendar series, focusing on the beauty of fungal biodiversity.

The next calendar is scheduled for April 2016, and will be handed out at the “Fungi and Global Challenges” Symposium (14–15 April) in Amsterdam. To this end we invite all those making photographs or micrographs to submit their most beautiful fungal illustrations. Photographs of fungi cultivated in the laboratory, or observed in nature will be considered. Illustrations should be identified by the species name. Images should be in landscape layout, at least 300 dpi (3600 x 2400 px) and in full colour.

The mycologist who submits the most beautiful picture (selected by a CBS panel), will receive one CBS publication of choice. All submissions will subsequently also be added to MycoBank.

The publication of the 2017 calendar is scheduled for April 2016 and the submissions for the 2017 calendar are welcome until 26 February, 2016.

SHOW US YOUR FUNGI!

Submissions can either be sent to p.crous@cbs.knaw.nl or r.samson@cbs.knaw.nl. For large files we recommend using www.wetransfer.com, dropbox, or any other service that will allow you to share large files.
ASIAN MYCOLOGICAL CONGRESS (AMC) 2015

The Asian Mycological Congress 2015 was held during 7–10 October 2015 at the Dr Shyama Prasad Mukherjee Indoor Stadium, located in the picturesque Goa University campus of verdant Goa, India. The Congress was declared open at a grand inaugural session by Shri Laxmikant Parsekar, Chief Minister of Goa, on the forenoon of 7 October 2015. The function was presided over by Sarish Shetye, Vice-Chancellor of Goa University. B.N. Johri, President of the Mycological Society of India, graced the occasion with an inaugural address. A special First-Day postal cover was released on the occasion with an inaugural address. A total of 205 presentations, comprising 112 oral and 93 poster presentations were made.

- A Special Interest Group, led by Daiske Honda and S. Raghukumar, discussed the ‘Straminipilan fungi’ during the Congress.
- A special and much-needed workshop on ‘conservation of fungi’ was led by K.V. Sankaran and Peter Buchanan. A large number of delegates attended this session, and more information is provided elsewhere in this issue (pp. (40)–(41)). The establishment of an Indian Society for Fungal Conservation was proposed at the workshop.
- An ‘industry-academia’ interactive session, held at Goa Chamber of Commerce, Panaji, during the Congress, was moderated by Shreekumar Suryanarayan, a renowned technocrat and former vice-President of BioCon, India.
- A popular lecture, ‘Five Microfungi that changed the World’, was delivered on 8th October 2015 by Keith Seifert, IMA President, at the Instituto Menezes Braganza auditorium, Panaji, Goa. A large number of people from all walks of life attended and enjoyed this wonderful public lecture.
- A total of 205 presentations, comprising 112 oral and 93 poster presentations were made.
- In all, 276 delegates attended the conference. Of these, 67 were from overseas countries such as Japan, Korea, Malaysia, Nepal, Philippines, Singapore, Indonesia, Iran, Egypt, and Vietnam in Asia, besides ones from The Netherlands, UK, Germany, North America, Canada, Australia, and South America. A large number of delegates were from various universities and scientific institutes in India.
- The delegates were treated to two grand dinners, one of which featured a cultural program of Goan folk dances and music.
- The Asian Mycologist Award was given to K.R. Sridhar of Mangalore University, India, while the Young Asian Mycologist Award was given to Baokai Cui of Beijing Forestry University in absentia.

While the delegates included several senior mycologists and young researchers, representatives from mycological societies of the UK, USA, Canada, and New Zealand also participated in the Congress. Notable overseas mycologists included Keith A. Seifert (IMA President), Pedro W. Crous (Director, CBS-KNAW Fungal Biodiversity Centre, The Netherlands) E.B. Gareth Jones (UK), Peter Buchanan (New Zealand), and national representatives of the Asian Mycological Association. Several leading Indian mycologists came to Goa and participated in the Congress. Unfortunately, no Chinese delegates could attend as a consequence of delays in obtaining visas, and also none came from Thailand.

The Asian Mycological Congress witnessed several particularly interesting papers, both in oral and poster forms. One of the highlights was the session on ‘Marine and Freshwater Mycology’ led with a keynote address by E.B. Gareth Jones. Several poster award presentations were given. Overall, AMC-2015 turned out to be an intellectual feast of a rare kind to mycologists from this part of the world. As curtains were drawn for the event on 10 October 2015, in a brief closing ceremony, hosts of the next AMC in 2017, the Mycological Society of Vietnam, extended a warm invitation to all delegates. As part of the Organizing Committee, we are very happy and delighted that the programme was most fruitful and the event a memorable one.

D. Jayarama Bhat and Chandralata Raghukumar
(lata_raghukumar@rediffmail.com)
Scenes from the Asian Mycological Congress (2015) in Goa.
**2ND IRANIAN MYCOLOGICAL CONGRESS (IMYC2)**

The Iranian Mycological Society (IMS) was founded in 2009 during the 19th Iranian Plant Protection Congress, with the purpose of encouraging and promoting the visibility of mycology in its widest sense in Iran. One of the most important programmes of IMS was to hold national mycological meetings to gather Iranian mycologists nationwide in order to enhance and strengthen mycological integration and collaboration between members. Iranian mycological congresses are set to happen every two years.

After the successful organization of the 1st Iranian Mycological Congress (IMyC1) in 2013, hosted by the University of Guilan (Guilan province, Rasht), the University of Tehran, in collaboration with the IMS, organized the 2nd Iranian Mycological Congress (IMyC2) on 23–25 August 2015; meetings were held in the College of Agriculture and Natural Resources in Karaj.

IMyC2 consisted of diverse programmes, and apart from oral and poster sessions, organized two post-congress workshops (on the “population genetics of fungi” and “spore production for edible mushroom cultivation”), side-meetings, excursions (including free tickets for the swimming pool and museum of zoology in the University), a session on improving mycology teaching in Iran, the general assembly of IMS members, as well as an exhibition which was well-attended by participants.

The oral sessions consisted of six keynote lectures and 30 plenary lectures, and in addition there were 207 poster presentations. The themes of the congress were: biodiversity of fungi of Iran, phylogeny and taxonomy of fungi, population genetics and genetic diversity of fungi, biology of fungi, fungal biocontrol agents and their application, mushrooms and industrial fungi and their application, and the role of mycorrhizal fungi, endophytic and lichens in natural ecosystems. An interesting keynote lecture was presented by Walter Gams (The Netherlands) entitled “Lumpers and splitters, past and present”.

During a ceremony at the General Assembly, the IMS presented Walter Gams with the Award of Excellence in recognition and appreciation of the quality, originality and quantity of his published research, support of many young Iranian mycologists, and services to the field of mycology; he was also elected as an honorary member of the Society.

The IMS also established a new award, the “Dr Hedjaroud’s Award”, to be made every two years to an Iranian mycologist who has established an outstanding mycological career, or acknowledge the contributions of its members. Ghorbanali Hedjaroud, who founded this award, is currently Vice-president of the IMS, and a retired professor of the University of Tehran. The first “Dr Hedjaroud’s Award” was presented to Djafer Ershad, a retired professor of the Iranian Research Institute of Plant Protection; for 50 years of fruitful services to the field of mycology in Iran.

The Congress was closed on the last day with a report from the IMyC2 President and some artistic presentations, including poetry (an amusing mycological poem) and Shahnameh-Khani (the epic of the Persian kings) presented by children.

Mounes Bakhshi and Rasoul Zare (mounesbakhshi@gmail.com)

---

**ESSENTIAL SKILLS FOR YOUNG MYCOLOGISTS**

Students attracted to a research career in mycology are often interested in approaches that can help build towards such a professional future. In light of this need, a workshop in mycological skills was held recently for students and early career researchers (ECRs) at the 2015 scientific meeting of the Australasian Mycological Society (AMS) in Canberra, Australia. The workshop was organized and chaired by the AMS student representative Susan Nuske, and featured four AMS members with different areas of mycological research expertise. The presenters were Ana Traven (Medical Mycology), Jeff Powell (Fungal Ecology), Diana Leemon (Applied Mycology), and Tom May (Fungal Systematics). Their brief was to outline what had inspired them to become a mycologist, the skills they had acquired which had been instrumental in their careers, and how young scientists could gain these skills.

The first speaker, Ana Traven (Monash University, Melbourne), had started her scientific career at the University of Zagreb in Croatia where she completed a PhD in Mary Sopta’s laboratory on transcriptional regulation in the model yeast, *Saccharomyces cerevisiae*. After a postdoctoral position at St Vincent’s Institute of Medical Research in Melbourne and a short postdoctoral visit to Aaron Mitchell’s lab, then at Columbia University, she now runs the Fungal Pathogens and Gene Expression Control Laboratory at the Faculty of Medicine, Nursing and Health Sciences in Monash University. Ana focuses her research on the molecular mechanisms of pathogenesis in *Candida albicans*. She became interested in working with fungi when she realised they were great model organisms for understanding fundamental biological questions. Ana highlighted that networking is key to building a scientific career and that it was important to stay in touch with friends and colleagues who may provide opportunities for collaboration at some stage. PhD and Masters students should appreciate that their supervisor is their mentor but also that ownership of the project should be taken and that they should strive to be co-owners.

Medical Mycologist, Ana Traven.
Scenes from the Iranian Mycological Congress (IMyC2) held in the College of Agriculture and Natural Resources of the University of Tehran in Karaj on 23–25 August 2015.
help with experimental difficulties should be actively sought, and not necessarily just via their formal supervisors. Ana encouraged students to be brave: to embrace new technologies, to move locations if professionally favourable (e.g. to leading laboratories and universities), to ask “what is the most exciting thing to work on?” and to not be afraid to change research questions and even fields. Although it was important to continue to take up new challenges, she also recommended building on expertise and knowledge already learnt. As a molecular mycologist she suggested students and ECRs acquire skills in quantitative biology, bioinformatics, cell-imaging, in vivo animal and clinical models, and also work with other fields (e.g. chemistry, immunology, structural biology) to answer scientific questions. To be successful she also advocated thinking about how best to frame the scientific question, to do things differently or better than others in the field and to keep in mind that key experiments, can also be simple experiments.

Jeff Powell (University of Western Sydney) explained he did not intend to be a mycologist, and indeed this was his plan C (behind plan A of becoming a vet, and plan B of working in insect nematology). He became interested in fungi when he completed a PhD in soil ecology with microbial ecologist John Klironomos at the University of Guelph, Canada. He carried out postdoctoral research in ecological bioinformatics with Matthias Rillig in Berlin before moving to the Hawkesbury Institute for the Environment, University of Western Sydney. His current research is largely focused on community dynamics in mycorrhizal and other root-associated fungal communities, attempting to understand the mechanisms underlying these processes, their effects on ecosystem properties, and how they are impacted by environmental change. Jeff is concerned that the different ecological strategies occupied by fungi are often not appreciated by people that are trying to model and manage ecosystems. He is excited about working with this challenge. Jeff agreed with Ana’s philosophy of being flexible in one’s career ambitions and also in developing expertise that sets one apart from the norm. He also believed that targeting research groups with people who are well respected (and not necessarily the biggest and most prestigious laboratories) is the way to strategize one’s career. Statistics and the statistical computing and graphic software programs like ‘R’ are important skills for students and ECRs to acquire in the fungal ecology area. Jeff maintains that statistics are your friend (and not your downfall!) and that they are the only way to confirm suspicions about patterns in data. The best time to learn programs like ‘R’ is as a student. He also stated that one should never be intimidated by new techniques and that the best way to learn these was to shadow someone who uses them (if they can do it, you can do it!). One should also be nice to taxonomists and ‘classical’ scientists as they know so much more about fungi than you do. He advocated continually reading the scientific literature (including publications on other taxa) to find answers to questions you are pursuing or to find out that your research is covering uncharted waters. Jeff said don’t be afraid to ask questions or to share ‘dumb’ ideas. Such approaches can lead to useful scientific discussions, may lead to new questions being asked, and are a useful networking tool. Good ideas for research should be followed up on, and results of investigations should be published before someone else gets there first.

Jeff responded to a number questions from the audience. When asked “how do you know what journal to publish in?”, he suggested that one’s supervisor could assist here, or use impact factors or the expertise of the editor as a guide. When asked “how did he find time to supervise”, he said you have to sacrifice something, which for him was actually getting into the laboratory himself. On this theme, he strongly recommended that postdoctoral staff assist with supervising students (formally or not) as this was good experience for such staff and might lead to a name on a publication. When asked “how do you balance teaching duties with research” he and others suggested that time management and prioritizing was key.

Diana Leemon (Queensland Department of Agriculture, Fisheries and Forestry) stated that she had had a fairly unconventional research career. She had initially worked as a research assistant in plant pathology and timber pathology at Queensland University, then trained and worked as a High School biology teacher, before having a family. She then commenced a career in applied mycological research as a scientist in the Queensland Department of Primary Industries. While leading livestock ectoparasite biocontrol projects, she also completed a PhD through Queensland University investigating the mechanisms of pathogenesis in entomopathogenic fungi. She is now a Principal Research Scientist at the Queensland Department of Agriculture, Fisheries and Forestry, where she coordinates and is actively involved in a range of projects researching the fungal biocontrol of agricultural pests, including whiteflies, macadamia weevils, nuisance flies, and the small hive beetles that can devastate beehives in eastern Australia. Diana never ceases to be both amazed and fascinated by fungi in terms of what they are, where you find them, what they do, and what we can use them for (e.g. food, medicines, industrial chemicals, biocontrol). She also appreciated that many taxa are easy to grow in a Petri dish and therefore make good experimental subjects. The things that were instrumental to Diana’s career included a range of practical lab skills (particularly microscopy), general biological knowledge, a passion for understanding fungi, a wide breadth of knowledge about fungi, communication and networking skills and an ability to look for opportunities. She felt that the skills that are essential for a young mycologist to gain in applied mycology included learning all you can about fungi, coming to understand the fungal organism, developing a wide range of lab skills with fungi, gaining a good knowledge of the area you are working in (e.g. livestock industries, plants) and developing good communication skills. To develop these skills students and ECRs should regularly attend conferences, work on building up one’s research network, choose good mentors and not be afraid to
ask for advice, help or work experience. Diana also felt that it is critical to understand the industry you are working in and build links with key stakeholders, to understand the goals of your industry funding body and to continuously be alert for research and employment opportunities.

The final speaker of the session was Tom May (Royal Botanic Gardens Victoria, Melbourne). Tom had had a childhood interest in natural history and was exposed to citizen science during a spell in Scotland as a teenager, where he participated in the UK Bumblebee Distribution Maps Scheme. He completed a PhD in mycology at Monash University, in the days when time was not so pressing for completion; and had the luxury of working on his target genus (Laccaria) but also spending much time in the field getting to know all groups of fungi. He has been employed at Royal Botanic Gardens Victoria as a mycologist since 1990 and through close connections with community groups, established Fungimap, a not-for-profit citizen science association dedicated to the study and appreciation of Australian fungi. From a research perspective, he was initially interested in the population genetics of fungi but became sidetracked into checklists and taxonomy and now how has an emerging role in international committees (such as that of Secretary to the Nomenclature Committee for Fungi). The availability of new molecular taxonomic techniques is now being used to answer many of his initial questions on fungal population genetics. Tom’s favourite thing about studying fungi (apart from spending time in the field looking at fungi in the natural environment) is the detective work involved in taxonomy – bringing together different pieces of information to solve problems, such as what are the limits of a species or working out which genus a species belongs in. Skills that are important for fungal systematists include microscopy, culturing and the abilities to learn one facet/skill well and to look out for emerging methods (e.g. genomics, bioinformatics and pipelines). Tom also advocated getting to know fungi in the field – indeed many model organisms in the laboratory have trophic roles and relationships (often symbiotic) in the natural environment. It is also be important to be aware of the whole fungus; both asexual and sexual morphs.

Tom stressed that fungal taxonomy is a science and not an art and that voucher specimens are crucial for research and should be lodged in fungaria and culture collections while type specimens are vital reference points for the naming of taxa. Taxonomy is crucial to many branches of mycology and correct species delimitation has major implications for the repeatability of research and for practical aspects such as drug resistance and treatment of patients. Tom suggested that students and ECRs assess their own emerging skills, which can assist in making decisions to specialize in an approach, a technique or a taxon or be more flexible in your research expertise, keeping in mind you don’t have to be good at everything. Also, ask yourself do you like managing projects or providing the key component of projects? He also recommends taking opportunities to teach and to attend conferences — where you should present your work well, look out for emerging research and take the opportunity to network and establish collaborations. Keep in touch with your friends and contemporaries. Remember, that heavy-metal t-shirt wearing guy sitting near you in the undergraduate lecture theatre may one day be a future leader.

In summary, networking is a key skill for young mycologists, and indeed this has been recognized as a key aspect of any scientific career. Be brave; present your work at conferences, ask those niggling questions, be vigilant for opportunities for collaboration or jobs. Remember to keep in contact with your friends and contemporaries; you never know when an opportunity for collaboration will pop up. Flexibility is also key. New technologies and techniques may be intimidating but by mastering them, they may also be the crucial to answering your research questions. Choose a good mentor and shadow them for the techniques they can teach you. At the same time it is also good to build on the expertise and knowledge you have already learnt. By developing proficiency and doing things differently and better than others in your field, this may set you apart to get that job in mycology!

Susan Nuske and John Dearnaley
Australasian Mycological Society
(susan.nuske@my.jcu.edu.au; John.Dearnaley@usq.edu.au)
This new award of the Mycological Society of America (MSA) recognizes people with non-traditional academic backgrounds who have made outstanding contributions to the field of mycology, or who have widely transmitted significant scientific or aesthetic knowledge about fungi to the general public. The award is named in honour of investment banker Robert Gordon Wasson (1898–1986) and his Russian wife Valentina Pavlovna Guercken (1901–1958). Together they published Mushrooms, Russia and History (1957), and amongst later works his Soma: divine mushroom of immortality (1968) is especially sought-after. Wasson is widely regarded as a founder of ethnomycology, and his investigations into mycolatry, and the traditional uses of neurotropic mushrooms, not least Psilocybe species in Central America.

Nominees for the award are judged on the basis of the impact and quality of their contributions and on their sustained commitment to the field of mycology.

The first award was presented to Paul Stamets, who founded Fungi Perfecti LLC in 1980, by MSA Past-President D Jean Lodge on 29 July 2015 during the MSA meeting in Edmonton, Canada. Located in Olympia, Washington State, Fungi Perfecti has grown to supply diverse mushrooms and mushroom related products worldwide (http://www.fungi.com/). Paul has also been involved in the discovery of beneficial medicinal attributes of various mushrooms, on which he holds several patents. Through his numerous publications, courses, and lectures, Paul has made an immense contribution to raising public awareness of not only their nutritional and medical benefits, but of their importance in the maintenance of the environment – not least through his inspiring Mycelium Running: how mushrooms can help save the world (2005). His work was recognized by the award of an honorary DSc by the The National College of Natural Medicine, Portland (OR), and it is pleasing to see him now also honoured by his mycological peers. We wish him well in his future campaigning on behalf of the fungi, aware that, as he observed in accepting the award: “We walk this mycelial path of life together but time is critically short for us to make a difference” (Inoculum 66 (5): 27 Sept. 2015).

Katharina Bickerich-Stoll, author of popular mushroom identification books, enjoyed her 100th birthday in her garden in Potsdam-Rehbrücke, Germany, on 24 June 2015. Katharina lived in the former East Germany (GDR) writing and illustrating her books with her own drawings. Her main text was first published with 60 of her coloured drawings (Bickerich-Stoll 1960) and ran to five editions, the last and fifth being issued in 1970. Others followed in 1980 (Bickerich-Stoll 1980, reprinted 1990) and 1981 (Bickerich-Stoll & Gottschlich 1981, second edition 1986). Her works have become documents of part of a contemporary history of achievements made under difficult conditions. Her books surely inspired those who could obtain copies to forage for mushrooms to supplement their diets under the harsh regime of the times.

René K. Schumacher kindly drew this event to our attention and provided the photographs.

Photo courtesy René K. Schumacher.
Andreas Bresinsky’s 80th

The mycologist and botanist Andreas Bresinsky, emeritus professor of Botany at the University of Regensburg, turned 80 on 19 January 2015. He was born in 1935 in Reval (now Tallinn, Estonia), and after World War II came to Augsburg (Germany) and later to Munich. In Munich he studied biology, chemistry, and soil science from 1954, supervised by Karl Mägdefrau (1907–1999), and was habilitated at the early age of 29. In 1973 he accepted an appointment as professor at the new University of Regensburg, where he held the chair in Botany II for nearly 30 years. During this period he established a mycological working group with different foci, including systematics, chemistry, ecology, and physiology. At the beginning of the new millennium he retired, when a major tribute to his achievements was prepared (Besl & Schönfelder 2000).

More than 200 scientific publications attest to the breadth of his interests and research in both mycology and botany. Within mycology, he focused mainly on two subjects. First, the distribution and ecology of fungi, initiating, for example, the nationwide mapping of selected fungal species. He also compiled an overview of the German Boletales and Agaricales, revised Britzelmayer’s Hymenomyceten aus Südbayern in a long series of papers in Zeitschrift für Pilzkunde from 1964–81 (together with Johann Stangl), provided a mycological survey of the two Bavarian national parks, and recorded the fungi of Regensburg. Second, he obtained international recognition for his studies on the systematics of basidiomycetes, especially Boletales, based on fungal chemistry (in collaboration with Wolfgang Steglich), karyology, and molecular biology. Together with his colleague Helmut Besl, he prepared the Colour Atlas of Poisonous Fungi (Bresinsky & Besl 1985, 1999), a most useful and well-illustrated book for pharmacists, physicians, and biologists. Andreas’ international reputation enabled him to attract the 4th International Mycological Congress (IMC4) to Regensburg in 1990, a spectacularly successful IMC attended by about 1500 mycologists from all over the world.

We wish him all the best in this special year, and join our tributes to those prepared by Kämmerer (2015).

Andreas’ wife Birgit kindly provided the photograph included here.

Evi Weber
(one of Bresinsky’s students)
(evimariaweber@gmail.com)

Renowned lichenologist Irwin Brodo turns Eighty

When the magnificent, 800 page book The Lichens of North America by Irwin Brodo and photographers Sylvia and Stephen Sharnoff appeared in 2001 (Brodo et al. 2000), a communal gasp of wonderment swept far and wide. The challenge had been monumental, the product a shining moment for lichenology. Since that time, Irwin Brodo hasn’t slowed down. He is celebrating his 80th birthday with a new book, Keys to Lichens of North America, which he describes as “a spiral-bound

Andreas Bresinsky at the Nanhua Mushroom Market in Yunnan, China, in August 2014, with Ganoderma sinense. Photo: Birgit Wittmann-Bresinsky.
workbook of over 400 pages including keys to 2045 species.” Irwin (Ernie) Murray Brodo is a proud Canadian, but he was born in New York City (7 November 1935). He grew up in the Bronx and attended City College in Manhattan as an undergraduate, notably attributing his acceptance in part to a letter written by alumnus Frederick Lascoff, owner of the Manhattan pharmacy where his mother was a bookkeeper. He obtained his MS degree in Biology from Cornell University, and PhD from Michigan State University under the supervision of his esteemed mentor Henry Imshaug. He was hired in 1965 by the National Museum of Canada (now the Canadian Museum of Nature), where he continues to work as research scientist emeritus on a lichen collection (CANL) which he himself made into one of the best in North America.

He has published approximately 100 scientific articles, including in-depth, meticulously researched studies of challenging genera such as Bryoria, Lecanora, and Ochrolechia — all gold standards for monographic research. These and many other scientific accomplishments, not least his pioneering 1968 work on the lichens of Long Island and the effects of air pollutants, are nested within a continuous mission of service to the public (e.g., the Ottawa Field-Naturalists’ Club and the Ottawa-Hull Chapter of the Canadian Parks and Wilderness Society) and to the scientific community. He was President of the International Association for Lichenology (IAL) for a four-year term and of the American Bryological and Lichenological Society for a two-year term, as well as Editor-in-Chief of *The Bryologist* for several years, to name only a few. He also taught courses at the graduate level in addition to giving a multitude of introductory workshops. His field-course on crustose lichens at the Eagle Hill Institute in coastal Maine has been a perennial favorite for students.

In 2013, in recognition of his distinguished and continuing career in lichenology, as well as his scientific leadership in the international biosystematics community, he was awarded the degree of *DSc honoris causa*, by Carleton University (Ottawa). Other honours include the Acharius Medal (1994) – the most prestigious award of the IAL – and both the Mary Elliot Award (1993) and Lawson Medal (2003) of the Canadian Botanical Association, recognizing his outstanding professional service and lifetime research contributions.

At eighty, Irwin Brodo continues to inspire and indeed to serve as a rallying force for upcoming generations of taxonomists and systematic biologists, ecologists, conservation biologists, naturalists, and others working on lichens. Happy Birthday Ernie, from some of those you have inspired!

Hubertus Antonius van der Aa celebrated his 80th birthday on 5 July 2015. Huub was appointed at the Centraalbureau voor Schimmelcultures (CBS) in Baarn in 1965, charged with becoming an expert in coelomycetes. In those early years he worked alongside the Director, Josef Adolf von Arx (1922–1988), who stimulated Huub’s interest in plant pathogenic fungi. Huub skillfully made series of sections by hand of specimens of mostly plant inhabiting coelomycetes. He isolated numerous fungi that were identified by von Arx and himself, some thousands of which are kept in the CBS collection today. He started his taxonomic studies of the speciose and poorly understood genus *Phyllosticta* in 1966, and his training skills were also soon recognized in CBS and he became involved in teaching the CBS Course of Mycology. He was a driving force in developing this course, which he led for many years. Numerous students came to Baarn from all over the world and benefited from his broad experience and deep insight into the ecology of many important fungi. I met no one who knows more of the life-cycles of plant-pathogenic fungi occurring in The Netherlands from personal observations in the field than Huub! This unique knowledge allowed him to contribute to the influential five volume *Netherlands Ecological Flora* (Weeda *et al*. 1985–1994), by describing many examples to illustrate the importance of fungi to plant species and communities in The Netherlands.

In 1973 he published his thesis and first monograph of the genus *Phyllosticta* van der Aa (1973). Based on the generic concept developed then, he continued the painstaking work of studying thousands of (especially type) specimens which finally lead to the publication of *A Revision of Species described in Phyllosticta*, co-authored by his Bulgarian colleague and friend Simeon Vaney (van der Aa & Vaney 2002),


François Lutzoni, Stephen Clayden, James Lendemer, Trevor Goward, David L. Hawksworth (francois.lutzoni@duke.edu)

Huub van der Aa at 80 years

Huub van der Aa in 2002.

Hubertus Antonius van der Aa
who sadly passed away recently (see p. 55 below). Because of the taxonomic confusion of *Phoma* with *Phyllosticta*, Huub also started a very successful collaboration with Gerhard Boerema and co-workers of the Plant Protection Service in Wageningen who worked on characterizing *Phoma* in pure culture over many years. Many papers on *Phoma* appeared, and although he did not co-author it, Huub made very significant contributions to the *Phoma Identification Manual: differentiation of specific and infra-specific taxa in culture* (Boerema et al. 2003).

Besides his projects in CBS, Huub also became a prominent student of the teratology of basidiomes and plant galls inflicted by fungi and insects; building up a unique collection that has been recognized for its diversity and quality of annotations. He also contributed to the two editions of a book on Dutch galls (Docters van Leeuwen 1982, Docters van Leeuwen & Roskam 2009).

Huub served as Treasurer of the IMA from 1983 until 1990. He received a royal decoration in 2014 recognizing his great contributions to the Baarn community, most notably his many years of activity for the Cantonspark Foundation, but certainly also for his many years of service to the Dutch Mycological Society (NMV), as Secretary between 1974 and 1984, Editor-in-Chief of *Coolia*, and as the Librarian for the society for 20 years. Huub was granted honorary membership of the NMV in 1997.

It is with deep regret that we record the death on 4 February 2015, of one of the most experienced tropical plant pathologists of the mid-20th century, at the age of 91 years. His aunt, Elizabeth Blackwell who later became a president of the British Mycological Society, was a great friend of Edward Mason (1890–1975), the first mycologists at the then Imperial Mycological Institute (IMI) at Kew. Paul visited the Institute while he still at school, and learnt to call Mason „Teddy“, something no other mycologist ever dared to. After graduating from the University of Cambridge, where he came under the influence of the distinguished plant pathologist Noel F. Robertson (1923–1999), Paul went to Sri Lanka in 1940. This was perhaps as a part of his military service, but there he evidently gained a taste for the tropics and an interest in the diseases of tropical crops. In the late 1940s Paul went to the Cocoa Research Institute Trinidad investigating what we now know as *Moniliophthora perniciosa* causal agent of witches’ broom disease, in which he had a long-term interest (Baker & Holliday 1957).

From 1955–60 Paul was a part of the colonial pool of plant pathologists, based at what had then become the Commonwealth Mycological Institute (CMI), and ready to go wherever there were disease problems of tropical crops. During that time he was seconded to Sarawak to work on the Phytophthora disease of black pepper. After apparently then serving as a lecturer at University of Hull, he returned to CMI in 1968 as editor of the *Review of Plant Pathology*, a post in which he continued until his retirement in 1983. He contributed reflections of his time at IMI to the Institute’s history (Aitchison & Hawksworth 1993).

Editing an abstract journal which involved him scanning the world’s research publications in plant pathology, placed him in a unique position from which to prepare major reference works. His *Fungus Diseases of Tropical Crops* (Holliday 1980) is an extraordinarily thorough synthesis of the then current state of knowledge, including copious references and nomenclatural information on the pathogens, and which can still be consulted with profit today. While undertaking his editing work, he meticulously maintained a file of 5 x 3 inch cards on which he wrote notes of terms and tid bits of anything he considered of interest. In the years after his retirement, these cards matured into *A Dictionary of Plant Pathology* (Holliday 1989, 1998). The second edition has around 14,000 entries, and provides an eclectic mix of information modelled on the *Dictionary of the Fungi* which was compiled on cards in a similar way by Geoffrey C. Ainsworth (1905–1998), for some years in an office next to his. Paul will long be remembered through his

Paul enjoying his retirement party at Kew, 1983.

Paul will long be remembered through his

Several former colleagues of Paul, notably Clive M. Brasier, Harry C. Evans, and Jim M. Waller, are thanked for information while preparing this note.
Simeon Vanev (1937–2015)

Simeon Vanev. Photo courtesy Simeon’s wife Dora and granddaughter Ina.

Simeon Vanev was born on 14 September 1937 in Bulgaria where he studied biological sciences and obtained his PhD. Simeon worked first at the Institute of Viticulture and Enology in Pleven (Bulgaria) and then at the Institute of Botany of the Bulgarian Academy of Sciences in Sofia, where he became deputy director. Simeon also taught at the Faculty of Biology at Sofia University „St. Kliment Ohridski” and was been a long-term collaborator with many researchers at CBS, where he stayed several times for long periods to study various fungi together with Huub van der Aa, Joost Stalpers, Andre Aptroot, Richard Summerbell, Gerard Verkley, Pedro Crous, Vincent Robert, and many others. He published numerous papers on genera including Agaricus, Amanita, Apiocarpella, Ascochyta, Asteromella, Botrytis, Cytospora, Discosia, Fusicoccum, Gyroerytella, Krombholzia, Leptodothiorella, Microsphaeropsis, Monochaetia, Mycoleptodiscus, Peronospora, Phomopsis, Phyllosticta, Pleurotus, Ramularia, Sclerotagonospora, Seimatosporium, Septoria, Stagonospora, and Uncinula, and authored or co-authored 146 new fungal names or combination. His first publications date from 1960 with a paper on a plant disease caused by Sclerotinia rici, while his last major contribution to mycology was the book revising Phyllosticta prepard with Huub van der Aa (van der Aa & Vanev 2002; see (54) above). After that milestone contribution to fungal taxonomy, Simeon remained active for several years, and continued to work from home or at CBS on a number of databases related to aphylophoraceous fungi, Aspergillus, and Penicillium, and also on MycoBank.

Simeon was a family man, and when we shared an office he often talked about his wife Dora (a German philologist), his daughter Nadya (Nikolova, a lawyer), and granddaughter Ina (an architect). He missed them very much when working at CBS, but his dedication to science and to his work helped him to continue with his tasks. He was also passionate about classical music, literature, mountain hiking, and sport. We often discussed the latter when national teams were playing against each other, or major sporting events were taking place. Our discussions often ending in laughter because of his sense of humor. Having him as a colleague was a blessing. He was always careful for others, generous, smiling, polite, willing to help and such a nice and easy character. Simeon passed away on 17 September 2015, but will always be remembered as a gentleman and a great scientist with a great heart. We miss you a lot Simeon.

Vincent Robert
(v.robert@cbs.knaw.nl)
Rust fungi are one of the most diverse groups of plant pathogens and their divergence was thought to mirror the evolution of their hosts. Recently ancestral rusts were hypothesized to have angiosperm hosts, which altered the long-held view of rust evolution. Estimates on the age of rust fungi range from 150–300 million years ago (Ma), however, this had not been tested with a molecular clock.

In the study by McTaggart et al. (2015), a molecular clock was calibrated to the evolution of rust fungi on species of *Acacia* (~20 Mya), which have a rich fossil record in Australia. The ancestral *Pucciniales* were calibrated to the ages of divergence for either angiosperms (up to 194 Ma), or to the hosts of the most ancestral species of rust on gymnosperms in the cupressophytes (up to 256 Mya). Two ribosomal DNA genes (LSU and SSU) and a mitochondrial gene (CO3) were used for phylogenetic reconstruction with Bayesian evolutionary analyses.

Rust fungi were recovered with a much younger age than previously hypothesized, with a mean age between 113–115 Ma (full range between 70–161 Ma), when calibrated to angiosperms or cupressophytes. This new estimate of age provides evidence that host jumps, rather than coevolution, were the main speciation events that drove the evolution of rust fungi. Genera of rust fungi likely arose from host jump events and then diversified by co-speciation or taxonomically small host-shifts. Perhaps there is more plasticity in the host range of rust fungi, and host expansions on novel host populations that have not developed resistance will be common; this has already occurred with taxa such as *Cronartium ribicola*, *Puccinia lagenophora*, and *P. psidii*.


Host-jumps drove rust evolution


Just how plant pathogenic and root-infecting fungi are able to respond and grow towards chemical stimuli from plants has remained obscure. Now David Turrà and colleagues from the Universidad de Córdoba in Spain have been able to elucidate this phenomenon in the case of *Fusarium oxysporum* and the roots of *Solanum lycopersicum* (Turrà et al. 2015). They studied the germination of microconidia in the presence of a range of compounds, and elegantly demonstrated that the fungus was able to grow towards the roots as a result of a response triggered by class III peroxidases secreted by the plant roots. This involved a mitogen-activated protein kinase (MAPK) and a transmembrane protein Ste2 in the fungal cell wall. Intriguingly, the Ste2 protein is a functional homologue of the sex pheromone α-receptor in *Saccharomyces cerevisiae*.

In addition, the group went on to demonstrate that hyphal growth towards nutrients, including sugars and amino acids, is controlled by a particular MAPK cascade. While it is unclear how widespread the phenomenon is in root-infecting fungi, the genes involved would appear to be conserved and they interpret plant-sensing in complex environments such as soil as an unexpected alternative role for the fungal pheromone machinery.


Fusarium oxysporum.
## FIFTY KEY EVENTS IN FUNGAL SYSTEMATICS

<table>
<thead>
<tr>
<th>Taxonomy</th>
<th>Year</th>
<th>Nomenclature (and organization)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fungi recognized as a separate kingdom</td>
<td>1753</td>
<td>Starting point for “botanical” nomenclature</td>
</tr>
<tr>
<td>Treatment of the known fungi started</td>
<td>1821</td>
<td></td>
</tr>
<tr>
<td>Pleomorphism in fungi recognized</td>
<td>1851</td>
<td></td>
</tr>
<tr>
<td>Asexual fungi classified separately</td>
<td>1870</td>
<td></td>
</tr>
<tr>
<td>Incompatibility as a species criterion</td>
<td>1910</td>
<td>Category of special forms introduced</td>
</tr>
<tr>
<td>Keys to all known fungal genera</td>
<td>1912</td>
<td>Separate naming of morphs permitted</td>
</tr>
<tr>
<td>Ascomycete ontogeny linked to ascus types</td>
<td>1915</td>
<td></td>
</tr>
<tr>
<td>Categories of conidiogenesis</td>
<td>1951</td>
<td></td>
</tr>
<tr>
<td>Parasexual cycle discovered</td>
<td>1953</td>
<td></td>
</tr>
<tr>
<td>Numerical taxonomy of fungi</td>
<td>1954</td>
<td>Registration of fungal names proposed</td>
</tr>
<tr>
<td>Scanning electron microscope</td>
<td>1956</td>
<td></td>
</tr>
<tr>
<td>Taxonomy of Fungi Imperfecti conference</td>
<td>1958</td>
<td>Type designations mandatory</td>
</tr>
<tr>
<td>Sexual-asexual synthesis conference</td>
<td>1964</td>
<td>International Mycological Association founded</td>
</tr>
<tr>
<td>Ascomycete Systematics international workshop</td>
<td>1966</td>
<td></td>
</tr>
<tr>
<td>Amplified fragment-length polymorphisms introduced</td>
<td>1967</td>
<td></td>
</tr>
<tr>
<td>Saccharomyces cerevisiae genome sequenced</td>
<td>1971</td>
<td></td>
</tr>
<tr>
<td>Cladistics used in mycology</td>
<td>1985</td>
<td>Later starting points for fungal nomenclature ended</td>
</tr>
<tr>
<td>rDNA fungal primers introduced</td>
<td>1986</td>
<td>Rules on naming pleomorphic fungi revised</td>
</tr>
<tr>
<td>Oomycota placed in kingdom Straminipila</td>
<td>1987</td>
<td>International Commission on the Taxonomy of Fungi founded</td>
</tr>
<tr>
<td>Phylogenetics</td>
<td>1988</td>
<td>Systema Ascomycetum launched</td>
</tr>
<tr>
<td>Assembling the Fungal Tree of Life project</td>
<td>1990</td>
<td></td>
</tr>
<tr>
<td>Molecularly based ordinal classification of Fungi</td>
<td>1991</td>
<td></td>
</tr>
<tr>
<td>Next-generation sequencing</td>
<td>1992</td>
<td></td>
</tr>
<tr>
<td>DNA Barcode primers for Fungi proposed</td>
<td>1993</td>
<td></td>
</tr>
<tr>
<td>1000 fungal genomes project launched</td>
<td>1994</td>
<td></td>
</tr>
<tr>
<td>Reference Sequences for higher fungal taxa issued</td>
<td>1995</td>
<td></td>
</tr>
<tr>
<td>Phylogenomics</td>
<td>2000</td>
<td>Index Fungorum available online</td>
</tr>
<tr>
<td>Oomycota placed in kingdom Straminipila</td>
<td>2001</td>
<td></td>
</tr>
<tr>
<td>MycoBank registration system launched</td>
<td>2004</td>
<td></td>
</tr>
<tr>
<td>Amsterdam Declaration on fungal nomenclature</td>
<td>2006</td>
<td></td>
</tr>
<tr>
<td>Separate naming of morphs of pleomorphic fungi ended</td>
<td>2011</td>
<td></td>
</tr>
<tr>
<td>English allowed as an alternative to Latin for diagnoses</td>
<td>2012</td>
<td></td>
</tr>
<tr>
<td>Electronic publication permitted for new names</td>
<td>2012</td>
<td></td>
</tr>
<tr>
<td>Registration mandatory for new fungal names</td>
<td>2013</td>
<td></td>
</tr>
</tbody>
</table>

INTRODUCTION: FUNGI

Fungi (Figs 1, 2) are a group of eukaryotes representing a unique kingdom in the Tree of Life (Kendrick 2001). Representatives from two other kingdoms, i.e. Oomycota in the Straminipila, and Myxomycota in the Prototaxa, have morphological similarities to the fungi and were traditionally dealt with by those studying fungi (mycology), which remains the case today. Members of the fungal kingdom are immobile and are defined by the formation of threadlike structures called hyphae that form the different tissues of the fungus, an absorptive metabolism and reproduction with spores of diverse shapes formed by morphologically different structures (Boddy & Coleman 2010). They represent diverse general groups commonly called mildews, moulds, lichens, mushrooms, rusts, smuts, cup fungi, and yeasts.

In nature, fungi occur in every niche imaginable, including ice fields, deserts, rocks, tropical forests, any form of water, soil, air, on diverse organisms such as plants, animals and arthropods, and in decaying organic matter. In these diverse substrates they have various ecological roles, such as breaking down organic matter for recycling, forming unique mutualistic relationships with their partners, or as pathogens killing or causing disease of their hosts. Fungi impact on humans as pathogens of humans, livestock and crops, contaminants of food and living environments, or they are instrumental in making bread, alcoholic beverages or medicine. The biodiversity of fungi and the complexity of their combined or individual functions, are too wide for individual mycologists to fully study in one lifetime.

Fungi are largely microscopic but have forms that are visible with the naked eye by trained as well as untrained eyes. Estimates put the number of species in South Africa alone to at least 172 000 species, taking into account a number of niches but not all (Crous et al. 2006). The estimated number of species in the world is considered to be at least 1.5 million and probably 3 million (Hawksworth 2012). These most likely are all underestimates due to the numerous niches inhabited by different communities of fungi such as soil and the guts of insects, and because studies continue to uncover yet more unknown species and even novel niches. This represents a taxonomic dilemma with an ever-dwindling number of systematists who are able to describe or at least characterize these fungi, even if using only molecular tools (Taylor & Hibbett 2013).

Fungi, together with numerous other microscopic organisms (Cowan et al. 2013), are often treated by users of species names such as ecologists, conservationists and quarantine authorities, as a black box representing a group of organisms impossible to deal with (Gryzenhout et al. 2012). This is true throughout the world, and the case in South Africa. This is compounded by the ecological impact of fungi being largely unstudied even if fundamental (Gryzenhout et al. 2010). Because this impact is difficult to measure, it is thus easily ignored and omitted from conventional ecological surveys. However, biodiversity, ecological surveys and the systematics of fungi are equally important to study as for other organisms such as plants and larger animals, and equally possible. Although funding available for biological surveys continues to be in a state of flux, biodiversity awareness for fungi has been increased in South Africa over the past two decades.

As with larger animals and plants, it is possible to engage with members of the public who are interested in various strange or unknown mushrooms they encounter. Larger fungi are incredibly photogenic, enigmatic to simply strange, and various beautiful books and websites exist (e.g. Lockwood 2002; http://www.taylorlockwood.com). Fungi are also important in the traditions of many human cultures (a field known as ethnomycology), including those of numerous African countries (Gryzenhout et al. 2012). It is thus possible to include fungi in greater ecological studies by scientists other than mycologists. However, in South Africa knowledge of our fungi is difficult to access, and the interest in our fungi is relatively low; further, the number of professional mycologists with the needed expertise, time and capacity, is critical. This commentary explores the possibility of engaging with the public to enhance the image of mycology and to also generate scientific data in an.
Fig. 1. Various types of larger fungi. (a) Three types of bracket fungi commonly found in forests; white arrow, the tropical cinnabar bracket (*Pycnoporus sanguineus*); grey arrow, the black cork polypore (*Trametes cingulata*); dotted arrow, the false turkey tail (*Stereum ostrea*). (b) *Anthracophyllum archeri*, a bracket fungus from Mpumalanga identified from an Australian field guide. (c) The gigantic *Macrocybe lobayensis* first discovered by a field guide user in the iSimangaliso Wetland Park, but remained unknown until the identity was obtained from an Australian mycologist. (d) An unknown fungus, still unidentified. (e) Minute basidiomes of a possible *Mycena* sp. on a rotting leaf.
effort to address these problems. It is my hope that this document will be useful to promote mycology among the public and circles where fungi are not as well-known.

**THE USEFULNESS OF FIELD GUIDES AND BIODIVERSITY DATA**

Field guides are vital for various biological disciplines. They do not only function to be used by laymen interested in what is in their garden or what was seen during a recent hike. They are equally sought after by professional biologists seeking to identify specimens, those exploring a different niche or geographical area, undertaking biodiversity and ecological impact assessments, or involved in multi-disciplinary investigations for instance where hosts of organisms, such as plants, must be identified. They are vital in the training of students and their research. More than one guide for a particular organism group is often useful to showcase other plants not previously included, to provide additional information and illustrations, or is more suited to different individual preferences.

Over the past decades a flurry of diverse nature guides has been published in South Africa. For some biological groups more than one specialized nature guide exists. These originate from scientific experts who are professional biologists in universities, museums or research institutes, or those working in the field, such as conservationists and ecological officers. Often, however, citizen scientists or groups doing these out of passion and as a hobby, also drive, write or significantly contribute to these guides. A good example in South Africa is the numerous publications brought out by the Lepidopterists’ Society for Africa, Lepsoc (http://www.lepsoc.org.za/publications/books-posters-ebooks/).

Unfortunately the same is not necessarily true for fungi. Two currently available field guides (Branch 2001, Gryzenhout 2010) can be obtained on the mushrooms of South Africa, and two previous guides (Levin et al. 1987, van der Westhuizen & Eicker 1994) exist but are out of print. However, these guides only contain a small fraction of the fungi known in South Africa. There is no current checklist of all, or even some, of the fungi occurring naturally, especially of native fungi and those that are not necessarily in a collection. Those professionals needing fungal biodiversity data for national biodiversity initiatives or where ecological data needs to be generated, often lament this state of affairs.

There are few focused, continuous and large-scale surveys with published data to add to our knowledge of the native fungi of South Africa, especially when compared to other biological disciplines. This is especially true for the larger fungi. However, surveys on specific fungal groups, often microscopic forms, are still undertaken by current researchers (e.g. Marincowitz et al. 2008). No professional mycologist is, however, in the position of being able to only undertake biodiversity surveys and describe fungi, as has been done in some other biological disciplines, since funds for this must always be channelled from applied research these mycologists are employed to do. Expertise and time for descriptions are dwindling. There are now very few mycologists who can devote significant portions of their time to assist citizen scientists in organized efforts to generate such data, even if the capacity and interest exists. These facts have been summarized by the South African Fungal Diversity Network (2013).

What are the reasons for this information dilemma? There is a limited number of mycologists in South Africa, and very few opportunities of positions. Existing research is often restricted or focused in certain research topics or groups of fungi, and a limited number of surveys can only cover a small portion of the fungal diversity of South Africa. Many fungi are also seasonal organisms that occasionally need specialized environmental conditions to produce sporophores, hence efforts to find more samples of scarce species can be serendipitous. This situation is compounded by fungi being so biologically and morphologically diverse, that the sampling and expertise to collect the full taxonomic diversity does not overlap. Collecting is often quite specialized, needing isolations and purifications on artificial growth medium that are complex and time consuming (Fig. 3b). No less than 21 specialists required to undertake an intensive fungal inventory have been recognized (Hawksworth et al. 1997), and diverse methods and approaches are required (Mueller et al. 2004).

Despite these challenges, the current state of mycology in South Africa at the moment is not that dire. Mycology has a rich history in South Africa with a dedicated National Collection and a number of internationally known and active mycologists (Rong & Baxter 2006). Information is generated and published in reputable international, scientific, and specialized journals. However, most of the current scientific expertise currently available in South Africa focuses more on the microfungi, particularly those concerned with tree diseases, while the larger fungi and lichens that can be included in nature guides, are poorly studied with needed macrofungal and lichenological expertise for assistance to the public becoming less.

**IS THIS GOOD OR BAD?**

Is it necessarily bad that there is not a consistent series of field guides to the fungi of South Africa and no current checklist? International cutting-edge research in mycology has been, and still is, being produced by South African mycologists. A very strong knowledge base on fungal diseases of plants, humans and animals exists with previous exploratory research on fungi reported (e.g. Crous et al. 2000, 2006). Furthermore, the systematics of some fungi in South Africa is published and highly developed. Since it is impossible to cover the entire diversity of fungal groups, it is in any case a daunting task to satisfactorily study all fungi in South Africa. The National Collection of Fungi is still active and continuously improving its collections, infrastructure, biodata, and initiatives. All of these activities indicate that some level of funding and effort can be used to produce data. Furthermore, a level of awareness has also begun in official circles regarding the importance and presence of fungi with the hopeful need to support mycological studies. A great deal of excellent mycological research in South Africa has focused, and still do, on applied issues such as plant and forest pathology, food safety, food mycology, industrial mycology, and the role of fungi in the health of humans and animals. Undoubtedly this is because that is where the funds are. The benefit of this is that mycologists know very well what the applications of their work are, and how exploratory work can be utilized, for instance, to create new opportunities in biotechnology, and aiding crop and food safety.

Unfortunately the pressure to deliver applied research and to successfully procure funds leaves little space for basic exploration and characterization of our rich mycological biodiversity. This includes those willing
to do it, while others are often simply not interested. This results in that although passionate about fungi, many mycologists do not have the time, capacity or funds for continued exploratory and descriptive work, or even to train and assist others to do so.

Past biodiversity studies of fungi have not necessarily been continued, and thus represent occasional and fragmented reports. Fungal data also need to be presented more in fields such as ecology, biodiversity, and conservation, where exploratory and biodiversity work is still being done. This lack of representation contributes to fungi being generally ill-funded compared with larger animals and plants, and that the presence and usefulness of fungal data are not understood or appreciated by non-mycologists. There are consequently very few service points devoted to assist users of fungal names in
The lack of elementary biodiversity data in the form of available checklists, comprehensive scientific literature such as monographs, databases and the more user-friendly nature guides, unfortunately does have implications. The continued lack of data for users, and their inability to understand, explore and note the presence of fungi, may contribute to fungi remaining obscure and unfunded. Without any type of list, fungi simply cannot be included in biodiversity databases. Whereas now we can still make a case that it is necessary to produce such data and continuously add to the deficit we already have by means of new surveys and publications, the time may come when this will not be enough anymore. The need to promote fungi may later depend more on the availability of data and visible efforts to expand this, than on the continued absence of data, because this may be seen as inactivity and unreliability of the community. The inability of users readily to obtain fungal data may later lead to continued exclusion of such data from ecological services, whereas continued initiatives, even if limited, would have promoted mycology more. Therefore, while currently there may be a heightened awareness of general biodiversity in South Africa, if mycology is not able to utilize this momentum it may be more difficult in future.

**CAN WE REMEDY THIS?**

Training of future mycologists passionate about fungi continues to be the duty of current professional mycologists. However, it is unlikely that the near future will see a boom in the number of mycologists able to undertake such work unless the importance of mycology becomes recognized in education circles and with the promise of adequate work opportunities. This foundation is crucial to ensure that a sound knowledge base remains available.

Currently very limited capacity exists to deal with gaps in our knowledge of South African fungi. Here, lichens (Fig. 2c–f; a mutualistic association between fungi and algae or cyanobacteria) and larger fungi such as mushrooms, are of most concern, because they are what the public observes and wonders about. Currently there is no mycologist (or lichenologist) active in research, describing new species, collecting throughout South Africa, and publishing works for non-mycologists. Other ways have to be sought to generate novel data, or even to collate existing data.

Active collaborations with experts based in other countries can be sought, and they can visit South Africa, collect and publish their data following the appropriate regulatory procedures. Many such experts have their own, successful public awareness initiatives and engagement with appropriate authorities. However, random visits will not necessarily meaningfully contribute to the dilemma of data, unless these visits are communicated, co-ordinated, and fed into our national initiatives.

Unless more professional mycologists can be trained, knowledgeable citizen scientists will be crucial to the generating of data. From my personal experience and interactions with members of the public (Fig. 3a), it is clear that there are resourceful, passionate, and knowledgeable people with an ardent interest in fungi. Many of them are excellent photographers that take meaningful photographs once they know what is important to look for. An example is the first report of _Entoloma virescens_ from South Africa, a bright blue mushroom better known from Australasia, by a citizen scientist (Carbutt & Gryzenhout 2011).

Often people who did not really know anything much about fungi, became intrigued once properly introduced to them. This is usually because they did not know that fungi were so common, diverse (especially when introduced to microfungi as well), ecologically important or impacting so much on the lives of humans. During “fungal walks” or when dealing with queries, it is unfortunately frustrating that the majority of finds are of unknown identity. However, this can be used to eloquently illustrate how little we know of our indigenous fungi, and the need to study them.

Members of the public contribute tremendously towards various biological sciences in South Africa, and examples include CREW, LepSoc and Toadnuts (Young 2010, Anon. 2013). Members of the public, either independently or together with experts, have been able to establish meaningful datasets useful for science. This situation can be applied to mycology, because there are plenty of success stories for amateur science initiatives in mycology in other countries, where the public contribute distribution and ecological data for target fungi (e.g. Fungimap in Australia; http://fungimap.org.au) and several local societies compile biodiversity data (e.g. the North American Mycological Association (NAMA); http://www.namyco.org).

However, without guidance, efforts will not be co-ordinated and focused, nor will these be available to the greater scientific community.

**CAN THE PUBLIC DO IT ON THEIR OWN?**

For fungi, expert assistance is unfortunately essential. Mycology is a rather unusual, unknown discipline not given full exposure at school and often not even at tertiary level. Students are usually captured only at post-graduate level. There are too few guides to invite extensive study of local fungi, and other field guides produced in other countries often have to be used because there simply is no complete guide for visual identification. The most comprehensive South African guide (van der Westhuizen & Eicker 1994) contains at most 160 species, a small percentage of the estimated species in South Africa). There are no checklists against which to compare findings, and few experts to compile these. It is usually also unfortunate that often a level of microscopy will be needed, for critical identifications of even many larger fungi.

The lack of a species name need not be a hindrance. In the most recent field guide, Gryzenhout (2010) used a system of grouping fungi into their common name groups. If a member of the public encounters a large fungus that is unknown and not found in any existing guide, it can at least be referred to one of these general groups. That is to some degree satisfying, since it is not then a completely unknown fungus anymore; that step may also facilitate further identification by using other guides, or by sending the specimen to an appropriate expert.

The most current field guide (Gryzenhout 2010) is set up in a standard format that should be conducive to add more species in future. The field guide also did not depend only on the author, but was a combined effort from members of a network the author initiated, and who sent useable photographs the author would not easily have been able to obtain on her own. Although mushroom guides are seen as low volume overturn publications by publishers, it is hoped that the latest publication can be
expanded by additional volumes containing different fungi, and not merely showing the same species as those in previous guides.

**WHAT WOULD THE AIM OF CITIZEN INVOLVEMENT BE?**

Efforts to produce future field guides and public involvement in mycology should not only be there to feed the curiosity of the public, even though that in itself is an admirable goal. Feedback and input from the public can be used to produce a source of sustainable data for mycology. The public have the potential, with suitable guidance, to collect distribution data and produce photographic records, which most likely would not be attainable otherwise. The most enthusiastic may even be trained to collect and process specimens that can be lodged at the National Collection of Fungi for future study.

This would be in vain if not used to produce a meaningful, sound database of names, records, ecological data, and images accessible in future. Ideally this should be linked to service current scientific questions, initiatives, or deficits in the field of mycology. It would also be used where mycology may service other disciplines such as ecology, conservation, or greater national goals such as the sustainability of agricultural and natural resources, or studies in climate change.

Field guides, e-guides, or at least a photographic database, will be essential to collect and coordinate accurate data. It is likely that material for future field guides can already be produced. Creating awareness and engagement will be important to
ensure that future publications will be economically feasible.

A number of interactive, online database tools are available where data and images can be compiled (Silvertown 2009). Many of these were created to aid citizen scientists and contribute to professional science. Examples relevant to South Africa include iSpot (The Open University; http://www.ispot.org.za), a citizen identification service or survey tool developed to share observations, to obtain identifications (with varying levels of confidence), and to catalogue data and locations. Data are linked to other internet-based biodiversity databases. Results can also be published or are citable. A South African page including fungi has been initiated, and currently all data contribute to the biodiversity initiatives of SANBI [South African National Biodiversity Initiative, http://www.sanbi.org/].

A novel Facebook page, Mushrooms of Southern Africa (https://www.facebook.com/groups/MushroomsSouthernAfrica/) has recently been initiated by the Animal Demographic Unit (http://adu.org.za/) at the University of Cape Town. This unit has already initiated several citizen science groups where a diverse community of members has successfully compiled biodiversity data for various biological groups. The Mushrooms of Southern Africa facebook page was initiated in the first half of 2014, and had already grown to a membership of 1938 by 18 September 2015. This indicates that there is a large interest in the public to learn more about fungi and to get sightings identified.

The Animal Demographic Unit contributes significantly to creating distribution maps of several types of organisms in southern Africa. Similarly, a Mushroom Map database is currently being developed for the future creation of distribution data, with the input from a number of enthusiasts. With known species lists compiled from currently available fungal field guides, it is thus possible to actively use members of this page to compile curated observational data for species.

CONCLUSION

Fungi should become more visible and be promoted more. Information regarding the fungi occurring in South Africa should be available for a variety of users. Knowledge should be built in such a way that it can easily be expanded and accumulate in future. Such knowledge should be scientifically sound, and its accessibility should be permanent.

With the help of the scientific mycological community, the public can contribute meaningfully and can be trained. Members of the public interested in fungi are hungry and enthusiastic, but they need a champion (or champions) to provide assistance and continued impetus. Hard work and commitment will be necessary, or at least the necessary environment that enables these activities. It may well be possible that through such activities, the foundation of mycology in South Africa may be strengthened and may take mycology to the next level where it can be more competitive with other biological disciplines.

REFERENCES


Six simple guidelines for introducing new genera of fungi


1 Department of Plant and Microbial Biology, University of California at Berkeley, Berkeley, CA 94720-3102, USA; corresponding author e-mail: ecvellinga@comcast.net
2 Department of Soil Quality, Wageningen University, P.O. Box 47, 6700 AA Wageningen, The Netherlands; corresponding author e-mail: thom.kuyper@wur.nl
3 Department of Biology, University of Washington, Seattle, WA 98195-1800, USA
4 Department of Biology, San Francisco State University, 1600 Holloway Avenue, San Francisco, CA 94132, USA
5 Institute of Systematic Botany, New York Botanical Garden, 2900 Southern Boulevard, Bronx, NY 10458, USA
6 Department of Botany, Institute of Biology, National Autonomous University of Mexico, 04510 Mexico City, Mexico
7 Department of Biology/Natural History Museum of Denmark, University of Copenhagen, Universitetsparken 15, 2100 Copenhagen Ø, Denmark
8 Royal Botanic Gardens Victoria, Birdwood Ave, Melbourne 3004, Australia
9 Center for Forest Mycology Research, Northern Research Station, USDA-Forest Service, Luquillo, PR 00773-1377, USA
10 Department of Ecology and Evolutionary Biology, University of Tennessee, Knoxville, TN 37996-1610, USA
11 Department of Biological Sciences, Eastern Illinois University, Charleston, IL 61920, USA
12 Département des Sciences Végétales et Fongiques, Faculté des sciences pharmaceutiques et biologiques, Université de Lille, 59006 Lille, France
13 Chicago Botanic Garden, 1000 Lake Cook Road, Glencoe, IL 60022, USA
14 Naturalis Biodiversity Centre, P.O. Box 9517, 2300 RA Leiden, The Netherlands
15 Department of Biology, University of Central Oklahoma, Edmond, OK 73034, USA
16 Ghent University, Department of Biology, K.L. Ledeganckstraat 35, 9000 Gent, Belgium

Abstract: We formulate five guidelines for introducing new genera, plus one recommendation how to publish the results of scientific research. We recommend that reviewers and editors adhere to these guidelines. We propose that the underlying research is solid, and that the results and the final solutions are properly discussed. The six criteria are: (1) all genera that are recognized should be monophyletic; (2) the coverage of the phylogenetic tree should be wide in number of species, geographic coverage, and type species of the genera under study; (3) the branching of the phylogenetic trees has to have sufficient statistical support; (4) different options for the translation of the phylogenetic tree into a formal classification should be discussed and the final decision justified; (5) the phylogenetic evidence should be based on more than one gene; and (6) all supporting evidence and background information should be included in the publication in which the new taxa are proposed, and this publication should be peer-reviewed.

Key words: basidiomycetes, molecular systematics, nomenclature, phylogenetics, taxonomy,

INTRODUCTION

In 2014 and the first six months of 2015 alone, more than 20 new genera in Boletaceae were proposed. Most of these new generic names encompass species that occur in North America and Europe and that have been called “Boletus” for a long time. The numbers for Agaricales are comparable: we counted around 25 new generic names published in that same period, most of them white-spored, with six new genera for species we used to call Clitocybe, five new genera in Lyophyllaceae, and three new ones in Psathyrellaceae. The largest genus by far, Cortinarius, was not affected. In contrast, probably some 6–7 genera have now been subsumed under Cortinarius. With many new generic names being introduced for well-known species, it comes as an even larger surprise to note that there were very few new genera described based on newly discovered species with unique morphological character combinations. Examples of the latter are Ceropocyces crocodilinus from the Rocky Mountains (USA) and Hymenoporah paraduxus from China (Baroni et al. 2014, Tkáček et al. 2015).

The underlying principle for recognition of a genus, or any taxonomic rank for that matter, is monophyly (Hennig 1950, 1965). In the past this was extremely difficult to demonstrate in fungi, as the number of morphological characters that could be used was limited. It was also unknown whether some of the characters that were used, such as the formation of sequestrate or gastroid basidiomes, had a low evolvability, justifying recognition as separate genera, or a high evolvability, which then downplays its relevance in a phylogenetic context.

The flood of these recent new generic names has mainly been prompted by molecular-phylogenetic research and the resulting phylogenetic trees. It has led to re-evaluation of characters; in many cases sequestrate and gastroid forms were shown to have higher evolvability than assumed, justifying the subsumption of such genera under existing genera; for example in Suillus (Kretzer & Bruns 1997), Cortinarius (Peintner et al. 2002), Lactarius (Eberhardt & Verbeken 2004, Kirk 2015), Russula (Lebel & Tonkin 2007), Leotia (Ge & Smith 2013), and Boletus (Nuhn et al. 2013). Lichenization had low evolvability in basidiomycetes, justifying recognition of Lichenomphalia as a separate genus for species that were previously placed in three genera (Redhead et al. 2002).

However, such phylogenetic analyses have to a smaller extent than was hoped solved the problems of genus delimitation and recognition. Analyses have shown that several well-known genera remain paraphyletic, such
as Boletus (Nuhn et al. 2013, Wu et al., 2014) and Psathyrella (e.g., Padamsee et al. 2008). Dealing with the issue of paraphyly then set the mycological community on two, rather divergent, paths. These divergent approaches mirrored old distinctions of splitters and lumpers. One pathway was to take small monophyletic groups as the basis for new genera, without too much concern about the remainder of the original genus. This is a practice clearly shown by the treatment of Boletus and Clitocybe. Separation of one small monophyletic group often set into motion a splitting snowball, as in Xerocomellus (Gelardi et al. 2015, Vizzini 2015). Unfortunately, recognition of such small genera sometimes contributed to the formation of paraphyletic genera, as in the case of Resupinatus which became paraphyletic because of the recognition of R. velminius in a separate genus Lignomyces (Petersen et al. 2015). The second approach was taken in Entoloma and Clitopilus (Co-David et al. 2009), where deliberately a broad genus concept was chosen (though other authors opted for smaller genera (e.g. Largent 1994, Kluting et al. 2014). Other examples are Amanita (Justo et al. 2010) and Cortinarius (Peintner et al. 2001) where sequestrate and gastroid species were included in genera with predominantly agaroid basidiomes. It is an interesting question how this divergence should be explained; certainly, the existing taxonomy of the group in question plays a big role. In the boletes, for instance, many previously published generic names for well supported genera, such as Lecinum and Strobilomyces, were already available, and the proposal of new genera was (consequently) considered acceptable.

**PRINCIPLE CONCERNS**

We find several of the recent trends in mycological taxonomic research on basidiomycete fungi disturbing:

(1) In several groups, the translation from a phylogenetic tree into a classification is taken into extremes, where every single clade is recognized as a separate genus. This does not increase insight in the evolutionary history of the group in question, only inflates the taxonomic framework. From a formal phylogenetic perspective it may not matter whether we have one family (e.g., Boletaceae), with more than a hundred genera, or whether we have one genus (e.g., Boletus s. lat.) with many infrageneric units, formally named or not. In the case of the boletes, inclusion of more sequences from more taxa may impact the phylogeny, as the resolution of the phylogeny of Boletaceae is low at many branches (Nuhn et al. 2013, Wu et al. 2014). We strongly advocate that different options are explored and discussed, instead of using a boilerplate model in which every monophyletic clade is translated into a genus.

(2) Several of the new genera are erected solely based on phylogenetic evidence provided by one or two gene regions, sometimes only nrITS sequences that do not lend themselves to higher level phylogenies (Bruns 2001).

(3) More and more rapid on-line, non-peer-reviewed publications appear without any supporting evidence for the newly described taxa.

We therefore have formulated and present here criteria by which the phylogenetic studies and the publications that present the data from those studies should be tested before being accepted.

**PROPOSED GUIDELINES**

Some of our proposed guidelines are so self-explanatory and obvious to us that it seems superfluous to present them here; nevertheless, examples that are in conflict with these recommendations are surprisingly easy to find. The examples we present show how easy it is to meet these standards. We realize that practical issues, such as lost original type specimens, or material that does not easily yield DNA sequences may interfere with perfection, but one should at least try, and discuss failures. We emphasize the importance of exploring different classification options and giving arguments for the proposed new taxonomies. The examples we present are taken from the literature on basidiomycete fungi, but could equally have been chosen from the ascomycete literature.

The first five criteria relate to the underlying science; the first two criteria are equally important, and we put less emphasis on the next three. Nevertheless, it goes without saying that contradiction of any of these criteria should be avoided. We recommend first of all that researchers use these guidelines, but also that reviewers and editors of taxonomic journals use these criteria in their assessments of submitted manuscripts.

The sixth criterion concerns the way the results are published and presented to the scientific world.

1. All genera that are recognized should be monophyletic, not only the one that is the focus of the study, but also the group from which it is separated and the group to which it is added (the reciprocal monophly criterion).

Examples:

(i) When Macrolepiota was split into a monophyletic core Macropleiota with M. proceras as its type species, and a second group containing M. rachodes, the latter was moved to Chlorophyllum that in itself was only monophyletic by also including Endoppychum agaricoides (Vellinga et al. 2003) and all necessary nomenclatorial changes were made (Vellinga 2002, Vellinga & de Kok 2002).

(ii) The genus Porpoloma was found to be highly polyphyletic and split into four genera (Sánchez-García et al. 2014). It was essential for the application of names to clades to sequence the type species of Porpoloma (see Recommendation 2).

Unfortunately, many studies do not reach this standard, as it is very easy to expel species from a genus, without taking into account the monophyly of the target group. Especially in cases of poor resolution in a phylogeny, erection of very small monophyletic genera will create more and ever more messy paraphyletic units, from which the next separation of a new genus can already be predicted.

2. The coverage of the phylogenetic tree has to be broad.

Coverage needs to be broad in terms of the:

(a) number of species – it is important to remember that a phylogenetic tree only gives information on those taxa that are included in it, and that all statements on relationships are relative, and not absolute, unless all known taxa are included; (b) geographic distribution of the taxa – a phylogeny based on species from temperate areas of North America and Europe, is not informative.
if the group in question is represented by many more species in tropical Africa, Asia, and Australia; and (c) the data base should include type species of all genera that are being included, as the placement of the type species decides which name to use for a genus.

Examples:
(i) The genus Anamika was shown to fall in the middle of Hebeloma when specimens from Australia were added to the phylogeny mainly based on Eurasian species (Rees et al. 2013).
(ii) Only by including the type species of Pachypleurium in the phylogenetic analyses, could the position of P. carbonicola and P. fanariophilum unambiguously be determined (Matheny et al. 2015).
(iii) The position of Marasmius sect. Hygrometrici was determined by analyses that explicitly included the types of the genus Marasmius (and hence of sect. Marasmius) and sect. Hygrometrici (Jenkinson et al. 2014).
(iv) The type of Rubinobolus always clusters with Chalciporus, hence the genus is subsumed into Chalciporus (Nuhn et al. 2013).

3. The branching of the phylogenetic trees should have sufficient and strong statistical support.

Weak support (or even absence of statistical support) of proposed new genera indicates that alternative classifications cannot be rejected. And so the advice is: ‘in dubiis abistine’, when in doubt refrain from proposing new genera.

Example:
Lenzites warneri occupies an isolated and unsupported position within Trametes s. lat.; Welte et al. (2012) refrained from introducing a new generic name for this species, as it also does not have discriminating morphological characters. Justo & Hibbett (2011) also did not recognize it as a separate genus either, but included it within their concept of Trametes, which is broader than that of Welte et al. (2012).

4. A list of options should be discussed, different options should be tested, and arguments for the final decision given.

Phylogenetic methods often allow, even in well-resolved trees, more than one formal classification with monophyletic groups. Therefore different options should be presented and discussed.

Examples:
(i) Justo & Hibbett (2014), and Justo (2014) discussed the different options for and consequences of recognizing ten or five genera or just one genus within Trametes s. lat., ultimately opting for a one-genus solution.
(ii) Halling et al. (2015) tested different options for the circumscription of Boletellus and delimitation of that genus in regard to Heimioporus; these authors included the species that form a grade at the base of the core Boletellus within that genus, instead of describing new genera for each clade.
(iii) Buyck et al. (2008) presented and tested 15 different options for the phylogeny of the different clades in Lactarius and Russula, before settling on the solution of breaking up Lactarius into two genera, Lactarius and Lactifluus, and recognizing Russula sect. Ochricompactae as a separate genus, Multifurca.
(iv) Lodge et al. (2014) discussed options for recognition of one or three genera for Gliophorus, Porpolomopsis, and Neoxygonycte.

5. The phylogenetic evidence has to be based on more than one gene, preferably protein coding genes in addition to gene regions of the SSU-ITS-nSSU repeat.

The different gene regions that are commonly used in basidiomycete classifications all have different evolutionary histories, and hence they have different resolving power at different levels. Phylogenies based on nrITS sequences have not only to be approached with a large dose of skepticism, but in fact should no longer be accepted as the basis for new genera. This marker is the universal barcode for fungal species recognition (Schoch et al., 2012), and it performs this role generally well because of sufficiently large variation between related species within many, but not all, fungal groups; the price we pay for that species-level accuracy is that nrITS is unalignable over more distant related taxa (Bruns 2001). In the past, it was debated what was better, to have more taxa/collections analysed or more genes for fewer taxa (Greybeal 1998); with lower sequencing costs and faster computers, the answer is: more taxa and more genes. However, phylogenies based on whole genomes are still rare and include only a small number of species (e.g. Dentinger et al. 2015); and of course, whole genome comparisons will be faced with the same issues as the phylogenies based on a hand-full of genes.

Example:
In an analysis of three loci (nLSU, nSSU, and rpb2 genes) from a wide range of taxa in the tricholomatoide clade, Sánchez-García et al. (2014) recognized the new genera Corneriella, Albamagister, Pogonoloma, and Pseudotricholoma all with full statistical support.

6. All supporting evidence and background information should be included in the publication in which the new taxa are proposed; and secondly, this publication should be peer-reviewed.

The first part of this guideline is prompted by the appearance of very short publications associated with one of the official taxon registration sites, without any supporting evidence nor illustrations, sometimes with a link to another, often personal, web site where the supporting information (e.g. a phylogenetic tree) can be found. Communication of the results of science is not a one-time event. A basic principle of science is that the results are verifiable by others. If in future researchers cannot go back to the whole set of data and information, that principle cannot be applied. This situation is the same as in experimental studies where the cultures used are not preserved thereby rendering the experiments unrepeatable, and in field reports where no vouchers are retained. Paper publications of the past provided and continue to provide a source of information that can always be consulted; putting that information on line in official library depositories such as JSTOR is an extra safe guard.

Peer review, though not waterproof, and always debated (see e.g., http://www.nature.com/nature/peerreview/debate/) is recommended as quality control before publication. The function of peer review in the case of taxonomic novelties is not to exercise censorship of taxonomic decisions, but to judge new genera against these principles to which the mycological community should adhere.
CONCLUSIONS

We encourage all mycologists undertaking phylogenetic studies around the generic level to adhere to these guidelines, and further invite editors and peer reviewers to bear them in mind when considering a work for publication.

In recognizing that these problems are wide-ranging in mycology and may frustrate communication within the subject, we commend a cautious approach to introducing changes at generic rank.

Finally, we welcome and foresee critical assessments of introductions of new taxa at all taxonomical levels, especially families and orders, in all groups of fungi.

REFERENCES


Justo A (2014) The taxonomy of Turkey Tails (Trametes) and related polypores: One genus or too many?: Mycophile 54 (4): 8–10.


An ex-type culture cannot always tell the ultimate truth

This note is prompted by the case of the generic name Ochroconis, a rather common genus of saprotrophic soil hyphomycetes, some of which grow occasionally on humans and fish. Von Arx, in the 1970s, being mainly interested in producing keys to identify fungal genera in culture morphologically, did not believe that Scolecobasidium terreum (E.V. Abbott 1927) with Y-shaped yellowish conidia, and other species of this genus with darker, unbranched conidia were congeneric, as proposed by Barron & Busch (1962). Therefore, he let his young staff member G. Sybren de Hoog describe a separate genus, Ochroconis, for the latter, larger group (de Hoog & von Arx 1974). In addition to mycologists who regarded the similar colony characters and rhexolytic conidial liberation of all species involved as significant (e.g. Ellis 1971, 1976, Domsch et al. 1980), molecular findings (Horré et al. 1999, Machouart et al. 2014) have clearly shown this generic separation to be unwarranted. In addition two faster-growing halophilic species, S. salinum and S. arenarium were included in the genus by Ellis (1976), but they are now rightly excluded again and classified in Paradendryphiella Woudenberg & Crous 2013 (Woudenberg et al. 2013). The probable ex-type culture of S. terreum, CBS 203.27, no longer sporulates and phylogenetically falls outside the genus. It no longer shows the features that the ex-type would be expected to support, a misfortune that sometimes happens with a culture after decades of preservation.

Other comparable cases are Acremonium domschii W. Gams 1971, a species originally described as closely related to the anamorph of the species now named Cosmospora viridescens (C. Booth) Gräfenhan 2011 (Gräfenhan et al. 2011), but lacking green pigmentation; the ex-type culture of Gams’ name, CBS 764.69, however, now shows a fungus unrelated to Cosmospora and therefore that species name was omitted in the cited paper. Monoccilium arcticola W. Gams 1971 was described by Gams (1971) as characterized by rather large conidia, but the ex-type culture, CBS 994.69, now has narrow conidia and is unrelated to other isolates accurately matching the description of this species. The dried type culture still shows the correct fungus and DNA sequences of the other isolates representing this species can be taken as correct.

Unfortunate consequences arise when a wrongly identified fungus is designated as an epitype of a species, as happened with Hypocrea farinosa Berk. & Broome 1851 for which Overton et al. (2000) designated an epitype without studying the ex tant holotype. It was left to Jaklitsch et al. (2008) to correct the situation and to resurrect the generic name Protocrea Petch 1937 for this fungus and its relatives, while Overton et al.’s fungus is now known as Hypocreopsis decipiens Jaklitsch et al. 2008.

Sybren de Hoog, in order to retain the generic name Ochroconis, let his student K. Sameritak declare Scolecobasidium a dubious genus, because the identity of the type was uncertain to them (Sameritak et al. 2014), in spite of many reliably named cultures of S. terreum being available all over the world, which clearly define the identity of this characteristic fungus. One of these cultures discovered by Domsch et al. (1980) was the CBS 510.71 ex-type of Humicola minima Fassat. 1967, whose author had not seen the characteristic Y-shaped conidia that were formed on certain media by the original culture. Without mentioning the source of this information, Sameritak et al. (2014) made the combination Ochroconis minima (Fassatová) Samer. & de Hoog 2014, which would have to replace Scolecobasidium terreum. Needless to say, Ochroconis has not been recognized by Seifert et al. (2011). In the era of production of lists of fungal names for protection, the responsible committee should not be misguided and recognize the incorrect name for this genus.

Thanks to David L. Hawksworth for helpful suggestions.


Walter Gams (walter.gams@online.nl)
It is pleasing to see the lack of field guides and texts in mycology in Africa is being increasingly addressed. Marja Härkönen is probably the most experienced ethnomycolologist and mycophagist active in East Africa, and has already provided a handbook of Tanzanian edible mushrooms (Härkönen et al. 2003). She has now collaborated with other specialists and forest pathologists in Zambia to produce this splendid and superbly illustrated guide. This new guide is based on extensive travels in Zambia in 2012–13, which included field work and also visits to local markets, in-depth interviews, and demonstrations by local cooks.

The book is, however, much more than an identification manual. Indeed, almost half is devoted to background material which can serve as a starting point to anyone wishing to learn more about fungi, and especially macromycetes. It starts with an introductions to the history of mushroom exploration in the country, including handwritten notes made by explorer David Livingstone in 1867, before moving to explanations of what fungi are. Their diverse biology is described, stressing mycorrhizal associations (with a useful list of trees with different mycorrhizal types), but also covering associations with termites, decomposition, wood-rots, and seasonality. The ecological treatment is supplemented by illustrations of different habitats, pointing out the importance of miombo woodland and the issue of introduced species in pine plantations. Methods of collection and examination are detailed, with well-illustrated accounts of characters used in macromycete identification, which while emphasising macroscopic features also includes an introduction to microscopic examination.

The core of the book is the species accounts which cover around 90 species. I was pleased to see these arranged in broad morphological groups rather than in any systematic one, and also the omission of all author citations following scientific names – with a single exception for the new species Hexagonia culmicola described in an Appendix. Each species account covers any vernacular names discovered, a succinct summary of key points, a description and information on habitat, edibility, and pertinent notes. The colour photographs can only be described as stupendous! The inclusion of locality details and fungarium reference numbers in each of the legends follows the best-practice often lacking in such guides. Photographs of some of the species have not been available before. Those of the 12 Amanita species, a genus favoured by Zambians, are especially stunning, the bright yellows of several are sure to render dishes made from them appealing to the eye. For mycophagists, 19 recipes obtained from local people are included; several do not specify the species to be used as local practice is evidently to use mixtures. They did not find evidence of recreational uses, but I wonder if inclusion of a more specific question than “other uses” in the list of questions used in interviews (p. 201) might have generated some different responses.

This book has been made possible through funds made available by the Development of Cooperation section of the Ministry for Foreign Affairs of Finland. They are to be congratulated on their foresight in supporting a work which is both educational and will encourage the use of sustainable food resources in Zambia. This parallels the support La Coopération Belge au Développement gave to production of the French guide to edible fungi in dense central African forests (Ndong et al. 2011; see IMA Fungus 2 (2): (65)–(66), December 2011). It is gratifying to see government development agencies recognizing the importance of mycology in Africa in this way, and hopefully this may stimulate others to emulate such initiatives.


This major text is designed to bring together in a single place the latest information on food borne human fungal pathogens, including mycotoxigenic fungi. At the same time it provides background to our knowledge of the fungi involved, especially in the light of molecular systematic studies. This combination is especially welcome as applied mycologists do not always have ready access to explanations of current methodologies and procedures for the naming and characterization of fungi. The underpinning chapters address classification and naming, phylogenetic analysis, DNA barcoding, metabolomics, systems biology, and genomics. That on phylogenetic analysis (Muszewska & Gnínski) is particularly understandable and thorough, and can
be commended as background reading to mycologists of all kinds starting to interpret or use such information.

The bulk of the book consists of 25 chapters, each devoted to presenting authoritative up-to-date accounts of particular fungal genera, prepared by a wide range of specialists. The range of fungi covered and the depth of the treatments is impressive, and includes some that often receive scant attention in texts on either food-borne or clinically important fungi. I found the chapter on "Alternaria" (Summerbell & Scott) particularly comprehensive: most of these fungi are now placed in Sarocladium, and they caution that all reports of S. strictum should be confirmed by sequence data — a problem I can relate to from the years I was responsible for identifying these fungi at the International Mycological Institute. Also treated in detail are Chaetomium (Hubka) where species concepts are still far from resolved, Curvularia (Guarter), Microascus (syn. Scopulariopsis) (Abbot), Pichia (Pasoeth) with a valuable summary of the current disposition of species, Trichoderma (Kubicek & Druzhinina) with much detail on the entolites, and Wallavia (Zajc et al.) This is all in addition to coverage of genera that feature most strongly in many texts, including notably Alternaria (two chapters), Apergillus (three chapters), Candida, Fusarium, mucoraceous fungi, Paecilomyces, Penicillium, Phoma, Rhodotorula, Trichoderma (Quindos et al.) including a synopsis of described species, and various other groups of yeasts.

The book closes with two more wide-ranging chapters, one on the development of antifungal vaccines (Ito), and the other on the extrolites, and (Kubicek & Druzhinina) with much detail on the current disposition of species, Trichoderma (Kubicek & Druzhinina) with much detail on the entolites, and Wallavia (Zajc et al.) This is all in addition to coverage of genera that feature most strongly in many texts, including notably Alternaria (two chapters), Apergillus (three chapters), Candida, Fusarium, mucoraceous fungi, Paecilomyces, Penicillium, Phoma, Rhodotorula, Trichoderma (Quindos et al.) including a synopsis of described species, and various other groups of yeasts.

The editors clearly had a vision of producing a particularly comprehensive and authoritative set of reviews. They were determined to get them even when the original invitees failed to deliver, and almost all contributors stepped up to the challenge and delivered chapters of the highest standards. The editors are to be congratulated also on the standard of their editing, the only slightly negative point being the poor quality of reproduction of some of the half-tone photographs and the paucity of coloured ones. This book merits a place in all laboratories dealing with aspects of food spoilage and clinically important fungi, and is also a source of much other information on the genera treated that will be pertinent to those working with them in other areas of applied mycology, especially biodeterioration, food spoilage, and post-harvest losses.


Meike, based at Goethe University Frankfurt am Main, has been teaching mycology in various Central American countries, particularly Panama, since her first assignment there in 2000. She soon found that textbooks produced by European and North American authors that illustrated and used temperate species as examples were far from ideal: “what was needed was a textbook tailored to their experience, with examples from their back yards” (p. vii). Anxious to address this situation, she secured funding from the German Academic Exchange Service and other sources, and has addressed this need spectacularly in this superbly illustrated book.

The structure is essentially systematic, in many cases down to family, and also with tables indicating diseases of tropical crops caused by species within particular genera, for example 14 species of Colletotrichum. Figures for the number of genera and species within orders are provided, which is most helpful as this will help readers appreciate just how species rich many of these are. For each order, information is provided under the subheadings etymology, systematics, geographical distribution, ecology, morphology, biochemical aspects, and importance for humans – positive and negative. Lucid diagrams and boxes elucidate particular aspects of structure, physiology, life-cycles, and ecology. Some boxes are particularly pertinent to tropical situations, for example that on symbioses with social insects, dealing with leaf-cutting ants and termites, extends over seven pages. Most of the diagrams are also published on the internet under creative commons licences to make them available as teaching material; a particular welcome gesture which will be a real boon to teachers.

I was pleased to see fruit(ing) body dropped in favour of ascoma and basidiome in the true fungal phyla, but then saddened to see those words re-appear in the chapters on fungi that do not belong in kingdom Fungi. Meike does not use the fungal terminations of rank names, such as “-mycota”, for example in Straminipila and Myxomycota because of an unfortunate misunderstanding; the word “fungi” (deliberately with a lower case) in the International Code of Nomenclature for
The most striking thing about the book, however, is the stunning full-cover photographs, mostly taken by the author, and combining field-shots with microscopic ones, disease symptoms (plant and human), habitat views, and in some cases scanning electron micrographs.

The Introduction includes three pages of references to works on tropical fungi, and each section also has an extensive bibliography for those wishing to take their interests in fungi further. It would, however, have been good to see those listings extended to cover more national checklists and guides, but that is a minor point.

The book is offered “as a basic tool for teachers and students of mycology at the bachelor, licenciatura, master, and PhD levels, especially to those living in areas with a tropical or subtropical climate” (p. x). It will, I believe, also be illuminating and stimulating to citizen scientists and naturalists in the tropics and perhaps encourage some of them to take up the study of fungi “in their back yards.” This will be especially facilitated by the foresight of the American Phytopathological Society (ASP) Press in publishing a translation in Spanish at almost half the price of the English version. This book is complemented by another Spanish text from APS Press, the monumental illustrated dictionary of mycology of algae, fungi, and plants (McNeill et al. 2012) covers all organisms traditionally treated as such and they are treated together in single alphabetical lists under “Fungi” in the Code’s Appendices (Wiersma et al. 2015). This matter should be addressed in any future edition.

With the explosion in recognition of species in Trichoderma, particularly during the last ten years, the need for an authoritative and up-to-date identification manual has become urgent. This welcome new spiral-bound book not only provides “faces” for selected species, but aims to provide an introduction for those wishing to exploit members of the genus in agriculture. It starts with a masterly overview of the genus. The ancestral species were evidently mycoparasitic, something now supported by whole-genome comparisons, with switches to saprobic life-styles and back in some lineages. While most records are as isolates from soil, it is pointed out that this masks their actual niches, many being found as endophytes or being restricted to particular fungi they parasitise. Biogeographical patterns are also becoming clearer, with well-known species names, such as T. vireide, emerging as Northern Hemisphere taxa. The literature abounds with what prove to be misidentifications under current species concepts, and there is a helpful table giving the correct names for 15 strains that have been exploited and produce particular products.

The agricultural applications are in disease suppression and biocontrol, and that they generally grow rapidly and are not harmful to humans facilitates their exploitation. A table extending over four pages lists commercial products and their applications, with references to key publications. Plants full colonised by a Trichoderma strain can be substantially different from uncolonised ones in responses to the environment and yield. A key factor in this is now known to be a stimulation of the plant’s immune system and increased resistance to other fungi. Genomic studies have increased our understanding of these processes remarkably in the last few years. Finding optimal effects may require matching strains and cultivars.

In order to encourage applications, a wide-ranging hands-on guide is provided dealing with culture selection, isolation, strain improvement, use of mixtures with other mycoparasites (e.g. Clonostachys rosea), tracking cultures using genetic markers, assays of activity and effectiveness, establishment of root colonisation.
formulation methods, and culture preservation (with details of the silica gel method), through to product registration. A separate chapter is devoted to methods of application in field and greenhouse situations, including soil, hydroponics, seed treatments, and sprays.

The major part of the work is devoted to systematics and identification. This includes the latest phylograms and a comprehensive listing of species in each clade, identification methods using molecular and chemical methods, media for isolation and growth, culture characteristics, and micromorphology. Forty-five of the 254 known species are selected for detailed treatment, and a synoptic key to these based on microscopic features precedes the species accounts. Each comprises a page of text with information on synonymy, the type, GenBank references, description, distinguishing characters, habitat, distribution, and selected literature. The facing pages are occupied by composites of half-tone photographs, but the quality of the reproduction can only be described as disappointing and not to the standard we have come to expect in APS publications.

While the emphasis is on species exploited in agriculture, this authoritative work will be a boon to all who work with and struggle with identifying members of the genus regardless of their fields. That is especially so as the first author is yet to be involved in the production of a comprehensive monograph of this rapidly expanding genus – perhaps that will be the next product of Gary’s “retirement”.

This is essentially a set of 12 glossy loose-leaf cards, printed on each side, with three holes on one side so they can be placed in a binder. It is intended as a new edition of the eight cards presented by Noyd (2000) under a different title. Although evidently intended as a class teaching aid, I was unclear how they could be used in practice as the copyright statement forbids any reproduction without permission from the publisher. Following two cards on overall phylogeny, the cards are categorized taxonomically, mostly with a dendrogram illustrated by rather thumbnail sketches on one side and a glossary on the other. The first two are on reproductive methods, and are followed by one headed “Deuteromycota” which uses family names such as Stilbaceae and terms such as “form-order” on the grounds that these are “embedded in the literature” rather than taking the opportunity to make clear these are obsolete and should not be used today. Brave attempts are made to capture the diversity of Ascomycota and Basidiomycota on a single page, but these are hardly adequate and there are several explanations that are misleading or inaccurate; far too many to start listing here. Some of the other phyla treated have a disproportionate amount of space featuring taxa of little relevance to plant pathologists (e.g. Geosiphon, slime moulds), and including ectomycorrhizas on a sheet headed “Glomeromycota” may suggest these belong there. The whole gives the impression of not having been critically reviewed by an experienced mycologist prior to publication, and cannot be commended; it is also very expensive for so few pages. I sadly have to conclude that it is best avoided.

International and regional meetings which are entirely mycological or have a major mycological component.

2016

4th European Congress of Medical Mycology
Ecology and Mycology: from the environment to the patient’s bed
14–16 February 2016
Tel-Aviv, Israel
Contact: magalim@diesenhaus.com

Tackling emerging fungal threats to animal health, food security and ecosystem resilience
7–9 March 2016
The Royal Society, London, UK
Contact: Discussion.Meetings@royalsociety.org
https://royalsociety.org/events/2016/03/emerging-fungal-threats/

Neurospora Information Conference
10–13 March 2016
Asilomar Conference Grounds, Pacific Grove, CA, USA
Contact: Not indicated
http://www.fgsc.net/asilmtg.html

13th European Conference on Fungal Genetics (ECFG13)
Bridging Fungal Genetics, Evolution and Ecology
3–6 April 2016
La Cité de Science et de l’Industrie, La Villette, Paris, France
Contact: info@ecfg13.org
http://www.ecfg13.org/

5th International Symposium on Fusarium Head Blight (ISFHB) and the 2nd International Workshop on Wheat Blast (IWWB)
6–10 April 2016
Costão do Santinho, Florianópolis, Brazil.
Contact: mauricio.ferndandes@embrapa.br
http://scabandblastofwheat2016.org/

9th International Symposium on Septoria Diseases of Cereals
7–9 April 2016
Paris, France
Contact: ccdm@curtin.edu.au

CBS Symposium: Fungi and Global Challenges
14–15 April 2016
Royal Academy of Arts and Sciences, Amsterdam, The Netherlands
Contact: info@cbs.knaw.nl
www.cbs.knaw.nl

8th International Association for Lichenology Symposium (IAL8)
Lichens in Deep Time
1–5 August 2016
Finnish Museum of Natural History, Helsinki, Finland
Contact: ial8@helsinki.fi
www/ial8.luomus.fi

Black Yeast Workshop
15–16 September 2016
Universita’ Degli Studi Della Tuscia, University of Tuscia, Viterbo, Italy
Contact: Laura Selbmann; selbmann@unitus.it
2017

29th Fungal Genetics Conference
March 14–19 March 2017
Asilomar Conference Grounds, Pacific Grove, CA
http://www.genetics-gsa.org/conferences/fungal_genetics.shtml

19th International Botanical Congress
23–29 July 2017 [Nomenclature Section 18–22 July 2017]
Shenzhen Convention and Exhibition Center, Shenzhen, Guangdong, China
www.ibc2017.cn/

2018

3rd International Conference on Basic and Applied Mycology
March 2018
Assiut Mycological Centre, University of Assiut, Assiut, Egypt
Contact: Abdelaal H. Moubasher; ahamaumc@yahoo.com

20th Congress, International Society for Human and Animal Mycology (ISHAM)
2–6 July 2018
RAI Amsterdam, Amsterdam, The Netherlands
Contact: info@congresscare.com
http://www.isham2018.org

11th International Mycological Congress (IMC11)
15–21 July 2018
San Juan, Puerto Rico
Contact: Sharon A. Cantrell Rodriguez; scantrel@suagm.edu

ERRATUM

Smith et al. (IMA Fungus 6 (2): 297–317, December 2015)

Page 312, Left column, Paragraph 5: Replace “Costatisporus caerulescens” by “Costatisporus cyanescens”.

This change has no nomenclatural consequences as the genus is monotypic, with a single valid species name, C. cyanescens, registered in MycoBank.

NOTICES

IMA Fungus is compiled by David L. Hawksworth (Facultad de Farmacia, Universidad Complutense de Madrid) on behalf of the Executive Committee of the International Mycological Association. All unsigned items in the journal are by, and may be attributed to, him.

Items for consideration for inclusion in all sections of the journal should be submitted to him at d.hawkworth@nhm.ac.uk.

Books for possible coverage in the Book News section should be mailed to: IMA Fungus, Milford House, The Mead, Ashtead, Surrey KT21 2LZ, UK.
Phylogenetic placement of *Itajahya*: An unusual Jacaranda fungal associate

Seonju Marincowitz, Martin P.A. Coetzee, P. Markus Wilken, Brenda D. Wingfield, and Michael J. Wingfield

1Department of Genetics, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, P.O. Box X20, Pretoria, 0028, South Africa
2Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, P.O. Box X20, Pretoria, 0028, South Africa; corresponding author e-mail: seonju.marincowitz@fabi.up.ac.za

Abstract: *Itajahya* is a member of *Phallales* (*Agaricomycetes*), which, based on the presence of a calyptra and DNA sequence data for *I. rosea*, has recently been raised to generic status from a subgenus of *Phallus*. The type species of the genus, *I. galericulata*, is commonly known as the Jacaranda stinkhorn in Pretoria, South Africa, which is the only area where the fungus is known outside the Americas. The common name is derived from its association with the South American originating *Jacaranda mimosifolia* trees in the city. The aim of this study was to consider the unusual occurrence of the fungus in South Africa, to place it on the available *Phallales* phylogeny and to test whether it merits generic status. Fresh basidiomes were collected during the summer of 2015 and sequenced. Phylogenetic analyses were based on sequence data for the nuc-LSU-rDNA (LSU) and ATPase subunit 6 (ATP6) regions. The results showed that *I. rosea* and *I. galericulata* are phylogenetically related. They are also clearly distinguished from other members of *Phallales* such as *Phallus* spp. and *Dictyophora* spp., and so our new data supports the raising of *Itajahya* to the generic level.

INTRODUCTION

The generic name *Itajahya* was introduced by Möller (1895) for a fungus discovered near the city of Blumenau in Santa Catarina state, Brazil. The peculiar name derives from that of the river Itajahy in that region. The fungus is an unusual and poorly known member of *Phallales*, and its taxonomic position has been debated purely on the basis of morphological features. The fungus resides in *Phallaceae*, a family encompassing members of the order with unbranched basidiomata. The family *Phallaceae* is distinguished from the only other family recognized in the order, *Clathraceae* (Chevallier 1826) in which the genera have branched basidiomata and are known as “lattice stinkhorns”. Recent developments in the taxonomy of the order based on DNA sequence data can be found in Hosaka et al. (2006) and Trierveiler-Pereira et al. (2014). *Itajahya* now includes four species, with *I. galericulata* as type species, and has, until recently, been treated as a subgenus of *Phallus* (Malençon 1984, Kreisel 1996).

Cabral et al. (2012) considered the taxonomic placement of *Phallus roseus*, a species treated by Malençon (1984) and Kreisel (1996) in *Itajahya*, as a subgenus of *Phallus*. Cabral et al. (2012) were able to collect fresh specimens of *P. roseus* in the Rio Grande do Norte of Brazil, and for the first time generated DNA sequence data for this poorly-known fungus. Their phylogenetic inference showed that *P. roseus* did not cluster with *Phallus* species, and they raised *Itajahya* to generic rank.

The decision of Cabral et al. (2012) to treat *P. roseus* in *Itajahya* was based on the fungus having a calyptra at the apex of its receptacle and the molecular datasets. A calyptra is also present in the type of the genus, *I. galericulata*. However, there was no sequence data then available for *I. galericulata* or the other two species of *Itajahya*: *I. hornseyi* described from Australia (Hansford 1954) and *I. argentina* from Argentina (Spegazzini 1898, 1927). The placement of *P. roseus* in *Itajahya* rested solely on morphological evidence. This could be contested given the poor understanding of the taxonomic value of morphological features in *Phallales*. For example, the data of Cabral et al. (2012) confirm earliest suggestions that the conspicuous indusium in *Dictyophora* is apparently not phylogenetically informative.

*Itajahya galericulata* was first described from southern Brazil where it is known from three states: Santa Catarina (Blumenau), Rio de Janeiro, and Rio Grande do Sul (Pelo-tas) (Möller 1895, Lloyd 1907). The fungus has also been recorded in Bolivia (Fries 1909). Long & Stouffer (1943) re-considered a fungus initially identified as *Phallus impudicus* collected during a field study in 1941 in arid regions of New Mexico and Arizona noting a resemblance to *I. galericulata*, but they considered it “improbable that a tropical wet climate plant could grow under such arid conditions”. However, their careful morphological characterization of specimens...
from Arizona and New Mexico led them to conclude that they were dealing with *I. galericulata*. Although they considered this possibility, they were unable to justify establishing a new species for the fungus based on the morphological characteristics.

Intriguingly, there is only one locality outside the Americas where *I. galericulata* is known: the city of Pretoria, South Africa. Here the fungus commonly occurs in association with *Jacaranda mimosifolia*, a tree that is abundantly planted as an ornamental in gardens and for lining streets. Indeed, Pretoria is commonly referred to as the “Jacaranda city” from the very large number of trees that cover it in a blanket of purple flowers in the Southern Hemisphere spring. While the occurrence of *I. galericulata* in Pretoria is unusual, it is perhaps more interesting that the fungus, known locally as the “jacaranda stinkhorn” (van der Westhuizen & Eicker 1994) occurs in a very close association with *J. mimosifolia*, a tree native in the area of Brazil and Bolivia where the fungus was first discovered. This suggests that *I. galericulata* was probably introduced into South Africa with these trees. Based on herbarium records, *I. galericulata* was first collected and recognized in Pretoria, South Africa, by Ethel M. Doidge on 21 January 1915.

During the summer of 2015, we were able to collect a number of fresh specimens of *I. galericulata* in Pretoria. This provided an opportunity to obtain DNA sequence data for this unusual genus of *Phallales* of which the taxonomy has been confused. The overall aim was to place the fungus in the available phylogeny of *Phallales*, to test the hypothesis of Cabral et al. (2012) that *Itajahya* deserves generic status, and to consider the unusual occurrence of this poorly known fungus in South Africa.

### MATERIALS AND METHODS

#### Collection of samples

Three basidiomes of *Itajahya galericulata* were collected in the Brooklyn and Hatfield residential areas of Pretoria in January 2015 (Table 1, Fig. 1). These typically appear in dry sandy soils after summer rainfall and they were consistently associated with the root systems of *Jacaranda mimosifolia* trees. Dried basidiomes and cultures were deposited in the National Collection of Fungi in South Africa (PREM), Roodeplaat, Pretoria, South Africa, and living cultures were deposited in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute, University of Pretoria, Pretoria, South Africa and in the CBS-KNAW Fungal Biodiversity Centre (CBS), Utrecht, The Netherlands.

#### DNA extraction, PCR and sequencing

Fragments of three basidiomes were used for DNA extraction following the protocol used by Cabral et al. (2012). The DNA was quantified on a NanoDrop spectrophotometer (NanoDrop Technologies, Wilmington, DE), before the nucLSU-rDNA (LSU) and ATPase subunit 6 (ATP6) gene regions were amplified using the LROR/LR5 (Vilgalys & Hester 1990) and ATP61/ATP62 (Kretzer & Bruns 1999) primer sets, respectively. Each gene region was amplified using the KAPA Taq PCR Kit (Kapa Biosystems, Cape Town) according to the appropriate protocols from Cabral et al. (2012). All successful PCR products were purified using the Zymo Research DNA Clean & Concentrator kit (Zymo Research Corporation, Irvine, CA).

Purified fragments were cloned into *Escherichia coli* using the pGEM-T Easy Vector cloning kit (Promega, Madison, WI) following the manufacturers’ instructions. Cloned inserts were amplified directly from colonies using a colony PCR reaction (Sambrook & Russell 2001). Amplification was achieved using the FastStart High Fidelity PCR System (Roche Diagnostics, Mannheim) with the SP6 (Butler & Chamberlin 1982) and T7 (Dunn *et al*. 1983) primer set. Each sample was purified using the Zymo Research DNA Clean & Concentrator kit and sequenced in both directions using the vector specific primers, the BigDye Terminator v. 3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) and an ABI PRISM 3100 Genetic Analyzer (Perkin Elmer, Warrington). Resultant electropherograms were imported into the CLC Main Workbench package (CLC Bio, Aarhus), trimmed for vector-specific and low quality sequence and subsequently assembled. One ATP6 and one LSU sequence from each basidiome was selected for phylogenetic analysis.

#### Phylogenetic analyses of sequence data

ATP6 and LSU datasets generated in this study incorporated sequences from the basidiomes collected in Pretoria as well as sequences obtained from GenBank for species included in the study of Cabral *et al*. (2012) (Tables 1–2). Multiple sequence alignments were made using the online version of MAFFT (Katoh & Standley 2013) with alignment strategy set to Auto. Character congruency between the ATP6 and LSU datasets was determined using the partition homogeneity test (PHT) implemented in PAUP (Swofford 2002) after excluding missing, ambiguously aligned and uninformative

---

**Table 1. Itajahya galericulata** samples collected during this study in the Pretoria area of South Africa with GenBank accession numbers.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>CMW 44299 = CBS 140330</td>
<td>PREM 61268</td>
<td>KR071850 KR071847</td>
</tr>
<tr>
<td>Sample 2</td>
<td>CMW 44300 = CBS 140331</td>
<td>PREM 61269</td>
<td>KR071851 KR071848</td>
</tr>
<tr>
<td>Sample 3</td>
<td>CMW 44301 = CBS 140332</td>
<td>PREM 61270</td>
<td>KR071852 KR071849</td>
</tr>
</tbody>
</table>

*Culture collection of the Forestry and Agricultural Biotechnology Institute (CMW), University of Pretoria, South Africa.

CBS-KNAW Fungal Biodiversity Centre (CBS), Utrecht, The Netherlands.

National collection of Fungi in South Africa (PREM), Roodeplaat, Pretoria, South Africa.
Fig. 1. Habitat and features of *Itajahya galericulata* in Pretoria, South Africa. A. *Jacaranda mimosifolia* trees flowering in Brooklyn, Pretoria. B. Basidiome emerged from the ground (PREM 61269). C. “Egg” dissected to show inner features. D. Flies attracted by putrid odour produced by the fungus. E. Basidiome emerged in a moist chamber in the laboratory (PREM 61268). F. Close-up of the apex of basidiome showing calyptra and gleba (PREM 61270). G. Colonies grown for 3 months on 2% Yeast-malt extract agar (CMW 44300 = CBS 140331). H. Basidia. I. Basidiospores. Bars: E = 1.5 cm, F = 1 cm, G = 2 cm, H–I = 10 μm.
datasets were determined with jModelTest (Darriba 2012) with models selected based on the Akaike Information Criterion (AIC). The selected substitution models were applied to the individual gene partitions (LSU: TIM2+I+G and ATP6: TPM2uf+I+G) in the combined dataset for the Bayesian analysis. Phylogenetic trees were generated with MrBayes (Huelsenbeck & Ronquist 2002) with the number of generations set to 4 million and using four Markov chain runs. The first 25% trees with low likelihood were discarded from each run after which the remaining trees from the individual runs were combined to obtain a consensus tree and posterior probability (PP) values. Effective sample size (ESS), as a measure of convergence, was analyzed in Tracer (http://tree.bio.ed.ac.uk/software/tracer/).

RESULTS

Phylogenetic analyses of sequence data

Using previously described primers, sequences for both the LSU and ATP6 regions were successfully amplified from all three basidiomes. The final LSU and ATP6 datasets included 691 and 667 characters, respectively. Of these, 135 characters were parsimony informative in the LSU dataset, while 214 characters in the ATP6 dataset were informative. In total 271 characters were included in the parsimony analysis after excluding missing, ambiguously aligned, and parsimony uninformative characters.

 Parsimony analyses yielded four most parsimonious trees with a tree length of 908 steps. The consistency and retention index values were 0.476 and 0.729, respectively. The topology of the consensus tree generated from Bayesian inference was congruent with the trees generated using parsimony. All nodes were supported with posterior probability (PP) values > 0.95, with the exception of the node shared by Dictyophora indusiata and Phallus hadriani (Fig. 2). Phylogenetic trees generated from the parsimony and Bayesian analyses placed the sequences from Pretoria in a strongly supported clade (bootstrap support = 100%, PP < 0.95). This clade formed a sister group to Itajahya rosea with high bootstrap support (96%) and posterior probability (PP < 0.95). Trees generated in this study placed sequences from species of the genera Dictyophora and Phallus in a monophyletic group supported by their posterior probability values (PP < 1), but somewhat marginal bootstrap value of 70%. This group was placed sister to I. rosea and the samples from Pretoria with PP = 1, but with low bootstrap support (64%).

DISCUSSION

This study provided DNA sequence data for a poorly-known yet taxonomically important member of Phallales. The data for two gene regions have shown clearly that Itajahya galericulata groups separately from species of Phallus and Dictyophora. They also support the results of Cabral et al. (2012) who raised Itajahya to generic status to accommodate Phallus roseus, which they transferred to Itajahya. Their placement in Itajahya was based on morphological features and sequence data of I. rosea, but not of the type species, I. galericulata. Our results confirm that I. rosea is

Table 2. GenBank accession numbers of taxa used for phylogenetic analyses.

<table>
<thead>
<tr>
<th>Species</th>
<th>GenBank accession no.</th>
<th>nucLSU</th>
<th>ATP6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anthurus archeri</td>
<td>DQ218624</td>
<td>DQ218913</td>
<td></td>
</tr>
<tr>
<td>Abarachium floriforme</td>
<td>JF968440</td>
<td>JF968438</td>
<td></td>
</tr>
<tr>
<td>Aseroe rubra</td>
<td>DQ218625</td>
<td>DQ218914</td>
<td></td>
</tr>
<tr>
<td>Clathrus chrysosmycelinus</td>
<td>DQ218626</td>
<td>DQ218915</td>
<td></td>
</tr>
<tr>
<td>Dictyophora duplicata</td>
<td>DQ218481</td>
<td>DQ218765</td>
<td></td>
</tr>
<tr>
<td>Dictyophora indusiata</td>
<td>DQ218627</td>
<td>DQ218917</td>
<td></td>
</tr>
<tr>
<td>Dictyophora multicolor</td>
<td>DQ218628</td>
<td>DQ218918</td>
<td></td>
</tr>
<tr>
<td>Gelopellis sp. 1</td>
<td>DQ218630</td>
<td>DQ218919</td>
<td></td>
</tr>
<tr>
<td>Gelopellis sp. 2</td>
<td>DQ218631</td>
<td>DQ218920</td>
<td></td>
</tr>
<tr>
<td>Ileodictyon cibarium</td>
<td>DQ218633</td>
<td>DQ218922</td>
<td></td>
</tr>
<tr>
<td>Ileodictyon gracile</td>
<td>DQ218636</td>
<td>DQ218925</td>
<td></td>
</tr>
<tr>
<td>Itajahya rosea</td>
<td>JF968441</td>
<td>JF968439</td>
<td></td>
</tr>
<tr>
<td>Kobayasia nipponica</td>
<td>DQ218638</td>
<td>DQ218926</td>
<td></td>
</tr>
<tr>
<td>Laternae triscapa</td>
<td>DQ218640</td>
<td>DQ218928</td>
<td></td>
</tr>
<tr>
<td>Lysurus borealis</td>
<td>DQ218641</td>
<td>DQ218929</td>
<td></td>
</tr>
<tr>
<td>Lysurus mokusin</td>
<td>DQ218507</td>
<td>DQ218791</td>
<td></td>
</tr>
<tr>
<td>Mutinus elegans</td>
<td>AY574643</td>
<td>AY574785</td>
<td></td>
</tr>
<tr>
<td>Phallophata alba</td>
<td>DQ218642</td>
<td>DQ218930</td>
<td></td>
</tr>
<tr>
<td>Phallus costatus</td>
<td>DQ218513</td>
<td>DQ218797</td>
<td></td>
</tr>
<tr>
<td>Phallus hadriani</td>
<td>DQ218514</td>
<td>DQ218798</td>
<td></td>
</tr>
<tr>
<td>Phallus ravenelli</td>
<td>DQ218515</td>
<td>DQ218799</td>
<td></td>
</tr>
<tr>
<td>Protuber a borealis</td>
<td>DQ218531</td>
<td>DQ218800</td>
<td></td>
</tr>
<tr>
<td>Protuber a canecens</td>
<td>DQ218645</td>
<td>DQ218932</td>
<td></td>
</tr>
<tr>
<td>Protuber a jamaicensis</td>
<td>DQ218647</td>
<td>DQ218933</td>
<td></td>
</tr>
<tr>
<td>Protuber a maracuja</td>
<td>DQ218518</td>
<td>DQ218802</td>
<td></td>
</tr>
<tr>
<td>Protuber a parvispora</td>
<td>DQ218648</td>
<td>DQ218934</td>
<td></td>
</tr>
<tr>
<td>Protuber a sabalunensis</td>
<td>DQ218649</td>
<td>DQ218935</td>
<td></td>
</tr>
<tr>
<td>Simblum sphaerocephalum</td>
<td>DQ218521</td>
<td>DQ218806</td>
<td></td>
</tr>
<tr>
<td>Trappea darkeri</td>
<td>DQ218651</td>
<td>DQ218938</td>
<td></td>
</tr>
</tbody>
</table>
phylogenetically related to the type species, *I. galericulata*, and the two species can be comfortably accommodated in the same genus.

There is relatively little information available on the biology of *Itajahya* species. As it was first discovered in tropical Brazil, the subsequent discovery of *I. galericulata* in dry sandy environments of New Mexico and Arizona was unexpected (Long & Stouffer 1943). This habitat contrasted with most *Phallales*, which occur on rich organic substrates in moist situations. *Itajahya galericulata* is found in Brazil either in clay banks of forest streams or among roots of dead trees where the soil is rich in decaying leaves (Möller 1895). In contrast, in South Africa it is typically found in dry sandy soil, although it occurs annually after rain and is possibly associated with litter of *J. mimosifolia*. Similarly, the Australian *I. hornseyi* is also reported from a sandy soil habitat (Hansford 1954). We consider that this genus of *Phallales*, unlike most other species in the order, has species adapted to tolerate in relatively dry conditions, although we acknowledge that there are some exceptions in *Phallus*; for example, in the UK *P. hadriani* characteristically occurs in sand dunes associated with marram grass (*Ammophila arenaria*) (Watling & Rothove 1989, Pegler et al. 1995).

The occurrence of *I. galericulata* in South Africa and particularly its association with *J. mimosifolia* trees is intriguing. That these trees originate in the area where the fungus was first described suggests the fungus was introduced into South Africa with these trees. The very close association between *J. mimosifolia* trees and its apparent absence from other environments in South Africa raises the question as to whether there might be a mutualistic relationship between them. The members of *Phallaceae* are, however, not known as mycorrhizal associates of trees, although *Phallus hadriani* could perhaps be mycorrhizal with *Ammophila* when in sand dunes (Hawksworth, pers. comm.). The form of the relationship between *J. mimosifolia* and *I. galericulata* remains unknown and requires further investigation for a better understanding of the dynamic ecology of the fungus.

**Fig. 2.** Cladogram based on parsimony and generated from combined nuclear LSU and ATP6 DNA sequence for *Itajahya galericulata* and other member species of *Phallales*. Bootstrap values greater than 60% are indicated on the tree branches. All nodes, except that indicated with an asterisk, have posterior probability values greater than 0.95.
If *I. galericulata* was, as we believe is the case, introduced into South Africa with *J. mimosifolia*, the trees would probably have had to be introduced as potted plants as opposed to seeds, though we cannot exclude the possibility that the fungus could be seed-borne. The legal global movement of rooted plants is very difficult under contemporary quarantine regulations, which seek to prevent the concomitant introduction of alien and potentially invasive organisms. At the time of the first discovery in South Africa in 1915, such care would not have been required. Although there is no evidence to suggest that *I. galericulata* is invasive, its establishment in South Africa illustrates the potential dangers associated with the global movement of living plants for planting out (Andjic et al. 2011).

We have no doubt that the fungus that we have treated as *I. galericulata* from South Africa is the same species as that described from Brazil. It would, however, be useful to compare specimens from these two areas phylogenetically; this would ideally require the collection of fresh specimens from Brazil. For the present, the DNA sequences for this species from South Africa provide a foundation for inclusion of the genus in phylogenetic studies of *Phallales*.

We hope this study will stimulate further interest in *Itajahya* and encourage mycologists to collect additional specimens from which DNA can be extracted and used in phylogenetic analyses. It would, for example, be particularly interesting to compare sequence data from specimens collected in New Mexico and Arizona with ones from South Africa and Brazil. Long & Stouffer (1943) considered whether their collections might represent a species different to *I. galericulata*, but they were unable to make a distinction based on morphology; DNA comparisons would enable us to assess whether the geographical separation had led to any genotypic differences between the populations.

ACKNOWLEDGEMENTS

We thank Martin Kemler for translating Möller’s 1895 paper, Mario Saparrat for providing Spegazzini’s papers, and Riana Jacobs for assisting with *Itajahya galericulata* specimens in the National Collection of Fungi in South Africa (PREM).

REFERENCES


Accepted Trichoderma names in the year 2015

John Bissett1, Walter Gams2, Walter Jaklitsch3, and Gary J. Samuels4

1Agriculture and Agri-Food Canada, Eastern Cereal and Oilseed Research Centre, 960 Carling Avenue, Ottawa, Ontario, K1A 0C6 Canada
2Molenweg 15, 3743 CK Baarn, The Netherlands
3Department of Systematic and Evolutionary Botany, Faculty Centre of Biodiversity, University of Vienna, Rennweg 14, A-1030 Vienna, Austria; and Institute of Forest Entomology, Forest Pathology and Forest Protection, Dept. of Forest and Soil Sciences, BOKU-University of Natural Resources and Life Sciences, Peter Jordan-Straße 82, 1190 Vienna, Austria
4United States Department of Agriculture, Agriculture Research Service, 10300 Baltimore Ave., Beltsville, MD 20705 United States. Present address: 321 Hedgehog Mt Rd., Deering, NH 03244, USA; corresponding author email: samuelspatty@gmail.com

Abstract: A list of 254 names of species and two names of varieties in Trichoderma with name or names against which they are to be protected, following the ICN (Melbourne Code, Art. 14.13), is presented for consideration by the General Committee established by the Congress, which then will refer them to the Nomenclature Committee for Fungi (NCF). This list includes 252 species, one variety and one form. Two new names are proposed: T. neocrasatum Samuels (syn. Hypocrea crassa P. Chaverri & Samuels), T. patellotropicum Samuels (syn. Hypocrea patella f. tropica Yoshim. Doi). The following new combinations in Trichoderma are proposed: T. brevipes (Mont.) Samuels, T. cerebriforme (Berk.) Samuels, T. latizonatum (Peck) Samuels, and T. poronioideum (A. Möller) Samuels.


Article info: Submitted: 26 June 2015; Accepted: 26 August 2015; Published: 29 September 2015.

INTRODUCTION

On 30 July 2011, the provision to permit different morphs of the same fungal species to bear separate names was ended at the XX International Botanical Congress (IBC) in Melbourne. This decision was retroactive, but names published before 1 January 2013, which would otherwise have been illegitimate, were ruled to nevertheless be published before 1 January 2013, which would otherwise be protected. Further, Art. 56.3 allows for the preparation of a list of names in moving, but also by consensus among users. In this article, the need for a list of names in moving the one name for one fungus nomenclature.

As regards whether Trichoderma or Hypocrea should be adopted for the genus, the ICN concluded that the choice between two names should be determined not only by priority of publication, but also by consensus among users. In this case Trichoderma Pers. 1794 was published earlier than Hypocrea Fr. 1825 and, pursuant to Art 14.13, a poll taken by the ICTF (International Commission on the Taxonomy of Fungi) International Subcommission on the Taxonomy of Trichoderma and Hypocrea (www.isth.info) indicated a strong preference to maintain Trichoderma over Hypocrea (Rossman et al. 2013). Having decided to give priority to Trichoderma (with an asexually typified type species) over Hypocrea (with a sexually typified type species), Art. 14.13 further allows for the presentation of a list of names in Trichoderma with name or names against which they are to be protected. Further, Art. 56.3 allows for the preparation of a list of names to be suppressed. The lists are to be presented...
to the General Committee established by the Congress, which then will refer them to the Nomenclature Committee for Fungi (NCF). Following approval by the appropriate committees, rejected names are to be treated as rejected under Art. 56.1 and may become available for use only by conservation under Art. 14. We have not presented a list of names to be suppressed (Art. 56.3) because any names of Trichoderma or Hypocrea that are not in current use (i.e. have not been cultured and/or their DNA sequences) can be epitypified and added to the list of names in use.

Subsequent to the Melbourne Congress, it emerged that in order to promote stability of names it was essential that listed names should be protected against unlisted names and not just listed names against which they were protected (Hawksworth 2014). This view was overwhelmingly supported by the 10th International Mycological Congress (IMC10) in Bangkok in 2014, which agreed that the lists be referred to as “Lists of Protected Names” (Redhead et al. 2014). There was little support at the Congress for having any lists of names not to be used, but if lists were prepared the Congress concluded they should be referred to as “Lists of Suppressed Names” to differentiate them from the existing lists of rejected names. Following discussions by the International Commission on the Taxonomy of Fungi (ICTF), formal proposals to modify the ICN to allow these strongly supported changes have now been made (Hawksworth 2015).

We have included in the present list of accepted names all those names in Trichoderma that are ‘in use’ as of the middle of 2015; thus the list includes those names that were ‘in use’ as of 1 January 2013, the date on which the revision to Art. 59 came into effect. With only a few exceptions noted in the current list, a name is considered to be ‘in use’ if it is represented by a culture and/or diagnostic DNA sequences that are deposited in GenBank (http://www.ncbi.nlm.nih.gov) in the belief that reliable identification of a species of Trichoderma can, with rare exceptions, only be achieved through comparison of a diagnostic sequence such as rpb2. Jaklitsch & Voglmayr (2015) have published the most complete phylogeny of the genus Trichoderma, based on rpb2.

A list of species of Trichoderma that are not currently in use as defined by our criteria is appended at the end. The identity of most of these species is unknown; many are illegitimate later homonyms, synonyms of other Trichoderma species, or are not species of Trichoderma. Many of the names found in this list can be placed in use by epitypification.

In the following, the nomenclature of the Trichodermal Hypocrea pairs is examined and the correct or preferential name for each species is presented. At least 400 species have been described as Hypocrea and only a small number of them have been accounted for in modern terms. Many are not actually species of Trichoderma. A number of Hypocrea names that did not have named Trichoderma asexual morphs were transferred into Trichoderma recently (Jaklitsch & Voglmayr 2014) and a few more names are added here because of their usage in recent literature.

Recent research has shown that a few reported links between a Trichoderma and a Hypocrea are incorrect. Most notable is the link between T. harzianum and H. lixii (Chaverri & Samuels 2002). Revision of the T. harzianum species complex has revealed that T. harzianum and H. lixii are distinct species and the new combination T. lixii was proposed (Chaverri et al. 2015).

In most cases the asexual and sexual morph names of Trichoderma species with named teleomorphs are based on different type specimens. Consequently, from a nomenclatural point of view they represent distinct and priorable species names. In a nomenclatural sense, the species having named teleomorphs fall into four groups which can be defined as follows:

(I) The Trichoderma name is older than the Hypocrea name and thus automatically has priority.

(II) Asexual and sexual morph names were proposed simultaneously and using the same epithet. In this case the Trichoderma name has priority in the genus Trichoderma.

(III) Asexual and sexual morphs share the same epithet but the Hypocrea name is older than the Trichoderma name. In these cases the older epithet cannot be adopted because it is already occupied in Trichoderma. Under Art. 11.4 of the ICN the next available name is to be adopted (Art. 11.4), and in these cases the next available name is always the Trichoderma name, which is adopted here.

(IV) The asexual and sexual morphs have different epithets and the sexual name is the older and should be adopted, but because of common usage it is preferable to maintain the younger Trichoderma name. Accordingly, several names have been proposed for conservation (Samuels 2014) but additional names remain to be conserved as proposed herein.

PROPOSAL FOR A PROTECTED GENERIC NAME IN HYPOCREALES

Rossman et al. (2013) proposed the protection or suppression of several generic names in Hypocreales. Since then, it has been found necessary to suppress two additional sexually-typified names against Trichoderma.

Trichoderma Pers. 1794 vs. Sarawakus Lloyd 1924 and Aphysistroma A.T. Martinez & G. Moreno 1986

Trichoderma Pers. 1794, typified by T. viride Pers. 1794, is an asexual morph-typified name and has priority over Sarawakus Lloyd 1924, typified by S. lycogaloides (Berk. & Broome) Lloyd 1924, and Aphysistroma A.T. Martinez & G. Moreno 1986, typified by A. stercorarium A.T. Martinez & G. Moreno 1986. Since 2008 (Jaklitsch et al. 2008, Jaklitsch 2011) it has been known that the type species of Aphysistroma clusters within Trichoderma and thus Aphysistroma should be considered a synonym of Trichoderma. Similarly, Jaklitsch & Voglmayr (2014b) have shown that the type species of Sarawakus clusters in Trichoderma and it too should be considered as a synonym of Trichoderma. Jaklitsch & Voglmayr (2014b) transferred S. lycogaloides and several additional species (see below) into Trichoderma and Jaklitsch & Voglmayr (2015) recombined A. stercorarium in Trichoderma. However, Art 57.2 of ICN stipulates that “an asexual morph-typified name that has priority is not to displace the teleomorph name(s) unless and until a proposal to reject the former under Art. 56.1 or 56.3 or to deal with the latter under Art. 14.1 or
14.13 has been submitted and rejected.” Until now, no such proposal has been made for the protection of *Trichoderma* over *Aphysiostroma* or *Sarawakus*, but as that provision in the ICN is proposed for deletion (Hawksworth 2015) this may not become necessary.

**ACCEPTED TRICHODERMA NAMES IN 2015**

Current ICN only permits names to be protected against listed names which otherwise would take precedence. While it is anticipated that the provisions will be changed to permit listed names to be protected against unlisted names (see above), the current mandate of the General Committee and the Nomenclature Committee for fungi is consider for protection only names where there are competing names. In order to facilitate the work of the Committees, the few names which require protection against competing names are prefixed by an asterisk (*), although most are already proposed for conservation. However, we wish all names published prior to 1 January 2013 and accepted here to be included in the eventual list of protected names as soon as that is permitted by the ICN.

The entries are presented here largely in the form that is likely to be adopted for publication in the eventual list of protected names, though for completeness we have not abbreviated the authors of names where there are more than two to ”& al.”, omitted “in” before journal titles, and retained the names of authors of papers in which they were published (”in” citations).

Typus: [specimen] (CEYLON, Nuwara Eliya], No. 5 (K, ex herb. Berkeley).
Ex-type culture: CBS 130628.
Representative sequences: tef1: AB646526; rpb2: AB646526.

Ex-type culture: IFO 30608 = G.J.S. 04-186 = CBS 124620.
Representative sequences: tef1: FJ467645, AF348098, KP008993; rpb2: AF348098, FJ442706.

Typus: [dry culture] (BPI 88109).
Ex-type culture: G.J.S. 99-227 = CBS 130755 = = IMI 393967.
Representative sequences: tef1: AF348093, FJ463327; rpb2: FJ442778, FJ442799.

Typus: [dry culture] (BPI 88109).
Ex-type culture: G.J.S. 04-186 = CBS 124620.
Representative sequences: tef1: FJ463301, FJ463401, AF469194; rpb2: FJ442691, FJ442726.

Typus: [dry culture] (BPI 748201).
Ex-type culture: G.J.S. 04-186 = CBS 124620.
Representative sequences: tef1: FJ467645, AF348098, KP008993; rpb2: AF345541, FJ442706.

Typus: [dry culture] (BPI 748204).
Ex-type culture: CBS 100526.
Representative sequences: tef1: FJ467645, AF348098, KP008993; rpb2: AF345541, FJ442706.

Representative sequences: tef1: AY937440.
Note: The culture derived from the original collection of this species has been lost. Another Japanese (Kagoshima) collection, not a paratype, was cultured by Doi and deposited as IFO 30608.

Typus: [specimen] (CEYLON, Nuwara Eliya), No. 5 (K, ex herb. Berkeley).
Ex-epitype culture: G.J.S. 01-265 = CBS 114787.
Representative sequences: tef1: DQ835494, KP008993; rpb2: AF545541, FJ442706.

Typus: [dry culture] (WU 29301).
Ex-type culture: CBS 114788.
Representative sequences: rpb1 = DQ835494, rpb2: DQ835494.

Typus: [specimen] (CEYLON, Nuwara Eliya), No. 5 (K, ex herb. Berkeley).
Ex-epitype culture: G.J.S. 01-265 = CBS 114787.
Representative sequences: tef1: DQ835494, KP008993; rpb2: AF545541, FJ442706.

Typus: [dry culture] (BPI 748204).
Ex-type culture: CBS 100526.
Representative sequences: tef1: FJ467645, AF348098, KP008993; rpb2: AF345541, FJ442706.

Typus: [specimen] (CEYLON, Nuwara Eliya), No. 5 (K, ex herb. Berkeley).
Ex-epitype culture: G.J.S. 01-265 = CBS 114787.
Representative sequences: tef1: DQ835494, KP008993; rpb2: AF545541, FJ442706.


Ex-type culture: CBS 122303 = TFC 2000-36.  
Representative sequences:  
te{1}: EU498312,  
te{2}: EU248617.  

Hypocrea albolutescens (≡) Hypocrea clavata (e.g. Jaklitsch, 2011), but S. clavata is an illegitimate later homonym of S. clavata (Scop.) Weber (Spicil. Fl. Goett. 3: 364, 1778; Pers., Comm. fung. clav.: 12. 1797 : (≡) Sphaeria clavata (Fr., Syst. Mycol. 2: 325. 1823.  

Ex-type culture: CBS 120535. Other representative cultures  
CBS 120572.  
Representative sequences:  
te{1}: DQ307502, DQ307503;  
te{2}: DQ307503.  

Trichoderma amazonicum (≡) Sphaeria alutacea (Scop.) Weber (Spicil. Fl. Goett. 3: pl 189. 1779) is sometimes given as a synonym of S. alutacea.  

Note: In the protologue for H. citrina var. americana the holotype is given as deposited in CUP and NY, and the specimen indicated as type of H. citrina var. americana in the hand of S. C. Canham is marked ‘EX CUP 38045.’ Because Susan Canham worked at NY, we presume that the NY portion is the portion she worked with and, accordingly, designate it here as lectotype.  

Trichoderma andinense (Samuels & O. Petrini)  
Samuels, Jaklitsch & Voglmayr, Mycotaxon 126: 146. 2013.  


Ex-type culture: G.J.S. 97-243 = CBS 133558.  
Representative sequences:  
te{1}: DQ 307502, DQ307503;  
te{2}: DQ307503.  

Trichoderma appalachiense Samuels & Jaklitch, in  

Typus: [specimen] BPI 746727.  
Ex-type culture: G.J.S. 97-243 = CBS 133558.  
Representative sequences:  
te{1}: DQ 307502, DQ307503;  
te{2}: DQ307503.  

Ex-type culture: CBS 119575 = ATCC 90237.  
Representative sequences:  
te{1}: GU956321,  
te{2}: JN175531.  


Typus: [dry culture] BPI 879770.  
Ex-type culture: CBS 125938.  
Representative sequences:  
te{1}: GU248412,  
te{2}: GU248411.  

Typus: [dry culture] BPI 746504.  
Ex-type culture: CBS 433.97.  
Representative sequences:  
te{1}: AF456907,  
te{2}: EU338326.
Typus: [dry culture] (WU 29208a).  
Ex-type culture: CBS 120632.  
Typus: [specimen] (WU 29280).  
Representative sequences: **tef1**: FJ860649, **rpb2**: FJ860546.

Typus: [specimen] (BPI 802854).  
Ex-type culture: G.J.S. 92-110 = CBS 548.92.  
Representative sequences: **tef1**: AF443942, AF443943, FJ463297; **rpb2**: FJ442745, FJ442735, FJ442777.

Trichoderma atrogalatinosum (Dingley) Jaklitsch & Voglmayr, Mycotaxon 126: 146. 2013.  
Typus: [specimen] (PDD 10471).  
Epitypus (**hic designatus, MBT 202235**): [metabolically inactive culture] NEW ZEALAND (CBS 237.63).  
Ex-type culture: CBS 237.63.  
Representative sequences: **tef1**: KJ871083, **rpb2**: KJ842201.  
*Note:* Hypocre atrogalatinosina had no ex-type culture but Joan Dingley sent either a culture or a specimen identified as *H. atrogalatinosina* to John Webster, who deposited a culture in CBS as CBS 237.63. The specimen from which this culture was isolated cannot be located. It was isolated from basidiomata of a species of *Bondarzewia* (*Russulales, Bondarzewiaceae*). No specific substrate is indicated for the type collection of *H. atrogalatinosina*. Thus it is reasonable to interpret the species *H. atrogalatinosina* as growing on basidiomata of *Hymenochaetales*. We interpret the culture derived from the *Fuscoporia* (CBS 237.63) as being representative of the species and we designate this metabolically inactive culture as epitype of *H. atrogalatinosina*. Additional soil isolations made in New Zealand (LU498, LU501, LU502, LU503, LU504, LU505) can be identified as this species.

Ex-type culture: CBS 142.95.  
Typus: [specimen] (BPI 748312).  
Ex-type culture: CBS 110086 = NBRC 10177 = ATCC MYA-2687.  
Representative sequences: **tef1**: AF456887, AF456891, AY376051, FJ80611; **rpb2**: EU341801, FJ806518.

Typus: [dry culture] (WU 29183a).  
Ex-type culture: CBS 119284.  
Typus: [specimen] (WU 29183).  
Representative sequences: **tef1**: FJ860613, **rpb2**: FJ860520.

Typus: [dry culture] (SHD-M 2663).  
(≡) Hypocre aureoviridis Plowr. & Cooke, Grevillea 8: 104. 1880.  
Epitypus (**vide Jaklitsch, ibid.: ENGLAND, Norfolk, Thetford, Thetford National Forest Park, close to Lynford, MTB 3530/1, 52°28'54" N, 00°41'01" E, elev. 30 m, on decorticated, well-rotted hardwood, 3–4 cm thick, soc. *Eutypa* spp., 13 Sep. 2004, W. Jaklitsch & H. Voglmayr (W.J. 2708) (K(M) 162235).  
Ex-type culture: CBS 120536.  
Representative sequences: **tef1**: FJ665431, FJ665432, FJ860615; **rpb2**: FJ179602, JQ 685882.

Typus: [dry culture] (WU 29193a).  
Typus: [specimen] (WU 29193).  
Ex-type culture: CBS 122494.  
Representative sequences: **tef1**: FJ860619, **rpb2**: FJ860525.

Typus: [dry culture] (BP) 870962B).  
Ex-type culture: CBS 119092.  
Typus: [specimen] (BPI 870962A).  
Representative sequences: **tef1**: DQ307561, **rpb2**: FJ442772.

Ex-type culture: CBS 121667.  
Representative sequences: **tef1**: AY225857, **rpb2**: AF545562.

Typus: [dry culture] (BPI 881029).
Ex-type culture: CBS 125733.
Representative sequences: \textit{tef1}: HQ342223, \textit{rpb2}: HQ342286.

Typus: [dry culture] (WU 29196a).
\( (=) \) Hypocrea bavarica Jaklitsch, Fungal Divers. 48: 125. 2011.
Typus: [specimen] (WU 29196).
Ex-type culture: CBS 120538.
Representative sequences: \textit{tef1}: FJ860620, \textit{rpb2}: FJ860526.

Typus: [dried culture] (CBS H21626).
Ex-type culture: CBS 137447 = UTHSC 1073 = FMR 12635.

Ex-type culture: CBS 109720.
Representative sequences: \textit{tef1}: EU338299, \textit{rpb2}: EU338317.

Trichoderma brevipes (Mont.) Samuels, \textit{comb. nov.} MycoBank MB812025
\( (=) \) Cordyceps brevipes Mont., Syll. Gen. crypt. 201. 1856.
\( (=) \) Podostroma brevipes (Mont.) Seaver, Mycologia 2: 61. 1910.
Typus: FRENCH GUIANA: [on decorticated wood], \textit{Lepri}eur 1073 (PC; isotypus BPI-Lloyd 715550).
Representative culture: G.J.S. 92-76 = NBRC 101780 = CBS 130011 = S195.
Note: Samuels & Lodge (1996) described the sexual and unnamed asexual morph of this species. The culture cited here is from a New Guinean collection (BPI 737810). This culture has not been sequenced. Because this species was originally described from tropical America and the only culture for which we have a culture was collected in New Guinea, we do not epitypify the species.

Typus: [metabolically inactive culture] (CBS 137272).
Ex-type culture: CBS 121130.
Representative sequences: \textit{tef1}: JQ685865, \textit{rpb2}: JQ685881.
Note: Ascospores did not germinate; DNA for sequencing was isolated from a stroma.

Typus: [dry culture] (WU 28233a).
Ex-type culture: CBS 121130.
Typus: [specimen] (WU 28233).
Representative sequences: \textit{tef1}: EU498316, \textit{rpb2}: EU498358.

Trichoderma caeruleus (Jaklitsch & Voglmayr) Jaklitsch & Voglmayr, Mycotaxon 126: 146. 2013 [2014].
Typus: [dried culture] (WU 31600) (ex-type culture CBS 130011 = S195).
Ex-type culture: CBS 130011
Representative sequences: \textit{tef1}: JN715621, \textit{rpb2}: JN715604.

Typus: [dry culture] (BPI 86396).
Ex-type culture: CBS 124369.
Representative sequences: \textit{tef1}: HQ342216, \textit{rpb2}: HQ342279.

Typus: [dry culture] (WU 29198a).
\( (=) \) Hypocrea calamagrostis Jaklitsch, Fungal Divers. 48: 186. 2011.
Typus: [specimen] (WU 29198).
Ex-type culture: CBS 121133.
Representative sequences: \textit{tef1}: FJ860622, \textit{rpb2}: FJ860528.

Typus: [metabolically inactive culture] (CBS 137272).
Representative sequences: \textit{tef1}: AF348107, AF348108.
Typus: [dried culture] (BPI 882292).
Ex-type culture: CBS 130629
Representative sequences: tef1: JN182283, JN175585;
rpb2: JN182312, JN175530.

Typus: [dry culture] (BPI 746700B).
Ex-type culture: G.J.S. 97-3 = CBS 119093
Typus: [specimen] (BPI 746700).

Typus: [dry culture] (BPI 870965).
Ex-type culture: DIS 320c = CBS 119055 = IMI 393638
Representative sequences: tef1: DQ289010, rpb2: KT028596.

Typus: [dried culture] (BPI 8543653).
Ex-epitype culture: G.J.S. 02-76 = CBS 114232 = DAOM 232830.
Ex-type culture: CBS 130010 = DAOM 232831 = ATCC MYA-3222.
Typus: [specimen] (WU 33325).

Typus: [specimen] (WU 33325).
Ex-type culture: CBS 130010

Trichoderma ceraceum  P. Chaverri & Samuels, Stud. Mycol. 48: 45. 2004 ['2003'].
Typus: [dry culture] (BPI 843654).
Typus: [specimen] (BPI 737722).
Ex-type culture: CBS 114245 = DAOM 232831 = ATCC MYA-3222.
Representative sequences: tef1: AY937437, rpb2: AF545508.

Typus: [dry culture] (BPI 843655).
Ex-type culture: G.J.S. 88-70 = CBS 114576,
Representative sequences: tef1: AY737738, rpb2: AF545510.

Trichoderma cerebriforme  (Berk.) Samuels, comb. nov.
MycoBank MB812055
Typus: "Hypocrea cerebriformis B. AUSTRALIA, M & B" (K, ex herb. M.C. Cooke 1885).
Representative culture: G.J.S. 85-245 = CBS 139045.
Representative sequences: tef1: KP109824.
Note: *Trichoderma cerebriforme* is distinctive because of the stout stipe and convoluted cap of the teleomorph. Similar species having stipitate/capitate stromata include *H. brevipes* Mont., *H. poronioidea* Möller, and *H. capitata* Samuels & Lodge (Samuels & Lodge 1996). *H. petersii* Berk. & M.A. Curtis and *H. peltata* (Jungh.) Berk. have large, centrally attached stromata, the stipe being greatly reduced. Doi (1976) reported but did not illustrate a *T. cf. longibrachiatum* like asexual morph for *T. cerebriforme* based on collections made in Peru. Rogerson et al. (1990) reported the species from central Brazil (Roraima). The culture cited above was reported by Doi (in Samuels et al., 1990) from an Indonesian specimen (BPI 881335 ex NY). The sequence cited above was derived from this Indonesian collection; it indicates that *T. cerebriforme* may be a member of the Viride clade. Whether any of these collections made outside of Australia are actually *T. cerebriformis* remains to be proven, but the name is being used and for this reason we place it in *Trichoderma*.
Trichoderma cerinum


Typus: [dry culture] (DAOM 230012).

Ex-type culture: CBS 230012.

Representative sequences: tef1: AY605802, AY937443; rpb2: KF134788.

Trichoderma chlorosporum


Typus: [dry culture] (BPI 843656).

Ex-type culture: G.J.S. 94-68 = CBS 114577.


Trichoderma citrinoviride

(≡) P. Chaverri & Samuels, Stud. Hypocrea cinnamomea

Typus: [dry culture] (BPI 843656).

Trichoderma compactum


Ex-type culture: CBS 121218.

Representative sequences: tef1: KF134798, rp2: KP115276, KF134789.

Trichoderma composticola


Typus: [metabolically inactive culture] (CBS 133497).

Ex-type culture: CBS 133497.

Representative sequences: tef1: KC285631, rp2: KC285754.

Trichoderma corneum

(Pat.) Jaklitsch & Voglmayr, Mycotaxon 126: 147. 2014.

(≡) Hypocre a corneum Fr., Elench. Fung. 2: 60. 1828.

Ex-type culture: None.


Trichoderma citrinum


Ex-neotype culture: CBS 853.70.

(≡) Trichoderma lacteum


Typus: [dried culture] (DAOM 167644).

(≡) Sphaeria lactea Fr., Obs. mycol. 1: 141 (1815).

Hypocrea lactea (Fr.) Fr., Summa veg. Scand. 2: 383. 1849.


Ex-epitype culture (T. lacteum): CBS 894.85.

Representative sequences: tef1: FJ860631, DQ835411; rp2: FJ179630, AF545561.
Typus: SRI LANKA, Hakgala, on decorticated wood, Mar. 1922 (K No. 6373).
Representative sequence: tef1: AY937431.
Note: Chaverri & Samuels (2003) redescribed this species based on their study of the types of H. cornea and H. cincta Petch. Sequences deposited in GenBank that were derived from Thai collections identified as H. cornea are diverse. Of the cultures linked to sequences, only the one cited above was deposited in CBS; it was derived from ascospores of BPI 745564, identified as H. cornea. Because the stroma of this collection is morphologically consistent with the type of H. cornea, and because of the proximity of Thailand to Tonkin H. cornea, and because of the proximity of Thailand to Tonkin, we designate this Thai collection as epitype of H. cornea.

Typus: [dry culture] (BPI 20041).
(=) Hypocrea dacrymycella Cooke & Plowr., Grevillea 12: 100. 1884. Typus: UK: Norfolk: Brandon, on Scotch pine (= Pinus sylvestris), 7 Nov. 1881, C.B. Plowright (K (m) 114743).
Ex-type culture: CBS 131486.
Typus: [specimen] (WU 33300).
Ex-type culture: CBS 118980.
Representative sequences: tef1: DF345342, rpb2: DF345347.

Typus: [specimen] (WU 29046).
Ex-type culture: CBS 121273.

Typus: [specimen] (WU 33300).
Ex-type culture: CBS 131486.

Typus: [dry culture] (BPI 843659).
(=) Hypocrea cremea P. Chaverri & Samuels, Mycologia 95: 1115. 2004 ["2003"].
Typus: [specimen] (BPI 1112894).
Ex-type culture: G.J.S. CBS 111146 = DAOM 231312 = ATCC MYA-2862.
Representative sequences: tef1: AY737736, rpb2: AF545511.

Typus: [dry culture] (WU 24041a).
Typus: [specimen] (WU 24041).
Ex-type culture: CBS 118980.
Representative sequences: tef1: DQ345342, rpb2: DQ345347.

Typus: [specimen] (WU 29042a).
Ex-type culture: None.
Representative culture: None.
(=) Hypocrea dacrymycella Cooke & Plowr., Grevillea 12: 100. 1884.
Typus: UK: Norfolk: Brandon, on Scotch pine (= Pinus sylvestris), 7 Nov. 1881, C.B. Plowright (K (m) 114743).
Epitypus (vide Jaklitsch, Stud. Mycol. 67: 40. 2009): [specimen] (WU 29042);
Typus: CZECH REPUBLIC: Central Bohemia, Mnichovice near Prague; on Picea abies in cavities of a stump, Nov. 1934; J. Velenovsky 29/1947 (PRM No. 153288).
Note: Trichoderma dactrymycellum has not been grown in pure culture. The asexual morph of T. dactrymycellum described by Jaklitsch (2009) is based on frequent association of the asexual morph with stromata agreeing with type material of H. dacrymycella. Gene sequences for this species reported by Jaklitsch (2009) were derived from stromata.
Typus: [specimen] (BPI 747356).
Ex-type culture: CBS 121307.

Typus: [dry culture] (WU 29225a).
Ex-type culture: CBS 120631.
Ex-epitype culture: CBS 120631.

(≡) Gliocladium deliquescent Sopp, Monogr. Penicillium: 89. 1912.
(≡) Sphaeria gelatinosa var. lutea Tode, Fungi Mecklenb. 2: 48. 1791, as ‘α’.
(≡) Hypocrea lutea (Tode : Fr.) Petch, J. Bot. 75: 231. 1937.
Ex-epitype culture: CBS 121131.

Note: Gliocladium viride Matr. is recognized to be an older facultative synonym of G. deliquescent Sopp (e.g., Jaklitsch 2011) but the existence of the name Trichoderma viride Pers. 1791 precludes transfer of the epithet to Trichoderma. At the rank of species, the name Gliocladium deliquescent has priority from 1912, while the sanctioned (Fries, Syst. Mycol. 2: 336. 1823) and older epithet 'lutea' dates from 1791, but only at an undefined infraspecific rank; at species rank it dates from 1937. However, because G. deliquescent (≡ T. deliquescent) is typified by an asexual morph, while H. lutea is typified by a teleomorph, and both are in current use, Art. 57.2 requires that a proposal to conserve H. lutea be submitted and rejected before adopting the older asexual morph-typified name (Samuels 2014).

Typus: [dry culture] "Typus anamorphosis T. dingleyae cultura sicca ex ascospora oriens PDD 83839" (PDD 83838).
Typus: [specimen] (PDD 83838).
Ex-type culture: CBS 119056.
Representative sequences: tef1: AF348117, DQ289008, J665467; rpb2: EU341803, KJ665257.

Typus: [dry culture] NEW ZEALAND "... holotypus asexual morphosis T. dorotheae cultura sicca ex ascospora oriens PDD 83839"") (PDD 83839).
Typus: [specimen] (PDD 83839).
Representative sequences: tef1: DQ307536, rpb2: EU248602.

Typus: [dry culture] (DAOM 23000).
Ex-type culture: DAOM 230007.

Typus: [specimen] (TUMH 40457).
Ex-type culture: TUF 100002 = CBS 133190.
Representative sequences: tef1: JX684011, rpb2: X238484.

Typus: [metabolically inactive culture] (CBS 130729).
Ex-type culture: DIS 217a = CBS 130729 = IMI 395208.

Typus: [dry culture] (WU 28237a).
Ex-type culture: CBS 120524.
Representative sequences: tef1: X238484, rpb2: EU498360.
Accepted names in *Trichoderma*

**Trichoderma erinaceus** Bissett, C.P. Kubicek & Szakács, *in* Bissett & al., Can. J. Bot. 81: 583. 2003. [as ‘*erinaceum*’, to be corrected as masculine for a noun in apposition; the hedgehog ‘*erinaceus*’ cannot be used as an adjectival].

Typus: [dry culture] (DAOM 230019).
Ex-type culture: DAOM 230019.
Representative sequences: tef 1: AY7508880, DQ109547; rpb 2: EU248603, EU248604.  
Note: Unidentified *Hypocreopsis rufa*-like stromata collected in Sri Lanka (BPI 871397, culture G.J.S. 02-103 = CBS 130688, 116238). In order to stabilize this name, we designate one of them as epitype.


Typus: [specimen] (WU 29250).
Ex-type culture: CBS 121276.
Representative culture: CBS 901.72.

Typus: [specimen] (WU 33367).
Ex-type culture: CBS 130013.


Typus: [dry culture] (BPI 878744).
Ex-type culture: CBS 130279.
Representative sequences: tef 1: EY883563, rpb 2: EY883558.


Typus: [dry culture] (BPI 167161).
Ex-type culture: CBS 130279.
Representative sequences: tef 1: EY605801, rpb 2: AF545545, AF545546.


Typus: [specimen] (WU 29250).
Ex-type culture: CBS 121276.
Representative culture: CBS 901.72.

Typus: [specimen] (WU 33367).
Ex-type culture: CBS 130013.


Typus: [dry culture] (BPI 878744).
Ex-type culture: CBS 130279.
Representative sequences: tef 1: EY883563, rpb 2: EY883558.


Typus: [dry culture] (BPI 882293).
Ex-type culture: CBS 130626.
Representative sequences: tef 1: EY605801, rpb 2: AF545545, AF545546.


Typus: [dry culture] (BPI 882293).
Ex-type culture: CBS 130626.
Representative sequences: tef 1: EY605801, rpb 2: AF545545, AF545546.


Typus: [dry culture] (BPI 878744).
Ex-type culture: CBS 130279.
Representative sequences: tef 1: EY883563, rpb 2: EY883558.


Typus: [dry culture] (BPI 882293).
Ex-type culture: CBS 130626.
Representative sequences: tef 1: EY605801, rpb 2: AF545545, AF545546.


Typus: [dry culture] (BPI 882293).
Ex-type culture: CBS 130626.
Representative sequences: tef 1: EY605801, rpb 2: AF545545, AF545546.

Ex-type culture: None.

Typus: [dry culture] (BPI 882294).
Ex-type culture: CBS 114246 = DAOM 232835.

Trichoderma fomiticola Samuels, in Samuels & Ismaiel, Mycol. Prog. 11: 234. 2011.
Typus: [dry culture] (BPI 871616).
Ex-type culture: G.J.S. 01-238 = CBS 124372.
Representative sequences: tef1: HQ342218, rpβ2: HQ342281.

Trichoderma foliicola (Jaklitsch & Voglmayr) Jaklitsch & Voglmayr, Mycotaxon 126: 149. 2014 ["2013"].
Typus: [specimen] (WU 36111) (ex-type culture CBS 130008 = Hypo 645).
Ex-type culture: CBS 130008.
Representative sequences: tef1: JQ685862, rpβ2: JQ685876.

Typus: [dry culture] (WU 29050a).
Typus: [specimen] (WU29050).
Ex-type culture: CBS 121136.

Typus: [dry culture] (BPI 872183).
Ex-type culture: G.J.S. 06-09 = CBS 120075.
Representative sequences: tef1: DQ307541, DQ841722; rpβ2: JN133561.

Typus: [dry culture] (BPI 747556).
Ex-type culture: CBS 114246 = DAO 232835.
Typus: [icon] Tab. 123 a-d, 124 a-f in Tode, Fungi Mecklenb. 2. 1791.


(≡) Sphaeria cupularis Fr., Linnaea 5: 539. 1830.
Representative sequences: tef1: AY391983, FJ179569; rpβ2: AY391924, FJ179604.

*Note:* The type of *T. gelatinosum* was derived from ascospores of a collection made in Austria; this collection is the epitype of *H. gelatinosa*. The culture from that specimen was deposited (CBS 114246) and sequenced (Chaverri & Samuels 2003). The name *T. gelatinosum* needs protection over *Sphaeria cupularis* as it is Tode’s “gelatinosa” not Chaverri & Samuels’ “gelatinosum” that was sanctioned, unless the proposal of Hawksworth & al. (2013; Hawksworth 2015) is adopted when the citation in *Trichoderma* would become “(Tode) P. Chaverri & Samuels”.

Typus: [dry culture] TNS-F-237181.
Ex-type culture: G.J.S. 95-137 = ATCC 208858 = IAM 13109.
Typus: [dry culture] (DAOEM 165773).
Representative sequences: tef1: AY 937423, rpβ2: JN175559.

Ex-type culture: CBS 130435.
Representative sequences: tef1: JN175583, rpβ2: JN175527.

Typus: [specimen] (WU 32187).
Ex-type culture: CBS 130009.

Typus: [dry culture] (BPI 882295).
Ex-type culture: CBS 130009.

Typus: [dry culture] (HGUPd0038).
Ex-type culture: HGUP 0038 = CBS 131803.
Representative sequences: tef1: JN215484, JX089585; rpb2: Q901400, JQ901401.


Ex-epitype culture: DAOM 167057.

Representative sequences: tef1: AF456911, AY750893; rpb2: AF545548.

*Note:* Bonorden did not cite a specific specimen in the protologue to *V. hamatum*, but his illustration (Fig. 117) can easily be interpreted as representing our modern concept of *T. hamatum*. Bissett (1991) noted the lack of type material and commented on Fig. 117, but he designated a neotype for this species. Under Art. 9.2 of ICN the illustration published with the protologue of *Verticillium hamatum* has to be adopted as lectotype of this species as it is a part of the “original material” in the sense of the ICN. The culture designated by Bissett (1991) as neotype for *T. hamatum* (DAOM 167057) should therefore be regarded as epitype of *V. hamatum*. Jaklitsch & Voglmayr (2014) described a teleomorph with yellow-brown to dull orange stromata and colorless ascospores.


Ex-neotype culture: CBS 226.95.

Representative sequences: tef1: AF348101, AF348100, AF348092; rpb2: AF545549.

*Note:* *Trichoderma harzianum* has been known to be a species complex for several years (Chaverri et al., 2003b; Druzhinina et al., 2010). Chaverri et al. (2015) recognized several taxonomic species in the complex.


Typus: [specimen] (WU 32168).

Ex-type culture: CBS 130540.


Typus: [specimen] (DAOM 33410).

Ex-type culture: CBS 133499.


Typus: [dry culture] (DAOM 230022).

Ex-type culture: DAOM 230022.

Representative sequences: tef1: EU280055, rpb2: DQ087239.

**Trichoderma hispanicum** (Jaklitsch & Voglmayr) Jaklitsch & Voglmayr, Mycotaxon 126: 149. 2013 [“2013”].


Typus: [specimen] (WU 31606) (ex-type culture CBS 130540 = S453).

Ex-type culture: CBS 130540.

Representative sequences: tef1: JN715659, rpb2: JN715600.

**Trichoderma hunua** (Dingley) Jaklitsch & Voglmayr, Mycotaxon 126: 149. 2013.


Ex-epitype culture CBS 238.63.

Representative sequence: tef1: AF401011.

*Note:* The original gathering of *H. hunua* was not cultured. Joan Dingley sent a subsequently collected specimen and/or culture (*Dingley No. 5*) of this species to John Webster, who deposited the culture in CBS (CBS 238.63). A dry culture was made and deposited in CBS (H 13531). We designate the dry culture as epitype. This culture has been sequenced and included in phylogenetic analysis. Thus the name *’Hypocrea hunua’* is in current use and representative sequences have been deposited in GenBank. However, the specimen from which the culture CBS 238.63 was derived cannot be located (PDD) and is presumed lost. Thus its identity as *H. hunua* is uncertain. However, the phylogenetic results with Webster/Dingley’s culture of this species (Kullnig-Gradinger et al. 2002) that is deposited in CBS is consistent with the morphology of part of the type that is now deposited in K (as IMI 50433). Sequences under this name are deposited in GenBank and Jaklitsch & Voglmayr (2014) have commented on its phylogenetic position in their list of *Trichoderma/Hypocrea* species based on this culture. Thus there is an established taxonomy and literature for *H./T. hunua*. Stability of this name is served by adopting an epitype as we do here, despite the uncertainty about the provenance of CBS 238.63.


Ex-type culture: CBS 275.78.

Representative sequences: tef1: AF348099, rpb2: FJ442725.

*Note:* Three dry cultures [CBS H-18863, CBS H-18864, CBS H-18865] were made from the same ex-type culture of *T. inhamatum* and deposited in the herbarium of CBS. We presume them all to be isotypes; accordingly, designate one of them as lectotype.


Typus: [dry culture] (BPI 745751B).

Typos: [specimen] (BPI 745751).
Ex-type culture: G.J.S. 97-88 = CBS 119059.
Representative sequences: tef1: AY376060, rpb2: EU241505.

Typos: [specimen] (WU 33354).
Ex-type culture: CBS 130639.

Typos: [specimen examined] (WU 33310).
Ex-type culture: CBS 132567.

**Trichoderma ivoriense** Samuels, *in* Samuels & Ismaiel, Mycol. Prog. 11: 237. 2011.
Typos: [dry culture] (BPI 881030).
Ex-type culture: CBS 125734.
Representative sequences: tef1: HQ342217, rpb2: HQ342280.

Typos: [dry culture] WU 29229a.
Typos: [specimen] (WU 292299).
Ex-type culture: CBS 120926.
Representative sequences: tef1: FJ860641, rpb2: FJ860540.

Ex-nectotype culture: G.J.S. 96-145 = CBS 100808 = ATCC 208860 = IMI 378807.
Note: The protologue of *T. koningbra* indicates that the type is a dry culture deposited in BPI (s.n.) however this material has evidently been lost. Accordingly we designate the metabolically inactive ex-type culture deposited as CBS 100808 as lectotype.

Ex-epitype culture: CBS 457.96.
Typos: [specimen] (BPI 745885).
Representative sequences: tef1: AY376054, DQ288991, KC285594, rpb2: FJ442761

Note: Because Lieckfeldt et al. (1998) did not locate a type specimen from Rifai for *T. koningii*, they designated a neotype (a dry culture, BPI 744887). However the illustration provided with the original description should have been designated as lectotype; thus BPI 744887 and its corresponding culture (CBS 457.96) are to be regarded as epitype and ex-epitype culture, respectively.

Typos: [dry culture] (BPI 802571B).
Ex-type culture: CBS 119075.
Representative sequences: tef1: AF4456910, DQ284966; rpb2: DO381954
Typos: [specimen] (BPI 802571).

Typos: [specimen] JAPAN (TNS-F-38436).
Ex-type culture: TAMA 0193 = NBRC 109640.
Representative sequences: tef1: AB807645, rpb2: AB807657.

**Trichoderma lacu wombantesense** (B.S. Lu, Druzhinina & Samuels) Jaklitsch & Voglmayr, Mycotaxon 126: 149. 2014 [”2013”].

**Trichoderma lanuginosum** Samuels, *in* Samuels & Ismaiel, Mycol. Prog. 11: 240. 2011.
Typos: [specimen] BPI 863853.
Ex-type culture: CBS 125718.
Representative sequences: tef1: HQ342221, rpb2: HQ342284.

**Trichoderma latizonatum** (Peck) Samuels, comb. nov.
MycoBank MB812057
(≡) **Hypocrea latizonata** Peck in Ellis & Everhart, N. Amer. Pyrenom. p. 79. 1892.
Typos: [specimen] “A very curious species sent from Ohio under the above name [H. lati-zonata], by Prof. A.P. Morgan. Parasitic on Cyathus striatus, Hoff.” (NYS Specimen f 1661).
Note: Sundberg & Kost (1989) redescribed this remarkable, distinctive, host-specific *Trichoderma* from North America. This unmistakable species has not been cultured or sequenced.

Typos: [specimen examined] (WU 33397).
Ex-type culture: CBS 30014.

(≡) Hypocrea lentiformis Rehm, Hedwigia 37: 193 (1898).
Typus: [specimen] (BRAZIL: Santa Catarina State: on decaying leaves of Euterpe, Aug. 1888, Ule) (isotypus HBG #812).
Representative sequences: tef1: AF469195, AF443931; rpb2: FJ442687, FJ442749.

Typus: [dry culture] WU 29231a.
Ex-type culture: CBS 122499.
Representative sequences: tef1: FJ179571, FJ179570; rpb2: FJ179605, FJ179606.

Trichoderma lieckfeldtiae Samuels, in Samuels & Ismaiel, Mycologia 101: 149. 2009.
Typus: [dry culture] BPI 878745.
Ex-type culture: CBS 123049.
Representative sequences: tef1: EU856326, rpb2: EU883526.

Trichoderma lixii (Pat.) P. Chaverri, in Chaverri & al., Mycologia 107: 578. 2015
Representative sequences: tef1: AF443938, FJ716622; rpb2: KJ665290.
Note: Chaverri & Samuels (2002) considered H. lixii to be the sexual morph of T. harzianum. A revision of the T. harzianum species complex (Chaverri et al., 2015), however, shows that T. lixii and T. harzianum are closely related but distinct species.

Typus: [dry culture] USA: Ohio: Hamilton County, Duck Creek, on wood, 12 Sep. 1961, W.B. Cooke 4576 (SHD-M).
Ex-type culture: CBS 816.68 = ATCC 18648.
Representative sequences: tef1: EU041591, rpb2: DQ087242.

Typus: [dry culture] (DAO M 177227-1a).
Ex-type culture: DAOM 177227-1a.
Typus: [specimen] (WU 29106).
Ex-type culture: CBS 120953.
Typus: [dry culture] (BPI 843660).
(≡) Hypocrea cuneispora P. Chaverri & Samuels, Mycologia 95: 1118. 2004 [“2003”].
Typus: [specimen] (BPI 1112864).
Representative sequences: tef1: AF534622; rpb2: AF545550.

Typus: [dry culture] WU 29236a.
Typus: [specimen] (WU 29236).
Ex-type culture: CBS 120537.

Typus: [dry culture] WU 29237a.
Typus: [specimen] (WU 29237).
Ex-type culture: CBS 123828.

(≡) Hypoxylon lycogaloides Berk. & Broome, J. Linn. Soc., Bot. 14: 120 (1873) [1875].
Typus: [specimen] (K(M) 177253; G.H.K. Thwaites 1090).
Ex-type culture: CBS 123493.
Representative sequences: tef1: KF134800, rpb2: KF134792.

Typus: [dry culture] WU 29021a.
Typus: [specimen] (WU 29021).
Ex-type culture: CBS 120540.
Representative sequences: tef1: FJ860625, rpb2: FJ860529.

Typus: [dry culture] (BPI 878377).
Ex-type culture: CBS 123052.
Representative sequences: tef1: EU248618, rpb2: EU248597.


Typus: UK: Aberdeenshire: Ballochbrae Forest, near Braemar, on Quercus leaf, Nov 1979, Abdullah (HAME 3704; IMI 266915 authenticated specimen).
Ex-type culture: IMI 266915 = HME 3704.
Representative sequence: *tef1*: AB685539.

Note: Although the oldest epithet for this species is *Pap. viridis*, in *Trichoderma* the epithet *‘viride’* is occupied. The next available epithet is *‘matsushimae’*, which we have adopted here.

**Trichoderma mediterraneum** Jaklitsch & Voglmayr, Stud. Mycol. 80: 70. 2015.
Typus: [specimen examined] (WU 33334).
Ex-type culture: CBS 125719.

Ex-type culture: DAOM 167069.
Typus: [specimen] (BPI 1109373).
Ex-type culture: DAOM 167069.

Typus: [dry culture] (DAOM 167069p).
Typus: [specimen] (BPI 1109373).
Ex-type culture: CBS 120539.

Typus: [dry culture] (WU29283a).
Ex-epitype culture: CBS 120539.

**Trichoderma neocrassum** Samuels, nom. nov.
Mycobank MB812058
Typus: [specimen] (BPI 843647).
Ex-type culture: CBS 114230.
Representative sequences: *tef1*: JN133572, *rpb2*: AY481587.

Note: Chaverri & Samuels (2003) reported that T. crassum Bissett and the newly described *H. crassa* were an asexual morph/teleomorph pair, but this link is not supported by an unpublished molecular phylogenetic analysis. *Hypocrea crassa* is distinct both from *T. crassum* and the closely related *T. virens* (J.H. Miller et al.) Arx.

Typus: [dry culture] (BPI 872182).
Ex-type culture: CBS 120070.

Typus: [dry culture] WU 29296a.
Representative sequences: *tef1*: DQ835449, DQ835450, DQ835478, DQ835479; *rpb2*: DQ835460, DQ835462.

Note: This species was originally described from Japan. The cultures cited above were cited by Overton et al. (2006) and were collected in the U.S.A. For this reason we do not designate either as an epitype.
Trichoderma neoefurum (Samuels, Dodd & Lieckfeldt)
Ex-type culture: CBS 134882.
Typus: [specimen] (BPI 749315).
Ex-type culture: CBS 134882.
Typus: [specimen] (BPI 8456813).
Trichoderma novae-zelandiae (Samuels & O. Petrini)
Ex-type culture: CBS 496.97 = CBS 639.92 = G.J.S. 81-265.
Typus: [specimen] (PDD 46792).
Ex-type culture: CBS 132574.
Typus: [specimen] (W U 31622).
Ex-type culture HMAS 245079 = CGMCC 3.17527.
Trichoderma orientale (Samuels & O. Petrini)
Ex-type culture: CBS 130428.
Typus: [specimen] (BPI 1109853).
Typus: [dry culture] (BPI 843692).
Ex-type culture: CBS 133299.
Typus: [dry culture] WU 29327a.
Ex-type culture: CBS 122126.
Trichoderma ochroleu cum (Berk. & Ravenel) Jaklitsch & Voglmayr, Mycotaxon 126: 150. 2014.
≡ Hypocre a ochroleuca Berk. & Rav. 1382, on trunks of Myrica cerifera, S[outh] C[arolina], HWR near S. luteovirens" (K, ISOTYPE NY 00965640).
Ex-type culture: None. Representative culture: CBS 119502.
Ex-type culture: None. Representative culture: CBS 119502.

Typus: [specimen] (WU 29110a).
Ex-type culture: CBS 120636.
Representative sequences: tef1: FJ179578, AY937444; rpb2: FJ179614.

Typus: [dry culture] (WU 30015).
Ex-type culture: CBS 125925.
Other cultures: CBS 130513, CBS 130853
Representative sequences: tef1: GQ354353, rpb2: HM182963.

Typus: [metabolically inactive culture] (CBS 133496).
Ex-type culture: CBS 133494.

Typus: [metabolically inactive culture] (CBS 136489).
Ex-type culture: CBS 136489.

Typus: [specimen] (WU 24029).
Ex-type culture: CBS 119321.
Representative sequences: tef1: DQ672610, rpb2: KC285763.
Note: Jaklitsch et al. (2006) incorrectly regarded the new sexual morph H. viridescens as identical with Eidamia viridescens.

Typus: [dry culture] WU 29107a.
Typus: [specimen] (WU 29107a).
Ex-type culture: CBS 122769.

Typus: [dry culture] WU 29110a.
Typus: [specimen] (WU 29110a).
Ex-type culture: CBS 120636.

Trichoderma par mastoi (Overton) Jaklitsch & Voglmayr, Mycotaxon 126: 151. 2014.
Typus: [specimen] (BPI 843639).
Ex-type culture: TFC 97-143.
Representative culture: CBS 121139.
Representative sequences: tef1: FJ860668, DQ834456 (exon); rpb2: FJ860567, DQ834463.

Trichoderma patella (Cooke & Peck) Jaklitsch & Voglmayr, Mycotaxon 126: 151. 2014.
Ex-epitype culture: CBS 110081.
Note: Dodd et al. (2002) identified several collections and cultures as H. patella. From among them we select here a collection from Maryland as epitype. Dodd et al. (2002) recognized two forms of H. patella, including f. patella and f. tropica Yoshim. Doi. In the present work we elevate f. tropica to species rank as T. patellotropicum.

Trichoderma patellotropicum Samuels, stat. nom. nov.
MycoBank MB812059
Ex-type culture: CBS 121139.
Note: Dodd et al. (2002) identified several collections and cultures as H. patella. From among them we select here a collection from Maryland as epitype. Dodd et al. (2002) recognized two forms of H. patella, including f. patella and f. tropica Yoshim. Doi. In the present work we elevate f. tropica to species rank as T. patellotropicum.

Typus: [dry culture] (BPI 870953).
Ex-type culture: CBS 118645.
Representative sequences: tef1: DQ109540, rpb2: FJ150787.
Trichoderma peltatum (Berk.) Samuels, Jaklitsch & Voglmayr, in Jaklitsch & Voglmayr, Mycotaxon 126: 151. 2014.


Typus: [specimen] (L00532089, Herb. L 910.250.1421).

Ex-type culture: None.


Note: Several species have been listed as synonyms of H. peltata (Samuels & Ismaiel 2011), but their types were collected over a wide, mainly Southern Hemisphere, geographic range. The type of Sphaeria peltata was collected in Indonesia. We do not have cultures from Indonesia, but sequences of Japanese collections are highly similar to those obtained from collections made in the USA, indicating the likelihood of a single species with a wide distribution. Druzhina et al. (2007) reported the isolation of ‘Hypocrea sp. MKZ-2007a” (tef1: EF392731, rp2b: EF392733) from human lung tissue; that fungus was T. peltatum.


Typus: [dry culture] (BPI 864092B).


Typus: [specimen] (BPI 864029A).

Ex-type culture: CBS 119051.

Other cultures: DAOM 165782, CBS 119507, CBS 124375, CBS 124739.


Note: Originally described from the USA (Tennessee), T. petersenii appears to be a cosmopolitan and common species.


Lectotypus (*hic designatus*, MBT 202324): [specimen] “Hypocrea pezizoides No. 308, Cent Province, Dec 1868, sent before but these specimens in better fruit” (K, Herb. Berk 1879).


Ex-type culture: None.

Representative cultures: G.J.S. 01-231 (lost), CBS 101131 = C.P.K. 775 = G.J.S. 97-83.

Representative sequences: tef1:AY225859, rp2b: JN715610, AF545564.

Note: There are two collections of *H. pezizoides* in Berkeley’s herbarium; they appear to be parts of the same gathering. The portion in the Lloyd herbarium comprises a single stroma, which is identical to the other parts of this number in Berkeley’s herbarium. Samuels (2014) proposed conservation of *H. pezizoides* over the older *T. pezizoideum* Wallr. Sequences of the representative cultures place this species in the Viride clade of *Trichoderma*. Sequences deposited in GenBank are diverse and may represent more than one species. The sequenced culture of one of the cited representative cultures cited here was derived from ascospores (specimen THAILAND, BPI 841389) germinating in asci, giving us a high degree of confidence of its identity. However that culture has been lost.


Typus: [dry culture] WU 29402a


Typus: [specimen] (WU 29402a).

Ex-type culture: CBS 119283.


Typus: [dry culture] (BPI 843665).


Typus: [specimen] (BPI 802817).

Ex-type culture: G.J.S. 92-81 = CBS 114637 = DAOM 232100 = ATCC MYA -3067.

Other culture: G.J.S. 92-123 = CBS 114071 = DAOM 232101 = ATCC MYA 3066.

Representative sequences: tef1: AY737745, AY391986; rp2b: AF545513, AY391927.


Ex-type culture: CBS 120927.


Ex-type culture: CBS 814.68.


Note: For comments see Lu et al. (2004).


Typus: [dry culture] (BPI 882296).

Ex-type culture: CBS 131292.
Trichoderma placentula
Jaklitsch, Fungal Divers. 48: 120. 2011.
Typus: [dry culture] (WU 29410a).
(≡) Hypocreopsis placentula Grove, J. Bot. (Lond.) 23: 133. 1885.
Ex-epitype culture: 120924.

Trichoderma pleuroti
S.H. Yu & M.S. Park, in Park & al., Mycobiology 34: 111. 2006; [as ‘pleurotum’].
Ex-type culture: CBS 124387.
Representative sequences: tef1: HM142382, EU279975; rpb2: HM142372.

Trichoderma pleuroticola
Ex-type culture: CBS 124383.
Representative sequences: tef1: HM142381, EU918160; rpb2: HM142371.

Typus: JAPAN: Otsuno, Kochi City, on bark, 3 May 1966, Y. Doi TNS-D-77 (TNS-F-190528).
Typus: [dry culture] (DAOM 167068).
Typus: [specimen] (PDD 77488, isotypus: BPJ 746610).
Ex-epitype culture: CBS 820.68.
Representative sequences: tef1: AY750866, FJ860661; rpb2: FJ179613, JQ685878.

Note: S.J. Hughes (1958: 812) studied the specimens of both S. polysporum and Aleurisma sporulosum (L.), placing both species in synonymy with T. sporulosum (ibid.: 820). He wrote the following about the type collection of S. polysporum (S.J. Hughes, hand-written notes deposited in DAOM): ‘[dots white or cream coloured now squashed on rotten bark].’ [looks like Trichoderma candidum (Sacc.) but no curly hypheae seen] [Trichoderma (white) no spirals].’ Because it was sanctioned by Fries, the name polysporum was subsequently given preference over sporulosum. Rifai (Mycol. Pap. 116: 21. 1969) considered Gams C306 to be typical of T. polysporum. Accordingly, we designate this metabolically inactive culture as epitype of Sporotrichum polysporum here. Bissett (1991) distinguished between T. polysporum and H. pachybasiosiodes on the basis of morphology. However, phylogenetic analyses (Lu et al., 2004; Jaklitsch, 2011) have demonstrated that cultures isolated directly from substrate and identifiable as T. polysporum cluster with cultures isolated from ascospores of specimens identifiable as H. pachybasiosiodes, including cultures studied by Bissett. Moreover, T. polysporum appears to represent a species complex that includes T. croceum and T. stellatum, which we include here as synonyms of T. polysporum (Lu et al., 2004; Jaklitsch & Voglmayr, 2015; Bissett unpubl.). Future study focused on this complex may resolve additional species, including some that today we consider as synonyms.

Trichoderma poroniiodeum (A. Möller) Samuels, comb. nov.
MycoBank MB812060
Ex-epitype culture: CBS 139046.
Representative sequences: tef1: KP109823.

Note: Samuels & Lodge (1996) described the sexual and unnamed asexual morphs of this distinctive species. DNA
sequences indicate that *T. poronioideum* is a member of the Viride clade, closely related to *T. asperellum*.

Typus: [specimen] (WU 33327).
Ex-type culture: CBS 131487.


Typus: [specimen] TNS-F-192712.
Ex-type culture: CBS 739.83.
Representative sequences: tef1: FJ860676, rpb2: FJ860574.

**Trichoderma protrudens** Samuels & P. Chaverri, in Degenkolb & al., Mycol. Prog. 7: 212. 2008.
Typus: [dry culture] BPI (878378).
Ex-type culture: CBS 1143340.
Representative sequences: tef1: HM920206, JQ797400, JQ797401, JQ797402; rpb2: AF545518.


Typus: [specimen] TNS-D-366 = TNS-F-223432.
Ex-type culture: CBS 119129.
Representative sequences: tef1: AY737744, AF534582; rpb2: AF545518.

Typus: [dry culture] (WU 29420a).
Ex-type culture: CBS 119129.

Typus: TNS-D-366 = TNS-F-223432.
Ex-type culture: TUFC 60104.
Representative cultures: CNU N109, CNU N334, TUFC 60440, TUFC 60753.
Representative sequences: tef1: HM920206, JQ797400, JQ797401; rpb2: HM920177, JQ797408, JQ797409.

Typus: [specimen] (TMI 8484) (ex-type culture TUFC 61490 = CBS 133191).
Ex-type culture: TUFC 61490 = CBS 133191.
Representative sequences: tef1: JX175588, rpb2: JX175535.


Typus: [dry culture] BPI 843664.
Ex-type culture: CBS 1143340.
Typus: [specimen] (BPI 842416).
Representative sequences: tef1: AY737744, AF534582; rpb2: AF545518.


Typus: [specimen] TNS-D-366 = TNS-F-223432.
Ex-type culture: TUFC 60104.
Representative cultures: CNU N109, CNU N334, TUFC 60440, TUFC 60753.
Representative sequences: tef1: HM920206, JQ797400, JQ797401; rpb2: HM920177, JQ797408, JQ797409.

Typus: [dry culture] (DAO 166162).
Ex-type culture: DAO 166162.
Representative sequences: tef1: AY750887, AF534624; rpb2: EU248613.

**Trichoderma pulvinatum** (Fuckel) Jaklitsch & Voglmayr, Mycotaxon 126: 152. 2013.

Trichoderma pyramidale

Typus: [metabolically inactive culture] (CBS 135574).
Ex-type culture: CBS 135574.

Trichoderma reesei

Ex-type culture: QM 6a = CBS 383.79.

Trichoderma rossicum

Typus: [dry culture] (DAOM 230011).
Ex-type culture: DAOM 230011.
Representative sequences: tef1:AY937441, rp2b:HC342288.

Trichoderma rosulatum

Ex-type culture: HMAS 244906.
Representative sequences: tef1:KF729984, rp2b:KF730005.

Trichoderma rubi

Typus: [specimen] (WU 33316).
Ex-type culture: CBS 127380.

Trichoderma rufobrunneum

Ex-type culture: HMAS 244907.
Representative sequences: tef1: KF729984, KF729989; rp2b: KF730010, KF730007.

Trichoderma sambuci

Typus: [specimen] (WU 29442).
Ex-type culture: CBS 119288.
Representative sequences: tef1: JF860685, rp2b: JF860578.

Trichoderma radmianii

(Samuels & P Chaverri) Jaklitsch & Voglmayr, Mycotaxon 126: 152. 2014.
Typus: [specimen] (BPI 1112859).
Ex-type culture: CBS 120895.
Representative sequences: tef1: FJ860687, EU338286; rp2b: FJ860580, EU338324.

Trichoderma rogersonii

Typus: [dry culture] (BPI 870964).
Typus: [specimen] (BPI 8709964A).
Ex-type culture: G.J.S. 04-158 = CBS 119233.
Representative sequences: tef1: DQ307563, J860690; rp2b: JN133566, FJ860583.
Typus: [dried culture] (BPI 872181).
Ex-type culture: CBS 120069.
Representative sequences: tef1: DQ841726, rpb2: EU252007.

Trichoderma semiorbis (Berk.) Jaklitsch & Voglmayr,

(≡) Sphaeria semiorbis Berk., J. Bot. (Hooker) 2: 146. 1840.
(≡) Hypocrea semiorbis (Berk.) Berk., in Hooker, Fl. Tasm. 2: 278. 16 Aug 1859.

Ex-epitype culture: CBS 130716.
Representative sequences: tef1: JN133576, rpb2: JN133567.

Note: The type locality of S. semiorbis is not known with certainty. Berkeley (1840: 146) described two fungi from the collection of William Jackson Hooker, Lentinus fasciatus and Sphaeria semiorbis. The Lentinus was listed previously as Lentinus villosus by Berkeley in an account of fungi from Van Diemen’s Land but he did not provide the provenance of the collection of S. semiorbis. In the protologue the only collecting information given is "On bark. Hab. unknown." We assume the original collection to have been made in Australia because the second known collection of this species is reported in Hooker’s Botany of the Antarctic Voyage, although even in this report the only clue to its origin is its collector, Ronald Campbell Gunn, who sent specimens from Tasmania to J.D. Hooker in Kew between 1830 and 1860. Dingley (1956) examined a collection from Tasmania in Kew which she assumed to be the type collection, providing a description of this specimen and referring New Zealand collections she had earlier listed as Hypocrea patella to this species. In her description she described perithecia containing ascii with mostly immature spores. However, as Berkeley himself noted in the protologue, the type collection of S. semiorbis is immature, lacking spores and asci. Dingley (1957) later described a Trichoderma assexual morph that was derived from her collections. She subsequently sent material to John Webster in Exeter. It is not known whether she sent a culture or a specimen from which Webster made a culture, but eventually a culture was deposited as CBS 244.63 with provenance ‘Dingley No. 12,’ New Zealand: Mohoka. This culture was redescribed by Bissett (1991) as the asexual morph of H. semiorbis under the number DAAOM 67636 = CBS 244.63. Bissett’s description of H. semiorbis is consistent with Dingley’s, and Dingley collections of H. semiorbis (PDD) are consistent with the type collection of S. semiorbis. However, the culture CBS 244.63 cannot be linked to any Hypocrea collection; there is no specimen of H. semiorbis in the Sheffield University Herbarium and none of the collections in PDD can be linked to a specimen or culture that Dingley (Dingley 12) sent to Webster. Thus a question remains as to the link between CBS 244.63, which is the only living culture that links Bissett and Dingley’s concepts of the species and for which DNA sequences have been deposited in GenBank, and H. semiorbis as typified. An epitype for S. semiorbis is needed. There are three Dingley collections of H. semiorbis in her herbarium (PDD), all made from the same place in April and May 1953 (NEW ZEALAND: Hawkes Bay: Upper Mohoka River, Kaimanawa Range, elev. 2000 ft, on Nothofagus fusca, J.M. Dingley s.n. (PDD 12751 (May 1953), PDD 12755 (31 May 1953), PDD 12756 (April 1953) but none of them can be linked to a living culture and thus none of them can serve as an epitype. The material sent to Webster (Dingley 12 = CBS 244.63) is derived from a Dingley collection of H. semiorbis that was made from the Mohoka River on Nothofagus sp., date unknown, and there is a culture in ICMP (ICMP 1693) that is derived from H. semiorbis collected by Dingley (Dingley 584) from the Mohoka River, from Nothofagus sp. in 1958, but the specimen from which this culture was derived cannot be located (PDD, SHU). DNA sequences (tef1, Samuels unpubl.) indicate that CBS 244.63 is the same species as ICMP 1693, but the question as to the identity of teleomorphic H. semiorbis remained open. A recent New Zealand collection from Nothofagus sp. and its culture complete this circle and permit stabilization of the name H. semiorbis by epitypification proposed above. DNA sequences derived from this specimen indicate that it is the same species as ICMP 1693 and CBS 244.63; morphologically the stromata agree well with the type collection of S. semiorbis and the Dingley collections of H. semiorbis in PDD cited above, and the asexual morph matches descriptions of the asexual morph of H. semiorbis in publications from Dingley and Bissett. Although we do not know the substratum of either of the collections of H. semiorbis reported by Berkeley, the type collection of S. semiorbis was possibly collected in Tasmania where Nothofagus is common and thus could have been the substratum of the type collection. All of Dingley’s collections were from Nothofagus. Finally, the recent New Zealand collection was made in the South Island, which has a south temperate climate similar to that of Tasmania. Bissett (1991) and Chaverri et al. (2003a) redescribed the Trichoderma asexual morph of H. semiorbis, the description in the latter reference is based in part on the epitype collection. Chaverri et al. (2003a) redescribed the teleomorph based on the three Dingley collections cited above. Hypocrea semiorbis is common on Nothofagus in New Zealand but is not known outside of Australasia.

Typus: [metabolically inactive culture] (CBS 133498).
Ex-type culture: CBS 133498.

Typus: [specimen] (WU 28698).
Typos: [dry culture] (WU 29227a)


Typos: [specimen] (WU 29227).

Ex-type culture: CBS 120922.
Representative sequences: tef1: AF443935, AF443936, AF443933; rpb2: FJ442757, AY391925, FJ442710.


Typos: [specimen] (BPI 1112907).

Ex-type culture: G.J.S. 02-84 = CBS 114248 = DAOM 232840.
Representative sequences: tef1: AY750889, rpb2: JN175528.


Ex-type culture HMAS 245077 = CGMCC 3.17528.


Typos: [dry culture] BPI 843649.

(≡) *Hypocrea sinoua* P. Chaverri & Samuels, Stud. Mycol. 48: 812004 [*"2004"*].

Typos: [specimen] (BPI 843649).

Ex-type culture: CBS 114247.
Representative sequences: tef1: AY737743, AY391997; rpb2: FJ179619.


Typos: [dry culture] (DAOM 183974).

Ex-type culture: DAOM 183974.
Representative sequences: tef1: AY50890, rpb2: FJ442694, KJ665348.


Typos: [specimen] SPAIN: Puerto de Samossierra, on cow dung, 16 Sep. 1982, G. Moreno (MA-Fungi 3059 [IJFMA-12]).

Ex-type culture: CBS 148.85 = ATCC 62321.


Typos: [dry culture] (BPI 744463B).

Ex-type culture: CBS 992.97 = ATCC MYA 2970 = DAOM 231834.


Typos: [specimen] (BPI 744463).

Representative sequences: tef1: DQ109546, rpb2: EU341805.


Ex-type culture: HMAS 244908.
Representative sequences: tef1: KF29990, KF29991; rpb2: KF30011, KF320012.


Typos: [dry culture] BPI 843667.


Typus: [*P. Chaverri, Samuels & Minnis*, Persoonia 34: 122. 2015].

Ex-type culture: DAOM 232840.
Representative sequences: tef1: AY737743, rpb2: AY391945.


Typus: [GERMANY] "... im Frühling, auf einem sehr faulen Stengel von *Chelidonium majus*. Am Mühberg bei Oestrich" (G).

Ex-type culture. None. Representative cultures: CBS 310.50, CBS 311.50, CBS 121272.

**Note**: This species is not known to produce an asexual morph.


Typus: [GERMANY] "... im Frühling, auf einem sehr faulen Stengel von *Chelidonium majus*. Am Mühberg bei Oestrich" (G).

Ex-type culture. None. Representative cultures: CBS 310.50, CBS 311.50, CBS 121272.

**Note**: This species is not known to produce an asexual morph.


Typus: [GERMANY] "... im Frühling, auf einem sehr faulen Stengel von *Chelidonium majus*. Am Mühberg bei Oestrich" (G).

Ex-type culture. None. Representative cultures: CBS 310.50, CBS 311.50, CBS 121272.

**Note**: This species is not known to produce an asexual morph.


Ex-type culture: DAOM 172827.


Typus: [specimen] (TNS.D-148 = TNS-F 191611; isotypus NY No. 01293246).


Typus: [dry culture] WU 29487a.

Ex-type culture: CBS 120929.


Typus: [dry culture] (WU 29481a).

Ex-type culture: CBS 119929.


Epitypus (vide Jaklitsch, ibid.): [specimen] AUSTRIA (WU 29481).


Typus: [dry culture] WU 29487a.


Ex-type culture: CBS 120929.


Typus: [specimen] (NY No. 01169121).

Ex-type culture: G.J.S. 85-228 (lost). Representative culture: G.J.S. 85-228 (lost).

Representative sequences: tef 1: FJ737730, AY392002; rpb 2: AY391954.

Note: There are no longer living cultures of this species available.


Isolectotypus: "Salem. 45" (PH 01107658); "Salem and Bethlehem" (K); "Salem nec Pennsylv." (BPI 801107, a microslide).

Ex-type culture: None.

Epitypus (hic designatus, MBT 202326): [specimen] AUSTRIA (WU 29493).

Ex-type culture CBS 119929.


Note: The original material of S. sulphurea is given as "1221. 75. S. sulphurea, L.v.S., rara in cortice insidens, Salem nec Pennsylv." The specimen PH 01107657 includes two parts. One is labeled presumably in Schweinitz’s hand as "Sphaeria sulphurea 1221 — 75 Syn. Fung., Salem" The other is labeled in hand as "Sphaeria sulphurea Schew. β parasitica Schw. 1221—75 Syn. Fung., Ohio." The printed label that contains these two specimens gives “Salem, Ohio." The specimen BPI 801107, from the Collins autograph collection (Shear & Stevens 1917a: 203), is labeled ‘North Carolina, Salem’ and is certainly part of the original material that was studied by Schweinitz. Unfortunately the packet is empty, save for a microscope slide. Schweinitz is known to have collected extensively both in Salem, North Carolina, and in Pennsylvania but he also travelled to Ohio, and he is known to have combined into one packet specimens of what he thought were the same species that were collected in different localities (Shear & Stevens, 1917a, b). We follow the Collins collection label in concluding that the original collection of
S. sulphurea was collected in Salem, North Carolina. The specimen in K is labeled ‘Salem and Bethlehem’ also is an isolecotype. We have not seen Schweinitz material of S. sulphurea from Pennsylvania.

Typus: [dry culture] BPI 843668.
(≡) Hypocrea surrotunda P. Chaverri & Samuels, Mycologia 95: 1134. 2004 [“2003”].
Ex-type culture: G.J.S. 95-93 = CBS 119058.
Ex-type culture: G.J.S. 95-1134 = CBS 114237 = DAOM 232843 = ATCC MYA-3232.
Representative sequences: tef1:AY737734, AF534954; rpb2: AF545540.

Typus: [dry culture] BPI 737694.
Ex-type culture: G.J.S. 95-93 = CBS 119058.
Representative sequences: tef1: DQ284973.

Typus: [dry culture] BPI 843669.
Ex-type culture: G.J.S. 97-174 = CBS 114233.

Typus: [metabolically inactive culture?] CHINA: Jiangxi: Guanshan Nature Reserve (28.2° N/114.3° E), isolated from old trunk of Taxus mairei, ZJUF0986 (China General Microbiological Culture Collection CGMCC 1672).
Ex-type culture: ZJUF0986 = CGMCC 1672.
Representative sequences: tef1: DQ859029, rpb2: DQ859032.
Note: In the protologue for T. taxi there is some confusion concerning its typification. The authors of this species do not specifically state that the holotype is a ‘metabolically inactive culture’ and the culture ZJUF0986 = CGMCC 1672 does not appear in the CGMCC on-line catalogue.

Typus: [dry culture] (BPI 843670).
Typus: [specimen] (BPI 745832).
Ex-type culture: G.J.S. 97-61 = CBS 114234 = DAOM 232842 = ATCC MYA-3233.
Representative sequences: tef1:AY737748, AY392005; rpb2: AY391957.

Typus: [dry culture] BPI 843671.
(≡) Hypocrea thelephoricola P. Chaverri & Samuels, Stud. Mycol. 48: 962004 [“2003”].
Typus: [specimen] (BPI 737702).
Ex-type culture: G.J.S. 95-135 = CBS 114237 = DAOM 232843 = ATCC MYA-3232.
Representative sequences: tef1: AY737735, AY392006; rpb2: AY391958.

Typus: [dry culture] (BPI 871726).
Ex-type culture: DIS 85f = CBS 119120 = IMI 393419 = ATCC MYA-3640.
Representative sequences: tef1: EU856321, rpb2: FJ007374.

Typus: [dry culture] (DAOM 178713a).
Ex-type culture: DAOM 178713a.
Representative sequences: tef1: AY750882, rpb2: AF545557.

Neotypus: [dry culture] WU 29508a.
Ex-epitype culture: CBS 121140.

Typus: [metabolically inactive culture] (CBS 134702).
Ex-type culture: CBS 134702.
Representative sequences: tef1: DQ307526, DQ672606; rpb2: KC285770.

Typus: CHINA: Yunnan: Menglia, Cuipingfeng tropical rain forest park, alt. 800 m, on dead bark of Castanopsis, 1 Oct 1993, P.G. Liu D’93-40 (HKAS 26198).
Ex-epitype culture: HMAS 244983.
Representative sequences: tef1: KF923286, rpb2: KF923313.
Note: Hypocrea tropicosinensis was described without
reference to a culture or to DNA sequences. Zhu & Zhuang (2015) identified, cultured and sequenced a collection that they identified as *H. tropicosinensis*, but they did not designate an epitype. In the interest of stabilizing this name, we designate their collection as epitype.

Typus: [specimen] JAPAN (TNS-F-38437).
Ex-type culture: TAM 2003 = NBRC 109641.
Representative sequences: tef1: AB807647, rpb2: AB807659.

Typus: [dry culture] (BPI 747361).
Ex-type culture: [dry culture] (BPI 881031).

Typus: [dry culture] PDD 88476.
Ex-type culture: DAOM 230013.
Representative sequences: tef1: HQ342219, rpb2: HQ342282.

Typus: [specimen] (BPI 747361).
Ex-type culture: G.J.S. 99-158 = CBS 119087.
Representative sequences: tef1: HQ342573, rpb2: DQ835517.

Typus: [specimen] (WU 31627).


Typus: [specimen] (BPI 737768).
Representative cultures: ATCC 13213 = CBS 249.59. ATCC 13308 = CBS 248.59.
Representative sequences: tef1: AY750891, AF534631; rpb2: AF545558.

**Note:** Miller et al. (1957) did not indicate a place of deposit for the types of their new species, *G. virens* and *G. flavofuscum*, and no herbarium material can be found for them. Neither did the authors specifically indicate a culture collection into which the type cultures were deposited. However the e-type cultures of *G. virens* and *G. flavofuscum*, respectively, were deposited by the authors in ATCC, which subsequently (1959) deposited them in CBS. Because these cultures that are deposited in at least two culture collections are part of the original gatherings, they may be interpreted as isotypes. Accordingly, we designate the metabolically inactive cultures that are stored in CBS for each of these names as their respective lectotypes.


Representative sequences: *tef* 1: KC285647, 2: GU198274.

Note: *Trichoderma viride* is the type species of *Trichoderma*. Its connection with *Hypocrea rufa*, though for a long time generally recognized, was fixed by the epitypification of Jaklitsch & al. (2006).


Neotypus (*vide* Jaklitsch et al. in Persoonia 31: 126. 2013): [icon] (Horne & Williamson in Ann. Bot. 37: 397, Fig. 5. 1923).


Representative sequences: *tef* 1: DQ672617, 2: EU711362.

Note: Jaklitsch & al. (2006) linked *T. viridescens* to the sexual morph *H. viridescens* Jaklitsch & Samuels. However in a revision of the *T. viridescens* complex Jaklitsch et al. (2013) concluded that *H. viridescens* is actually the sexual morph of *T. paraviridescens* and that *T. viridescens* was linked to an unnamed sexual morph.


Typus: [metabolically inactive culture] (CBS 133495).

Ex-type culture: CBS 133495.

Representative sequences: *tef* 1: KC285705, 2: KC285772.


Typus: [specimen] (WU 31628).

Ex-type culture: CBS 132569.

Representative sequences: *tef* 1: KJ665772, 2: KC285692.


Typus: [dry culture] (WU 25711a).


Typus: [specimen] (WU 25711).

Ex-type culture: CBS 117711.

Representative sequences: *tef* 1: DQ086146, 2: FJ179622.


Ex-type culture: YMF 1.00169 = CBS 121219.

Representative sequences: *tef* 1: AY94182, 2: GU198274.

Note: *Podostroma yunnanense* is distinct from *T. yunnanense*. Apparently *P. yunnanense* has not been cultured or sequenced.


**Trichoderma aeruginosum** Chevall., Fl. Gén. Env. Paris: 54. 1826; nom. illegit. (Art. 53.1); non *T. aeruginosum* Link, 1816.

Note: MycoBank gives *T. aeruginosum* Chevall. as a synonym of *T. violaceum* Oudem., but without explanation. In the original description of the latter species, no mention is made of *T. aeruginosum*. The protologue of *T. aeruginosum* Chevall. does not permit speculation as to the identity of the species.

**Trichoderma album** Preuss, Linnaea 24: 141. 1851.


**Trichoderma brassicae** Schum., Enum. Pl. 2: 235. 1803.


Trichoderma collae (Schwein.) Sacc., Syll. Fung. 4: 60. 1886.


(≡) Trichoderma polysporum.


Note: When it was originally described a verticillum-like Trichoderma asexual morph was described for this species but there was no indication that a culture had been placed in a public collection, although a culture was said to have been kept in the collection of P.-G. Liu at HKAS. There are no sequences deposited in GenBank for this species. Thus we do not include T. corrugatum among the ‘accepted’ species of Trichoderma at this time.


(≡) Trichoderma longipile.


(≡) Hypocreopsis discoidea (Berk. & Broome) Sacc., Michelia 1:322. 1878.

Typus: Ceylon, on dead leaves of Zingiber (K, ex herb. Berk.).

Note: The holotype specimen consists of a piece of stiff paper with two pieces of leaf glued to it. There are scale insects on the leaf pieces. Five 3 mm diam, orange stromata are glued separately to the paper. Each stroma is a hemispherical aggregate comprising 100 or more cespitose, orange perithecia. Ascospores are filiform and remain entire in the ascus. The ascal apex is typical of the Clavicipitaceae.


(≡) Trichoderma strictipile.

Trichoderma flavum Sommerf., Suppl. Fl. Lapp.: 312. 1826.


Note: When H. fomitopsis was described, a Trichoderma asexual morph having pachybasium-type branching and hyaline/white conidia was described for the species. However no indication was given that a culture had been deposited in a public collection. The authors did not sequence the culture. An unpublished sequence (18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence) was deposited in GenBank (JF905628) by N Fan (College of Life Sciences, Nanjing Agricultural University, No. 1 Weigang Xuanwu District, Nanjing, Jiangsu 210095, China) for a cellulose degrading culture identified as H. fomitopsis. Because there is no indication of how the culture was identified, and no specimen or culture number was indicated with the deposit, and because sequence of the RNA gene cluster are too highly conserved to distinguish Trichoderma species, the identity of this accession is in doubt. We do not include T. fomitopsis among the names of Trichoderma that we accept as being in use at this time.


(≡) Trichoderma citrinum.

Note: Hughes (1958) did not offer an opinion on the application of this name.


Trichoderma lateritio-roseum Libert, in Cooke, Grevillea 8: 83. 1880.

(≡) Pyrenium lignorum Tode, Fung. mecklenb. 1: 33. 1790.
Note: Trichoderma lignorum is generally considered a synonym of T. viride.


Note: The ex-type culture of this variety (= CBS 316.31) is morphologically T. harzianum.

Trichoderma minimum (Speg.) Gunth. Müller, Wiss. Z. Humboldt-Univ. Berl. 14: 775. 1965; as “minima”.

Note: Rifai (1969) synonymized T. minutum Bain. under T. polysporum, but Bain.’s pl. XIV, figs. 14–16 do not appear to be T. polysporum and in fact might not even be a Trichoderma.


(≡) Sporotrichum narcissi Tochinai & Shimada, Trans.

(≡) Trichoderma harzianum s. lat. (fide Rifai, 1969).
Ex-type culture CBS 316.31.


(≡) Trichoderma pseudonigrovirens.


(≡) Trichoderma ghanense.


Trichoderma pezizoideum Wallr., Fl. crypt. Germ. 2: 246. 1833; nom. rej. prop. (Art. 53, voted example 11); non T. pezizoides.
Note: Samuels (2014) proposed rejection of this name.

(≡) Pyrenium lignorum [var.] β. aureum Tode, Fungi Mecklenb. Sel. 1:33. 1790.
Note: This synonymy is from Hughes (1958). Hughes incuded this name in square brackets with the annotation: “Quid?” The identity of T. pyrenium (Tode) Pers. is not known.

Trichoderma pyrenium Schumach., Enum. pl. 2: 235. 1803; nom. illegit. (Art. 53.1); non T. pyrenium (Tode) Pers.

Trichoderma racemosum McAlpine, Fungus Dis. Stone-fruit Aust.: 105. 1902.

(≡) Trichothecium roseum (Pers.) Link (fide Hughes, 1958).

(≡) Hyphelia spadicea (Schwein.) Fr., Syst. mycol. 3(1): 212. 1829.
Accepted names in *Trichoderma*


(≡) *Trichoderma polysporum*.

(≡) *Trichoderma polysporum*.

**Trichoderma subsulphureum** (Syd.) Jaklitsch & Voglmayr, Mycotaxon 126: 153. 2013.  
Typus: “Kisantu, 1907 (H. Vanderyst).”  
Ex-type culture. None. Representative culture: None.  
**Note:** Overton et al. (2006) did not locate the type specimen of *H. subsulphurea* in S, and the protocol did not include illustrations. Overton et al. (2006) identified a recent (2002) Japanese collection as *H. subsulphurea*, from which they obtained a culture and DNA sequences. Although Overton et al. (2006) reported that the specimen (Overton M 141) had been deposited in BPI, there are no specimens of *H. subsulphurea* or *Hypocrea* specimens collected in Japan in 2002 in BPI. Moreover, the culture from the Overton specimen has apparently been lost. Thus *T. subsulphureum* is only known from DNA sequences of doubtful origin deposited in GenBank (ITS DQ835509, tef1 DQ835492, rpb2 DQ835552).


**Trichoderma todica** Sokoloff & Toda, nom. inval. (Art. 32.1).  
**Note:** This name has not been effectively published; it is referred to as ’sp. nov NRRL 3091’ in U.S. patent no. US 3323996 A, which is *T. ghanense* referred to as ‘sp. nov NRRL 3091’ in U.S. patent no. US 9: 143. 1975.

**Note:** Hughes (1958) did not offer an opinion on this species.


(≡) *Trichoderma aeruginosum* Chevall., *Fl. Gén. Env. Paris* 1: 54. 1826; nom. illegit. (Art. 53.1); non *T. aeruginosum* Link 1816.  
**Note:** MycoBank indicates that the illegitimate name *T. aeruginosum* Chevall. is *T. violaceum*, but without explanation.  
Conidia of *T. aeruginosum* are described as ‘… presque globuleuses, violacé-tendre à l’état isolé, violacé-noirâtre en masse, apiculées à la base, et appliquées par moyen de cette petite prôférence à l’entour du sommet de la hyphé, lequel par là semble cinglé d’un anneau colorié.’ This description suggests that the fungus is not a *Trichoderma*.

**Trichoderma viride** Schumach., *Enum. Pl.* 2: 235. 1803; nom illegit. (Art. 53:1); non *T. viride* Pers. 1791.


**ACKNOWLEDGMENTS**

We greatly appreciate John Wiesemara (USDA, ARS) for his advice and encouragement in preparing this list, and for the many copies of articles that he provided. Lisa Castlebury and the staff of The Systematic Mycology and Microbiology Laboratory (USDA, ARS) very kindly located, prepared and sent many cultures from their collection to CBS. Keith Seifert (Agriculture Canada, Ottawa) provided us with S.J. Hughes’ valuable annotations of *Trichoderma* species and specimens. Wen-ying Zhuang (Academia Sinica, Beijing) provided very helpful comments in her review of this work, and copies of important articles. David L. Hawksworth’s advice and his editorial efforts were significant and appreciated.

**REFERENCES**


Bissett et al.


New sequestrate fungi from Guyana: Jimtrappea guyanensis gen. sp. nov., Castellanea pakaraimophila gen. sp. nov., and Costatisporus cyanescens gen. sp. nov. (Boletaceae, Boletales)

Matthew E. Smith¹, Kevin R. Amses², Todd F. Elliott³, Keisuke Obase¹, M. Catherine Aime⁴, and Terry W. Henkel²

¹Department of Plant Pathology, University of Florida, Gainesville, FL 32611, USA
²Department of Biological Sciences, Humboldt State University, Arcata, CA 95521, USA; corresponding author email: Terry.Henkel@humboldt.edu
³Department of Integrative Studies, Warren Wilson College, Asheville, NC 28815, USA
⁴Department of Botany & Plant Pathology, Purdue University, West Lafayette, IN 47907, USA

Abstract: Jimtrappea guyanensis gen. sp. nov., Castellanea pakaraimophila gen. sp. nov., and Costatisporus cyanescens gen. sp. nov. are described as new to science. These sequestrate, hypogeous fungi were collected in Guyana under closed canopy tropical forests in association with ectomycorrhizal (ECM) host tree genera Dicymbe (Fabaceae subfam. Caesalpinioideae), Aldina (Fabaceae subfam. Papilionoideae), and Pakaraimaea (Dipterocarpaceae). Molecular data place these fungi in Boletaceae (Boletales, Agaricomycetes, Basidiomycota) and inform their relationships to other known epigeous and sequestrate taxa within that family. Macro- and micromorphological characters, habitat, and multi locus DNA sequence data are provided for each new taxon. Unique morphological features and a molecular phylogenetic analysis of 185 taxa across the order Boletales justify the recognition of the three new genera.

Key words: Boletineae Caesalpinioideae Dipterocarpaceae ectomycorrhizal fungi gasteroid fungi Guiana Shield

Article info: Submitted: 31 May 2015; Accepted: 19 September 2015; Published: 2 October 2015.

INTRODUCTION

Gasteroid fungi comprise a diverse, artificial assemblage of fungi within Agaricomycetes (Basidiomycota) that are functionally united in their enclosed hymenial development and lack of ballistospory. This informal group includes such charismatic macrofungi as puffballs, earthstars, false earthstars, earthballs, bird’s nest and cannonball fungi, stinkhorns, and false truffles (Ingold 1965, Miller & Miller 1988). These fungi have once been treated as a cohesive taxonomic unit (e.g. class Gasteromycetes) with the assumption that basidiomycotes were rarely lost in basidiomycete evolutionary history (e.g. Coker & Couch 1928) or that the sequestrate state was ancestral, predating the evolution of basidiomycete (e.g. Singer 1971). Other authors regarded sequestrate basidiomycetes as a polyphyletic assemblage based on morphological and developmental evidence (e.g. Rejinders 1963, 2000, Heim 1971, Moore 1988). Application of molecular techniques in mycology has since corroborated the latter view by discovering new sequestrate taxa in numerous family and genus-level lineages in Agaricomycetes, demonstrating that the sequestrate basidioma form has independently evolved multiple times (e.g. Bruns et al. 1989, Mueller & Pine 1994, Hibbett et al. 1997, Miller et al. 2000, Peinter et al. 2001, Miller & Aime 2001, Binder et al. 2006, Lebel & Tonkin 2007, Henkel et al. 2010, Gube & Dorfert 2012, Lebel & Syme 2012, Ge & Smith 2013). Some sequestrate fungi resulted from recent, isolated evolutionary events that led to one or a few sequestrate species within a clade of non-sequestrate relatives (e.g. Kretzer & Bruns 1997, Martin et al. 2004, Giachini et al. 2006, Smith et al. 2006, Henkel et al. 2010) whereas other sequestrate clades of earlier origin have speciated and radiated across the globe (e.g. Grubisha et al. 2002, Binder & Hibbett 2006, Hosaka et al. 2006, Lebel et al. 2015). Understanding of the multiple origins and taxonomic affinities of sequestrate fungi has provided insight into the evolutionary forces that drastically alter basidiomycete form, function, and basidiospore dispersal (Thiers 1984, Kretzer & Bruns 1997, Rejinders 2000, Trappe & Claridge 2005, Albee-Scott 2007).

Knowledge of the diversity and distributions of sequestrate fungi has progressively advanced for some regions of the world (e.g. Bougher & Lebel 2001, Montecchi & Sarasini 2001, Trappe et al. 2009), but tropical sequestrate fungi remain especially poorly known. While some epigeous sequestrate fungi have recently been documented from the Brazilian Amazon (e.g. Cabral et al. 2014), Mueller et al. (2007) estimated that ~30 species of hypogeous sequestrate taxa are currently described from the Neotropics with approximately 200 species remaining unknown to science. Recent studies in the Guiana Shield region of northeastern South America have revealed a diverse assemblage of sequestrate fungi in remote, primary tropical rain forests dominated by...
ectomycorrhizal (ECM) species of *Dicymbe* (Fabaceae subfam. Caesalpinioideae), *Aldina* (Fabaceae subfam. Papilionoideae), and *Pakaraimaea* (Dicterocarpaceae). These include epigean and hypogean taxa from diverse basidiomycetes and ascomycetes, including ECM-forming genera such as *Hysterangium* (*Hysterangiales*), *Scleroderma* and *Tremellogaster* (*Boletales*), and *Pseudotulostoma* and *Elaphomyces* (*Eurotiales*), as well as non-ECM genera such as *Protuberia* (*Hysterangiales*), *Guyanagaster* (*Agaricales*), and *Geastrum* (*Geastrales*) (Miller et al. 2001, Henkel et al. 2010, 2012, Castellano et al. 2012, Henkel unpubl.).

Within *Boletaceae* (*Boletales*), numerous genera of sequestrate fungi have been recognized from various world regions, including: the widely distributed North Temperate *Chamonixia* and *Octaviania*; Australasian *Rossbeevea*; South-East Asian *Durianella*, *Spongiforma*, and *Rhodactina*; tropical African *Mackintoshia*; and Australian *Soliciassus* and *Royoungia* (Binder & Breinski 2002, Desjardin et al. 2008, 2009, Lebel et al. 2008, 2009, Lebel et al. 2012, Orihara et al. 2012a, b, Moreau et al. 2013, Trappe et al. 2013). However, despite a high diversity of non-sequestrate, epigean *Boletaceae* species in certain regions of the lowland Neotropics (e.g. Singer et al. 1983, Henkel et al. 2012, 2015) there are very few reports of sequestrate *Boletaceae* from the region (Mueller et al. 2007, Tedersoo & Smith 2013). Here we rectify this situation by describing three new monotypic genera of sequestrate *Boletaceae* from the Pakaraima Mountains of Guyana. These fungi were collected from closed-canopy, wet rainforests dominated by ECM trees, an infrequent habitat type in the lowland Neotropics (Henkel 2003, Degagne et al. 2009, Smith et al. 2013). Molecular data from the ITS and 28S rDNA, *RPB*1, and *RPB*2 loci, along with morphological features, indicate that these Guyanese sequestrate fungi are members of *Boletaceae* but are evolutionarily distinct from all other described genera and species within the family.

**MATERIALS AND METHODS**

**Collections**

Collections were made during the May–July rainy seasons of 2009, 2012, and 2015 from forests of the Upper Potaro River Basin, within a 15 km radius of a permanent base camp at 5°18’04.8” N 59°54’40.4” W, 710 m a.s.l. The collection sites were dominated by ECM *Dicymbe corymbosa* or co-dominated by ECM *D. corymbosa*, *D. alstonii*, and *Aldina insignis* (Smith et al. 2011, Henkel et al. 2012). Additional Guyana collections were made during Dec.–Jan. of 2010–2011 and June of 2012 from the Upper Mazaruni River Basin within a six km radius of a base camp at 5°26’21.3” N and 60°04’43.1” W, at 800 m a.s.l. Forests at this site were co-dominated by ECM *Pakaraimaea dipterocarpacea* and *D. jenmani* (Smith et al. 2013).

Descriptions of macromorphological features were made from fresh material in the field. Colours were described subjectively and coded according to Kornerup & Wanscher (1978), with colour plates noted in parentheses. Fresh collections were dried using silica gel. Preserved specimens were later examined and imaged using an Olympus BX51 microscope with light and phase contrast optics. Rehydrated fungal tissues were mounted in H2O, 3 % potassium hydroxide (KOH), and Melzer’s solution. For basidiospores, basidia, hyphal features, and other structures in at least 20 individual structures were measured for each specimen examined. Length/width Q values for basidiospores are reported as Qr (range of Q values over “n” basidiospores measured) and Qm (mean of Q values ± SD). Scanning electron micrographs (SEM) of basidiospores were obtained with a FEI Quanta 250 scanning electron microscope using 20 kV. Type and additional specimens were deposited in the following herbaria: BRG, University of Guyana; HSU, Humboldt State University; PUL, Kriebel Herbarium, Purdue University; and NY, New York Botanical Garden.

**DNA extraction, PCR amplification, and sequencing**

DNA extractions were performed on basidiomata tissue from types and additional specimens using the modified CTAB method (Gardes & Bruns 1993) or a Plant DNAeasy mini kit (QIAAGEN, Valencia, CA). PCR and DNA sequencing of the nuc rDNA region encompassing the ITS 1 and 2, along with the 5.8S rDNA (ITS), nuc 28S rDNA D1–D2 domains (28S), the gene for RNA polymerase II largest subunit (*RPB*1) and second largest subunit (*RPB*2) followed the protocols and used the primers of Dentinger et al. (2010), Smith et al. (2011), and Wu et al. (2014). Newly generated sequences were edited in Sequencher v. 5.1 (Gene Codes, Ann Arbor, MI) and deposited in GenBank (Table 1).

**Taxa used, sequence alignment, and phylogenetic analysis**

ITS ribosomal DNA sequences from each new species were initially subjected to BLASTn queries against GenBank in order to explore their putative phylogenetic relationships. In order to further assess their phylogenetic affinities, we used Maximum Likelihood (ML) of a concatenated dataset based on 28S, *RPB*1, and *RPB*2 sequences of diverse *Boletaceae* with additional *Boletales* taxa as outgroups. The analysis included original sequence data and additional sequences of 185 taxa from GenBank for representative species from infrafamilial clades across the family *Boletaceae* based on recent phylogenetic studies (e.g. Nuhn et al. 2013, Wu et al. 2014, 2015, Henkel et al. 2015). The type species and/or key representative taxa were included for as many epigean, non-sequestrate *Boletaceae* genera or undescribed genus-level clades as possible (sensu Wu et al. 2014), contingent on their 28S, *RPB*1, and *RPB*2 sequences being available in GenBank. Representatives from numerous sequestrate *Boletaceae* taxa were also included, even if sequences were available only for one gene region. Sequences of 28S, *RPB*1, and *RPB*2 were compiled in separate nucleotide alignments of 1131 base pairs (28S), 978 base pairs (*RPB*1), and 903 base pairs (*RPB*2) using MEGA5 software (Tamura et al. 2011) and aligned with the aid of MAFFT v. 7 (Katoh & Standley 2013). The Gblocks software package (Talavera & Castresana 2007) was used to exclude ambiguous portions of the alignment, producing a final aligned dataset of 729 base pairs for 28S, 709 base pairs for *RPB*1, and 693 base pairs for *RPB*2. *Boletinellus meruloides*, *Gyrodon lividus*, *Gyroporus castaneus*, *Phlebopus portentosus*, *Paragyrodon sphaerosporus*, *Suillus aff. granulatus*, and S. aff.
Table 1. Taxa and GenBank accession numbers for sequences used in the phylogenetic analysis. If a taxon appeared in a collapsed clade in Fig.1, the collapsed clade is indicated on the right. Guyanese taxa described here are in bold. Unavailable sequences for individual taxa are indicated by —.

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Voucher ID</th>
<th>Location</th>
<th>GenBank accession number</th>
<th>Collapsed clade in Fig. 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Afroboletus luteolus</td>
<td>00-436</td>
<td>Africa</td>
<td>KF030238</td>
<td></td>
</tr>
<tr>
<td>Auroboletus gentilis</td>
<td>MG372a</td>
<td>Brancciano, Lazio, Italy</td>
<td>KF112344, KF11257, KF112741</td>
<td>Xerocomoideae</td>
</tr>
<tr>
<td>Auroboletus moravicus</td>
<td>MG374a</td>
<td>Brancciano, Lazio, Italy</td>
<td>KF112421, KF11259, KF112745</td>
<td>Xerocomoideae</td>
</tr>
<tr>
<td>Auroboletus roxanae</td>
<td>DS 626-07</td>
<td>Chestnut Ridge, NY, USA</td>
<td>KF030311, KF030381, —</td>
<td>Xerocomoideae</td>
</tr>
<tr>
<td>Auroboletus thibetanus</td>
<td>HKAS 76655</td>
<td>Dêqên, Yunnan, China</td>
<td>KF112420, KF112626, KF112752</td>
<td>Xerocomoideae</td>
</tr>
<tr>
<td>Austroboletus fuscoporus</td>
<td>HKAS 75207</td>
<td>China</td>
<td>JX889720, JX889721, —</td>
<td>Austroboletus s.s. clade</td>
</tr>
<tr>
<td>Austroboletus gracilis</td>
<td>112/96</td>
<td>MA, USA</td>
<td>DQ534624, KF030358, —</td>
<td>Austroboletus s.s. clade</td>
</tr>
<tr>
<td>Austroboletus aff. mutabilis</td>
<td>HKAS 53450</td>
<td>Chenzhou, Hunan, China</td>
<td>KF112487, KF112573, KF112768</td>
<td>Austroboletus s.s. clade</td>
</tr>
<tr>
<td>Austroboletus sp.</td>
<td>HKAS 57756</td>
<td>Fuzhou, Jiangxi, China</td>
<td>KF112383, KF112569, KF112764</td>
<td>Austroboletus s.s. clade</td>
</tr>
<tr>
<td>Baorangia bicolor</td>
<td>HKAS 59624</td>
<td>Yunnan, China</td>
<td>KF112485, KF112570, KF112765</td>
<td>Austroboletus s.s. clade</td>
</tr>
<tr>
<td>Baorangia pseudocalopus</td>
<td>HKAS 75739</td>
<td>Shenlongjia, Yunnan, China</td>
<td>KJ184558, KJ184564, KM605179</td>
<td>—</td>
</tr>
<tr>
<td>Binderobolus segoi</td>
<td>Henkel 8035</td>
<td>Region 8 Potaro-Siparuni, Guyana</td>
<td>LC043078, LC043079, —</td>
<td>—</td>
</tr>
<tr>
<td>Boletellus ananas</td>
<td>NY 815459</td>
<td>Puntarenas, Costa Rica</td>
<td>JQ924336, —, KF112760</td>
<td>Xerocomoideae</td>
</tr>
<tr>
<td>Boletellus ananas</td>
<td>TH 8819</td>
<td>Region 8 Potaro-Siparuni, Guyana</td>
<td>HQ161853, HQ161822, —</td>
<td>Xerocomoideae</td>
</tr>
<tr>
<td>Boletellus chrysenteroides</td>
<td>3838</td>
<td>North Collins, NY, USA</td>
<td>KF030312, KF030383, —</td>
<td>Xerocomoideae</td>
</tr>
<tr>
<td>Boletellus dicymbophilus</td>
<td>TH 8840</td>
<td>Region 8 Potaro-Siparuni, Guyana</td>
<td>HQ161852, HQ161821, —</td>
<td>Xerocomoideae</td>
</tr>
<tr>
<td>Boletellus aff. emodensis</td>
<td>HKAS 52678</td>
<td>Sanming, Fujian, China</td>
<td>KF112426, KF112621, KF112757</td>
<td>Xerocomoideae</td>
</tr>
<tr>
<td>Boletellus exigus</td>
<td>TH 8809</td>
<td>Region 8 Potaro-Siparuni, Guyana</td>
<td>HQ161862, HQ161831, —</td>
<td>Xerocomoideae</td>
</tr>
<tr>
<td>Boletellus longicollis</td>
<td>HKAS 53398</td>
<td>Chenzhou, Hunan, China</td>
<td>KF112376, KF112625, KF112755</td>
<td>Xerocomoideae</td>
</tr>
<tr>
<td>Boletellus mirabilis</td>
<td>HKAS 57776</td>
<td>Lijiang, Yunnan, China</td>
<td>KF112360, KF112624, KF112743</td>
<td>Xerocomoideae</td>
</tr>
<tr>
<td>Boletellus piakaii</td>
<td>TH 8077</td>
<td>Region 8 Potaro-Siparuni, Guyana</td>
<td>HQ161861, HQ161830, —</td>
<td>Xerocomoideae</td>
</tr>
<tr>
<td>Boletellus shichianus</td>
<td>HKAS 76852</td>
<td>Fuzhou, Jiangxi, China</td>
<td>KF112419, KF112562, KF112756</td>
<td>Xerocomoideae</td>
</tr>
<tr>
<td>Boletellus aff. shichianus</td>
<td>HKAS 56317</td>
<td>Dêqên, Yunnan, China</td>
<td>KF112363, —, KF112753</td>
<td>Xerocomoideae</td>
</tr>
<tr>
<td>Boletellus sp.</td>
<td>HKAS 53375</td>
<td>Sanming, Fujian, China</td>
<td>KF112364, KF112567, KF112748</td>
<td>Xerocomoideae</td>
</tr>
<tr>
<td>Boletellus sp.</td>
<td>HKAS 53376</td>
<td>Sanming, Fujian, China</td>
<td>KF112365, KF112566, KF112744</td>
<td>Xerocomoideae</td>
</tr>
<tr>
<td>Boletellus sp.</td>
<td>HKAS 58713</td>
<td>Dali, Yunnan, China</td>
<td>KF112428, KF112623, KF112759</td>
<td>Xerocomoideae</td>
</tr>
<tr>
<td>Boletellus sp.</td>
<td>HKAS 59536</td>
<td>Baoshan, Yunnan, China</td>
<td>KF112427, KF112622, KF112758</td>
<td>Xerocomoideae</td>
</tr>
<tr>
<td>Boletellus sp.</td>
<td>HKAS 74783</td>
<td>Nuijiang, Yunnan, China</td>
<td>KF112468, KF112612, KF112771</td>
<td>Xerocomoideae</td>
</tr>
<tr>
<td>Boletellus sp.</td>
<td>HKAS 74888</td>
<td>Baoshan, Yunnan, China</td>
<td>KF112413, KF112568, KF112747</td>
<td>Xerocomoideae</td>
</tr>
<tr>
<td>Boletinellus meruloides</td>
<td>AFTOL-ID 575</td>
<td>MA, USA</td>
<td>AY684153, DQ435803, —</td>
<td>Boletales outgroup taxa</td>
</tr>
<tr>
<td>Boletus abruptibulbus</td>
<td>4588</td>
<td>Cape San Blas, FL, USA</td>
<td>KF030302, KF030388, —</td>
<td>Xerocomoideae</td>
</tr>
<tr>
<td>Taxon</td>
<td>Voucher ID</td>
<td>Location</td>
<td>GenBank accession number</td>
<td>Collapsed clade in Fig. 1</td>
</tr>
<tr>
<td>-----------------------</td>
<td>-----------------------------</td>
<td>---------------------------</td>
<td>--------------------------</td>
<td>----------------------------</td>
</tr>
<tr>
<td>Boletus aereus</td>
<td>BM 06-559</td>
<td>Yunnan, China</td>
<td>KF102156</td>
<td>Boletus clade</td>
</tr>
<tr>
<td>Boletus aoki</td>
<td>BM 06-548</td>
<td>Yunnan, China</td>
<td>KF102103</td>
<td>Boletus clade</td>
</tr>
<tr>
<td>Boletus edulis</td>
<td>ATOL-713</td>
<td>MA, USA</td>
<td>KF102036, KF102037</td>
<td>Boletus clade</td>
</tr>
<tr>
<td>Boletus pallidus</td>
<td>DS-45-4</td>
<td>USA</td>
<td>KF102102, KF102103</td>
<td>Boletus clade</td>
</tr>
<tr>
<td>Boletus pulchriprous</td>
<td>HKAS 52289</td>
<td>Sonoma Co., CA, USA</td>
<td>KF102028, KF102029, KF102030</td>
<td>Boletus clade</td>
</tr>
<tr>
<td>Boletus molybdii</td>
<td>MBC-043</td>
<td>Yunnan, China</td>
<td>KF102031</td>
<td>Boletus clade</td>
</tr>
<tr>
<td>Boletus riparius</td>
<td>CUA-040</td>
<td>Yunnan, China</td>
<td>KF102032</td>
<td>Boletus clade</td>
</tr>
<tr>
<td>Boletus variipes</td>
<td>CUA-039</td>
<td>Yunnan, China</td>
<td>KF102033</td>
<td>Boletus clade</td>
</tr>
<tr>
<td>Boletus spp.</td>
<td>MB 06-559</td>
<td>Yunnan, China</td>
<td>KF102156</td>
<td>Boletus clade</td>
</tr>
<tr>
<td>Boletus sp.</td>
<td>ATOL-713</td>
<td>MA, USA</td>
<td>KF102036, KF102037</td>
<td>Boletus clade</td>
</tr>
<tr>
<td>Boletus sp.</td>
<td>DS-45-4</td>
<td>USA</td>
<td>KF102102, KF102103</td>
<td>Boletus clade</td>
</tr>
<tr>
<td>Boletus spp.</td>
<td>HKAS 52289</td>
<td>Sonoma Co., CA, USA</td>
<td>KF102028, KF102029, KF102030</td>
<td>Boletus clade</td>
</tr>
<tr>
<td>Boletus spp.</td>
<td>MBC-043</td>
<td>Yunnan, China</td>
<td>KF102031</td>
<td>Boletus clade</td>
</tr>
<tr>
<td>Boletus spp.</td>
<td>CUA-040</td>
<td>Yunnan, China</td>
<td>KF102032</td>
<td>Boletus clade</td>
</tr>
<tr>
<td>Boletus spp.</td>
<td>CUA-039</td>
<td>Yunnan, China</td>
<td>KF102033</td>
<td>Boletus clade</td>
</tr>
<tr>
<td>Boletus spp.</td>
<td>MB 06-559</td>
<td>Yunnan, China</td>
<td>KF102156</td>
<td>Boletus clade</td>
</tr>
<tr>
<td>Boletus sp.</td>
<td>ATOL-713</td>
<td>MA, USA</td>
<td>KF102036, KF102037</td>
<td>Boletus clade</td>
</tr>
<tr>
<td>Boletus sp.</td>
<td>DS-45-4</td>
<td>USA</td>
<td>KF102102, KF102103</td>
<td>Boletus clade</td>
</tr>
<tr>
<td>Boletus spp.</td>
<td>HKAS 52289</td>
<td>Sonoma Co., CA, USA</td>
<td>KF102028, KF102029, KF102030</td>
<td>Boletus clade</td>
</tr>
<tr>
<td>Boletus spp.</td>
<td>MBC-043</td>
<td>Yunnan, China</td>
<td>KF102031</td>
<td>Boletus clade</td>
</tr>
<tr>
<td>Boletus spp.</td>
<td>CUA-040</td>
<td>Yunnan, China</td>
<td>KF102032</td>
<td>Boletus clade</td>
</tr>
<tr>
<td>Boletus spp.</td>
<td>CUA-039</td>
<td>Yunnan, China</td>
<td>KF102033</td>
<td>Boletus clade</td>
</tr>
<tr>
<td>Boletus spp.</td>
<td>MB 06-559</td>
<td>Yunnan, China</td>
<td>KF102156</td>
<td>Boletus clade</td>
</tr>
<tr>
<td>Boletus sp.</td>
<td>ATOL-713</td>
<td>MA, USA</td>
<td>KF102036, KF102037</td>
<td>Boletus clade</td>
</tr>
<tr>
<td>Boletus sp.</td>
<td>DS-45-4</td>
<td>USA</td>
<td>KF102102, KF102103</td>
<td>Boletus clade</td>
</tr>
<tr>
<td>Boletus spp.</td>
<td>HKAS 52289</td>
<td>Sonoma Co., CA, USA</td>
<td>KF102028, KF102029, KF102030</td>
<td>Boletus clade</td>
</tr>
<tr>
<td>Boletus spp.</td>
<td>MBC-043</td>
<td>Yunnan, China</td>
<td>KF102031</td>
<td>Boletus clade</td>
</tr>
<tr>
<td>Boletus spp.</td>
<td>CUA-040</td>
<td>Yunnan, China</td>
<td>KF102032</td>
<td>Boletus clade</td>
</tr>
<tr>
<td>Boletus spp.</td>
<td>CUA-039</td>
<td>Yunnan, China</td>
<td>KF102033</td>
<td>Boletus clade</td>
</tr>
<tr>
<td>Boletus spp.</td>
<td>MB 06-559</td>
<td>Yunnan, China</td>
<td>KF102156</td>
<td>Boletus clade</td>
</tr>
<tr>
<td>Boletus sp.</td>
<td>ATOL-713</td>
<td>MA, USA</td>
<td>KF102036, KF102037</td>
<td>Boletus clade</td>
</tr>
<tr>
<td>Boletus sp.</td>
<td>DS-45-4</td>
<td>USA</td>
<td>KF102102, KF102103</td>
<td>Boletus clade</td>
</tr>
<tr>
<td>Boletus spp.</td>
<td>HKAS 52289</td>
<td>Sonoma Co., CA, USA</td>
<td>KF102028, KF102029, KF102030</td>
<td>Boletus clade</td>
</tr>
<tr>
<td>Boletus spp.</td>
<td>MBC-043</td>
<td>Yunnan, China</td>
<td>KF102031</td>
<td>Boletus clade</td>
</tr>
<tr>
<td>Boletus spp.</td>
<td>CUA-040</td>
<td>Yunnan, China</td>
<td>KF102032</td>
<td>Boletus clade</td>
</tr>
<tr>
<td>Boletus spp.</td>
<td>CUA-039</td>
<td>Yunnan, China</td>
<td>KF102033</td>
<td>Boletus clade</td>
</tr>
<tr>
<td>Boletus spp.</td>
<td>MB 06-559</td>
<td>Yunnan, China</td>
<td>KF102156</td>
<td>Boletus clade</td>
</tr>
<tr>
<td>Boletus sp.</td>
<td>ATOL-713</td>
<td>MA, USA</td>
<td>KF102036, KF102037</td>
<td>Boletus clade</td>
</tr>
<tr>
<td>Boletus sp.</td>
<td>DS-45-4</td>
<td>USA</td>
<td>KF102102, KF102103</td>
<td>Boletus clade</td>
</tr>
</tbody>
</table>
| Boletus spp.          | HKAS 52289                  | Sonoma Co., CA, USA       | KF102028, KF102029, KF102030 | Boletus clade te...
### Table 1. (Continued).

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Voucher ID</th>
<th>Location</th>
<th>GenBank accession number</th>
<th>Collapsed clade in Fig. 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caloboletus sp.</td>
<td>HKAS 53353</td>
<td>Sanming, Fujian, China</td>
<td>KF112410</td>
<td>Caloboletus clade</td>
</tr>
<tr>
<td>Castellanae pakaraimophila</td>
<td>Henkel 9514</td>
<td>Region 7 Cuyuni-Mazanuni, Guyana</td>
<td>KC155381</td>
<td></td>
</tr>
<tr>
<td>Chalciporus piperaeus</td>
<td>MB 04-001</td>
<td>MA, USA</td>
<td>DQ534648</td>
<td></td>
</tr>
<tr>
<td>Comerobolus indecorus</td>
<td>HKAS 63126</td>
<td>Qiongzhou, Hainan, China</td>
<td>KF112440</td>
<td></td>
</tr>
<tr>
<td>Costassaleae caeruleans</td>
<td>Henkel 9061</td>
<td>Region 8 Potaro-Siparuni, Guyana</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Costassaleae caeruleans</td>
<td>Henkel 9067</td>
<td>Region 8 Potaro-Siparuni, Guyana</td>
<td>LC053662</td>
<td></td>
</tr>
<tr>
<td>Crocinobolus rufaureus</td>
<td>HKAS 53424</td>
<td>Chenzhou, Hunan, China</td>
<td>KF112435</td>
<td></td>
</tr>
<tr>
<td>Cyanobolus pulvulentus</td>
<td>9606</td>
<td>West Newton, MA, USA</td>
<td>KF030313</td>
<td></td>
</tr>
<tr>
<td>Cyanobolus sp.</td>
<td>HKAS 52601</td>
<td>Yunnan, China</td>
<td>KF112469</td>
<td></td>
</tr>
<tr>
<td>Durianellula rambutaniispera</td>
<td>REH 8692</td>
<td>Malaysia</td>
<td>EU293063</td>
<td></td>
</tr>
<tr>
<td>Exsudoporus frostii</td>
<td>BDCR 0418</td>
<td>San Gerardo, San José, Costa Rica</td>
<td>HQ161855</td>
<td></td>
</tr>
<tr>
<td>Exsudoporus frostii</td>
<td>NY 815462</td>
<td>San Gerardo, San José, Costa Rica</td>
<td>JQ024342</td>
<td></td>
</tr>
<tr>
<td>Frostiella russelli</td>
<td>BD391</td>
<td>Anoka, MN, USA</td>
<td>HQ161874</td>
<td></td>
</tr>
<tr>
<td>Guyanapora albipodus</td>
<td>Henkel 8848</td>
<td>Region 8 Potaro-Siparuni, Guyana</td>
<td>LC043081</td>
<td></td>
</tr>
<tr>
<td>Gymnogastria boletoides</td>
<td>REH 9455</td>
<td>SE Queensland, Australia</td>
<td>JX89673</td>
<td></td>
</tr>
<tr>
<td>Gyrodon lividus</td>
<td>REG G11</td>
<td>Bavaria, Germany</td>
<td>AF098378</td>
<td></td>
</tr>
<tr>
<td>Gyroponus castaenus</td>
<td>HKAS 76672</td>
<td>Harbin, Heilongjiang, China</td>
<td>KF112478</td>
<td>Boletales outgroup taxa</td>
</tr>
<tr>
<td>Hanya chromapes</td>
<td>HKAS 50527</td>
<td>Dêqên, Yunnan, China</td>
<td>KF112437</td>
<td>Boletales outgroup taxa</td>
</tr>
<tr>
<td>Heimioporus japonicus</td>
<td>HKAS 52237</td>
<td>Chuxiong, Yunnan, China</td>
<td>KF112347</td>
<td>Xerocomoideae</td>
</tr>
<tr>
<td>Hellogaster columellifer</td>
<td>TNS-F-11696</td>
<td>Kyoto, Japan</td>
<td>EF183541</td>
<td></td>
</tr>
<tr>
<td>Imelaria badia</td>
<td>HKAS 74714</td>
<td>Marburg, Germany</td>
<td>KF112375</td>
<td>Imelaria clade</td>
</tr>
<tr>
<td>Imelaria sp.</td>
<td>HKAS 52557</td>
<td>Ninger, Yunnan, China</td>
<td>KF112374</td>
<td>Imelaria clade</td>
</tr>
<tr>
<td>Jimtrappea guyanensis</td>
<td>Henkel 9163</td>
<td>Region 8 Potaro-Siparuni, Guyana</td>
<td>LC053660</td>
<td></td>
</tr>
<tr>
<td>Lanmaoa angustisspera</td>
<td>HKAS 74752</td>
<td>Gongshan, Yunnan, China</td>
<td>KM605139</td>
<td>Lanmaoa clade</td>
</tr>
<tr>
<td>Lanmaoa asiatica</td>
<td>HKAS 54094</td>
<td>Kunming, Yunnan, China</td>
<td>KF112353</td>
<td>Lanmaoa clade</td>
</tr>
<tr>
<td>Lanmaoa asiatica</td>
<td>HKAS 63603</td>
<td>Nanhua, Yunnan, China</td>
<td>KM605143</td>
<td>Lanmaoa clade</td>
</tr>
<tr>
<td>Lanmaoa carminipes</td>
<td>MB 06-061</td>
<td>Erie Co., NY, USA</td>
<td>JQ327001</td>
<td>Lanmaoa clade</td>
</tr>
<tr>
<td>Lanmaoa flavonuba</td>
<td>HKAS 74765</td>
<td>Nujiang, Yunnan, China</td>
<td>KF112322</td>
<td>Lanmaoa clade</td>
</tr>
<tr>
<td>Leccinellum coristum</td>
<td>Buf 4507</td>
<td>unknown</td>
<td>KF030347</td>
<td></td>
</tr>
<tr>
<td>Leccinum aurantiacum</td>
<td>HKAS 63502</td>
<td>Kunming, Yunnan, China</td>
<td>KF112444</td>
<td>Leccinum s.s. clade</td>
</tr>
<tr>
<td>Leccinum monticola</td>
<td>HKAS 76669</td>
<td>Yanbian, Jilin, China</td>
<td>KF112443</td>
<td>Leccinum s.s. clade</td>
</tr>
<tr>
<td>Leccinum aff. scabrum</td>
<td>HKAS 57266</td>
<td>Qamdo, Tibet, China</td>
<td>KF112442</td>
<td>Leccinum s.s. clade</td>
</tr>
</tbody>
</table>
Table 1. (Continued).

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Voucher ID</th>
<th>Location</th>
<th>GenBank accession number</th>
<th>Collapsed clade in Fig. 1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>28S</td>
<td>RBP1</td>
</tr>
<tr>
<td>Leccinum subglabripes</td>
<td>72206</td>
<td>Jefferson, NH, USA</td>
<td>KF030303</td>
<td>KF030374</td>
</tr>
<tr>
<td>Leccinum varicolor</td>
<td>HKAS 57758</td>
<td>Lijiang, Yunnan, China</td>
<td>KF112445</td>
<td>KF112591</td>
</tr>
<tr>
<td>Macintoshia persica</td>
<td>Trappe 28216</td>
<td>Zimbabwe</td>
<td>KC905034</td>
<td>—</td>
</tr>
<tr>
<td>Mucilgopus castaneiceps</td>
<td>HKAS 75045</td>
<td>Nujiang, Yunnan, China</td>
<td>KF112382</td>
<td>—</td>
</tr>
<tr>
<td>Mycena amethystea congelensis</td>
<td>v99-105</td>
<td>Mashonaland, Zimbabwe</td>
<td>LC053665</td>
<td>—</td>
</tr>
<tr>
<td>Neoboletus brunnneissimus</td>
<td>HKAS 52660</td>
<td>Kunming, Yunnan, China</td>
<td>KF112314</td>
<td>KF112432</td>
</tr>
<tr>
<td>Neoboletus magnificus</td>
<td>HKAS 57475</td>
<td>Jianchuan, Yunnan, China</td>
<td>KM605137</td>
<td>KM605161</td>
</tr>
<tr>
<td>Neoboletus aff. luridiformis</td>
<td>HKAS 55440</td>
<td>Deqên, Yunnan, China</td>
<td>KF112315</td>
<td>KF112499</td>
</tr>
<tr>
<td>Neoboletus s.s. clade</td>
<td>HKAS 53369</td>
<td>Sanming, Fujian, China</td>
<td>KF112323</td>
<td>KF112509</td>
</tr>
<tr>
<td>Neoboletus thibetanus</td>
<td>HKAS 76851</td>
<td>Changjiang, Hainan, China</td>
<td>KF112321</td>
<td>KF112433</td>
</tr>
<tr>
<td>Octavia japonimontana</td>
<td>KPM-NC-0017812</td>
<td>Okayama Prefecture, Japan</td>
<td>JN378486</td>
<td>—</td>
</tr>
<tr>
<td>Paragyrodon sphaerosorus</td>
<td>MB 06-066</td>
<td>Iowa, USA</td>
<td>GU187593</td>
<td>—</td>
</tr>
<tr>
<td>Phellinus erubescens</td>
<td>HKAS 77032</td>
<td>Longnan, Jiangxi, China</td>
<td>KP658467</td>
<td>KP658471</td>
</tr>
<tr>
<td>Phlebopus portentosus</td>
<td>php1</td>
<td>Africa</td>
<td>AF336260</td>
<td>FJ536606</td>
</tr>
<tr>
<td>Phlebopus aff. portentosus</td>
<td>HKAS 52855</td>
<td>Yunnan, China</td>
<td>JQ928622</td>
<td>KF112647</td>
</tr>
<tr>
<td>Phylloporus imbricatus</td>
<td>HKAS 68642</td>
<td>Nuijiang, Yunnan, China</td>
<td>KF112398</td>
<td>KF112637</td>
</tr>
<tr>
<td>Phylloporus luxiensis</td>
<td>HKAS 75077</td>
<td>Chuxiong, Yunnan, China</td>
<td>KF112490</td>
<td>KF112636</td>
</tr>
<tr>
<td>Phylloporus pelletieri</td>
<td>Pp1</td>
<td>Bavaria, Germany</td>
<td>AF456818</td>
<td>KF030390</td>
</tr>
<tr>
<td>Phylloporus rubrobranarius</td>
<td>HKAS 52552</td>
<td>Ninger, Yunnan, China</td>
<td>KF112391</td>
<td>—</td>
</tr>
<tr>
<td>Porphyrellus holophaeus</td>
<td>HKAS 74894</td>
<td>Baoshan, Yunnan, China</td>
<td>KF112474</td>
<td>KF112554</td>
</tr>
<tr>
<td>Pseudoboletus parasiticus</td>
<td>xpa1</td>
<td>Bavaria, Germany</td>
<td>AF050646</td>
<td>KF030394</td>
</tr>
<tr>
<td>Pulveroboletus aff. ravenii</td>
<td>HKAS 53351</td>
<td>Sanming, Fujian, China</td>
<td>KF112406</td>
<td>KF112542</td>
</tr>
<tr>
<td>Pulveroboletus sp.</td>
<td>HKAS 57665</td>
<td>Deqên, Yunnan, China</td>
<td>KF112409</td>
<td>KF112544</td>
</tr>
<tr>
<td>Pulveroboletus sp.</td>
<td>HKAS 58860</td>
<td>Dali, Yunnan, China</td>
<td>KF112408</td>
<td>KF112543</td>
</tr>
<tr>
<td>Pulveroboletus sp.</td>
<td>HKAS 74933</td>
<td>Baoshan, Yunnan, China</td>
<td>KF112407</td>
<td>KF112545</td>
</tr>
<tr>
<td>Retiboletus griseus</td>
<td>HKAS 63590</td>
<td>Dali, Yunnan, China</td>
<td>KF112417</td>
<td>KF112537</td>
</tr>
<tr>
<td>Retiboletus nigerimus</td>
<td>HKAS 59699</td>
<td>Chuxiong, Yunnan, China</td>
<td>JQ928627</td>
<td>JQ928592</td>
</tr>
<tr>
<td>Retiboletus aff. ornatipes</td>
<td>HKAS 63548</td>
<td>Lijiang, Yunnan, China</td>
<td>KF112416</td>
<td>KF112536</td>
</tr>
<tr>
<td>Rossbeevera vittatissima</td>
<td>OSC 61484</td>
<td>New South Wales, Australia</td>
<td>JN378506</td>
<td>—</td>
</tr>
<tr>
<td>Rossbeevera yunnanensis</td>
<td>HKAS 70601</td>
<td>Gejiu, Yunnan, China</td>
<td>KC552051</td>
<td>—</td>
</tr>
</tbody>
</table>
### Table 1. (Continued).

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Voucher ID</th>
<th>Location</th>
<th>GenBank accession number</th>
<th>Collapsed clade in Fig. 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Royoungia boletoides</td>
<td>AWC 4137</td>
<td>Victoria, Australia</td>
<td>DQ534663</td>
<td>—</td>
</tr>
<tr>
<td>Tylopilus aff. balloui</td>
<td>HKAS 59700</td>
<td>Chuxiong, Yunnan, China</td>
<td>KF112458</td>
<td>KF112619</td>
</tr>
<tr>
<td>Rubroboletus dupainii</td>
<td>JAM 0607</td>
<td>Butner, NY, USA</td>
<td>KF030251</td>
<td>KF030361</td>
</tr>
<tr>
<td>Rubroboletus latisporus</td>
<td>HKAS 80358</td>
<td>Chongqing, China</td>
<td>KP055023</td>
<td>KP055026</td>
</tr>
<tr>
<td>Rubroboletus sinicus</td>
<td>HKAS 56304</td>
<td>Deqin, Yunnan, China</td>
<td>KJ605873</td>
<td>KJ619482</td>
</tr>
<tr>
<td>Rubroboletus sinicus</td>
<td>HKAS 68620</td>
<td>Nuijiang, Yunnan, China</td>
<td>KF112319</td>
<td>KF112504</td>
</tr>
<tr>
<td>Rugiboletus brunneporus</td>
<td>HKAS 83209</td>
<td>Linzhi, Xizang, China</td>
<td>KM605134</td>
<td>KM605158</td>
</tr>
<tr>
<td>Rugiboletus extremiorientalis</td>
<td>HKAS 63635</td>
<td>Chuxiong, Yunnan, China</td>
<td>KF112403</td>
<td>KF112535</td>
</tr>
<tr>
<td>Rugiboletus extremiorientalis</td>
<td>HKAS 76663</td>
<td>Neixiang, Henan, China</td>
<td>KM605135</td>
<td>KM605159</td>
</tr>
<tr>
<td>Rugiboletus aff. extremiorientalis</td>
<td>HKAS 68586</td>
<td>Dali, Yunnan, China</td>
<td>KF112402</td>
<td>KF112534</td>
</tr>
<tr>
<td>Singeroxomus inundabilis</td>
<td>Henkel 9199</td>
<td>Region 8 Potaro-Siparuni, Guyana</td>
<td>LC043087</td>
<td>LC043088</td>
</tr>
<tr>
<td>Singeroxomus rubriflavus</td>
<td>Henkel 9585</td>
<td>Region 8 Potaro-Siparuni, Guyana</td>
<td>LC043093</td>
<td>LC043094</td>
</tr>
<tr>
<td>Sinooboletus duplicatoporus</td>
<td>HKAS 50498</td>
<td>Ninger, Yunnan, China</td>
<td>KF112361</td>
<td>KF112561</td>
</tr>
<tr>
<td>Solioicoccus polychromus</td>
<td>REH 9417</td>
<td>Fraser Island, Australia</td>
<td>JQ287643</td>
<td>—</td>
</tr>
<tr>
<td>Spongiforma thailandica</td>
<td>DED 7873</td>
<td>Khao Yai Nat. Park, Thailand</td>
<td>EU685108</td>
<td>KF030387</td>
</tr>
<tr>
<td>Strobilomyces aff. seminudus</td>
<td>HKAS 59461</td>
<td>Baoshan, Yunnan, China</td>
<td>KF112479</td>
<td>KF112606</td>
</tr>
<tr>
<td>Strobilomyces strobilaceus</td>
<td>AFTOL-716</td>
<td>MA, USA</td>
<td>AY684155</td>
<td>—</td>
</tr>
<tr>
<td>Strobilomyces aff. venumculosus</td>
<td>HKAS 55389</td>
<td>Ninger, Yunnan, China</td>
<td>KF112461</td>
<td>KF112604</td>
</tr>
<tr>
<td>Suillellus amygdalinus</td>
<td>112605ba</td>
<td>Mendocino Co., CA, USA</td>
<td>JQ269966</td>
<td>KF030360</td>
</tr>
<tr>
<td>Suillellus aff. amygdalinus</td>
<td>HKAS 57262</td>
<td>Qamdo, Tibet, China</td>
<td>KF112316</td>
<td>KF112501</td>
</tr>
<tr>
<td>Suillus aff. granulatus</td>
<td>HKAS 57622</td>
<td>Chuxiong, Yunnan, China</td>
<td>KF112429</td>
<td>KF112645</td>
</tr>
<tr>
<td>Suillus aff. luteus</td>
<td>HKAS 57748</td>
<td>Lijiang, Yunnan, China</td>
<td>KF112430</td>
<td>KF112646</td>
</tr>
<tr>
<td>Sutorius australiensis</td>
<td>REH 9280</td>
<td>Fraser Island, Qld, Australia</td>
<td>JQ327005</td>
<td>—</td>
</tr>
<tr>
<td>Sutorius eximius</td>
<td>REH 9400</td>
<td>Ulster County, NY, USA</td>
<td>JQ327004</td>
<td>—</td>
</tr>
<tr>
<td>Sutorius aff. eximius</td>
<td>HK S56291</td>
<td>Chuxiong, Yunnan, China</td>
<td>KF112400</td>
<td>KF112585</td>
</tr>
<tr>
<td>Sutorius aff. eximius</td>
<td>HK S52672</td>
<td>Kunming, Yunnan, China</td>
<td>KF112399</td>
<td>KF112584</td>
</tr>
<tr>
<td>Tylopilus felleus</td>
<td>HKAS 54926</td>
<td>Marburg, Germany</td>
<td>KF112411</td>
<td>KF112575</td>
</tr>
<tr>
<td>Tylopilus microsporus</td>
<td>HKAS 59661</td>
<td>Yunnan, China</td>
<td>KF112450</td>
<td>KF112614</td>
</tr>
<tr>
<td>Tylopilus otsuensis</td>
<td>HKAS 53401</td>
<td>Chenzhou, Hunan, China</td>
<td>KF112449</td>
<td>KF112613</td>
</tr>
<tr>
<td>Tylopilus porphyrosporus</td>
<td>HKAS 76671</td>
<td>Yanbian, Jilin, China</td>
<td>KF112482</td>
<td>KF112611</td>
</tr>
<tr>
<td>Tylopilus aff. rigens</td>
<td>HKAS 53388</td>
<td>Sanning, Fujian, China</td>
<td>KF112405</td>
<td>KF112539</td>
</tr>
<tr>
<td>Tylopilus plumbeoviolaceoides</td>
<td>HKAS 50210</td>
<td>Yunnan, China</td>
<td>KF112431</td>
<td>KF112576</td>
</tr>
<tr>
<td>Tylopilus plumbeoviolaceus</td>
<td>MB 06-056</td>
<td>Chestnut Ridge, NY, USA</td>
<td>KF030350</td>
<td>KF030359</td>
</tr>
<tr>
<td>Taxon</td>
<td>Voucher ID</td>
<td>Location</td>
<td>GenBank accession number</td>
<td>Collapsed clade in Fig. 1</td>
</tr>
<tr>
<td>-----------------------------</td>
<td>------------</td>
<td>---------------------------</td>
<td>--------------------------</td>
<td>---------------------------</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>28S</td>
<td>RBP1</td>
</tr>
<tr>
<td>Tylopilus violatichtus</td>
<td>HKAS 50208</td>
<td>Jinghong, Yunnan, China</td>
<td>KF112472</td>
<td>KF112620</td>
</tr>
<tr>
<td>Tylopilus virens</td>
<td>HKAS 76678</td>
<td>Lianshan Yi, Sichuan, China</td>
<td>KF112438</td>
<td>KF112582</td>
</tr>
<tr>
<td>Tylopilus sp.</td>
<td>HKAS 46334</td>
<td>Deqên, Yunnan, China</td>
<td>KF112471</td>
<td>KF112581</td>
</tr>
<tr>
<td>Tylopilus sp.</td>
<td>HKAS 50229</td>
<td>Yunnan, China</td>
<td>KF112423</td>
<td>KF112574</td>
</tr>
<tr>
<td>Tylopilus sp.</td>
<td>HKAS 53367</td>
<td>Sanming, Fujian, China</td>
<td>KF112439</td>
<td>KF112615</td>
</tr>
<tr>
<td>Tylopilus sp.</td>
<td>HKAS 55438</td>
<td>Deqên, Yunnan, China</td>
<td>KF112404</td>
<td>KF112538</td>
</tr>
<tr>
<td>Tylopilus sp.</td>
<td>HKAS 74925</td>
<td>Baoshan, Yunnan, China</td>
<td>KF112473</td>
<td>KF112577</td>
</tr>
<tr>
<td>Tylopilus sp.</td>
<td>HKAS 74928</td>
<td>Baoshan, Yunnan, China</td>
<td>KF112483</td>
<td>KF112583</td>
</tr>
<tr>
<td>Veloporphyrellus alpinus</td>
<td>HKAS 57490</td>
<td>Lijiang, Yunnan, China</td>
<td>KF112380</td>
<td>KF112555</td>
</tr>
<tr>
<td>Xanthoconium affine</td>
<td>BD217</td>
<td>Giles, VA, USA</td>
<td>HQ161854</td>
<td>HQ161823</td>
</tr>
<tr>
<td>Xanthoconium purpureum</td>
<td>BD228</td>
<td>Macon, NC, USA</td>
<td>HQ161864</td>
<td>HQ161833</td>
</tr>
<tr>
<td>Xanthoconium separans</td>
<td>DPL 2704</td>
<td>TX, USA</td>
<td>KF030329</td>
<td>KF030385</td>
</tr>
<tr>
<td>Xanthoconium stramineum</td>
<td>3518</td>
<td>Gainesville, FL, USA</td>
<td>KF030353</td>
<td>KF030386</td>
</tr>
<tr>
<td>Xerocomellus chrysenteron</td>
<td>xch1</td>
<td>Bavaria, Germany</td>
<td>AF050647</td>
<td>KF030365</td>
</tr>
<tr>
<td>Xerocomellus cisanpinus</td>
<td>AT2005034</td>
<td>Upsala, Uppland, Finland</td>
<td>KF030354</td>
<td>KF030367</td>
</tr>
<tr>
<td>Xerocomellus aff. rubellus</td>
<td>HKAS 51239</td>
<td>Nyingchi, Tibet, China</td>
<td>KF112425</td>
<td>KF112550</td>
</tr>
<tr>
<td>Xerocomellus zelleri</td>
<td>REH 8724</td>
<td>Redwood Nat. Park, CA, USA</td>
<td>KF030271</td>
<td>KF030366</td>
</tr>
<tr>
<td>Xerocomus cyanibrunnescens</td>
<td>TH 8821</td>
<td>Region 8 Potaro-Siparuni, Guyana</td>
<td>HQ161866</td>
<td>HQ161835</td>
</tr>
<tr>
<td>Xerocomus aff. macrobbii</td>
<td>HKAS 56280</td>
<td>Chuxiong, Yunnan, China</td>
<td>KF112418</td>
<td>KF112541</td>
</tr>
<tr>
<td>Xerocomus magniporus</td>
<td>HKAS 56000</td>
<td>Qamdo, Tibet, China</td>
<td>KF112392</td>
<td>KF112632</td>
</tr>
<tr>
<td>Zangia erythrocephala</td>
<td>HKAS 75046</td>
<td>Nuijiang, Yunnan, China</td>
<td>KF112414</td>
<td>KF112579</td>
</tr>
</tbody>
</table>

1For the new taxon Costatisporus caeruleicans, original data for 28S was derived from specimen Henkel 9067, and for RBP1 and RBP2 from Henkel 9061, and concatenated prior to analysis. These two specimens are conspecific morphologically and have identical ITS sequences.
New sequestrate Boletaceae from Guyana

RESULTS

BLASTn queries and phylogenetic analysis
ITS BLASTn queries of each of the new taxa on GenBank indicated affinities with Boletaceae at the family level, but were uninformative at the genus level, with none of the searches exceeding 89% similarity with any ITS sequences in GenBank. The ML analysis of the combined 28S, RPB1, and RPB2 dataset produced a phylogram (-ln = 57979.037507) with overall topology similar to that of previously published studies (e.g. Wu et al. 2014) (Fig. 1). The new Guyanese taxa were placed within Boletaceae, but none were nested within previously described genera, including the boletoid sequestrate genera Chamonixia, Durianella, Gastroboletus, Gastroleccinum, Heligaster, Mackintosha, Mycoamaranthus, Octaviania, Rossbeevera, Royoungia, Sollioccusas, or Spongiforma.

TAXONOMY

Jimtrappea T.W. Henkel, M.E. Smith & Aime, gen. nov.
MycoBank MB812359 (Figs 2–3, 4A)

Etymology: The genus is named in honour of Dr. James "Jim" Trappe, the world’s foremost authority on sequestrate fungi.


Type species: **Jimtrappea guyanensis** T.W. Henkel et al. 2015.

**Jimtrappea guyanensis** T.W. Henkel, M.E. Smith & Aime, sp. nov.
MycoBank MB812360 (Figs 2–3, 4A)

Etymology: Guyana and –ensis (Latin adj. B) = adjectival suffix indicating origin or place; referring to the country of known occurrence of the species.

Description: Reminiscent of a Tylopilus species, but lacking a stipe and with a loculate gleba. Peridium pale, delicate. Basidiospores pinkish to reddish brown, smooth, and cystidio strongly dextrinoid in Melzer’s solution.

**Type**: **Guyana**: Region 8 Potaro-Siparuni: Pakaraima Mountains, Upper Potaro River Basin, ~10 km southeast of a base camp at 5°18'04.8" N 59°54'40.4" W, near Tadang camp, 20 cm deep within lateritic soil under *Dicymbe corymbosa*, *D. altsonii*, and *Aldina insignis*, 29 Dec. 2009, Henkel 9163 (BRG 41210 – holotype; HSU G1115, NY 02460742 – isotypes). GenBank accession numbers ITS and 28S: JN168684, LC053660; RPB1: LC053661.

**Description**: Basidiomata subglobose to ovate and irregularly lobed, occasionally appearing fused, (6–)11–21 mm tall, (3–)8–29 mm broad, subfirm to soft and gelatinous with age; surface off-white to pale cream (4A1–4A2, 5A2) throughout, unchanging with pressure or slightly browning, with occasional hemic stains, glabrous macroscopically, under hand lens a tightly appressed hyphal mat; base subtended by delicate white hyphal cords and occasionally concolourous ectomycorrhizas. Peridium in longitudinal section extremely thin (< 0.25 mm), light creamish white, single-layered, delicate, separable. Gleba nearly white (6A1) initially, with age light pink (6A2–6A3) to greyish pink (6B2–6B3), eventually variably darker pink (6C4–7C4, 7D5, 8B3–8B4), unchanging with exposure, moist, spongy, under hand lens of compact, folded locules that gelatinize with maturity; in longitudinal section columella a short basal structure 1–4 mm wide, off-white, gelatinous, opaque; upward-radiating sterile veins short (1–2 mm), less evident at maturity. Odour faintly fragrant, clay-like. Taste slightly bitter, astringent. Macrochemical reactions not obtained.

*Peridium* 94–200 μm thick, single-layered, of interwoven repent hyphae, these laterally branching, uninflated to inflated, 1–7 μm wide, occasionally swollen at septa, with irregular extracellular encrustations, granulose-guttulate, hyaline in KOH and H₂O, interspersed with golden brown, opaque conductive hyphae; terminal cells cylindrocapitate, infrequently subventricose, or rarely cylindrical or with distinct angles, occasionally with short side branches, 7–81 μm long, 2–16 μm wide at apex, 2–7 μm centrally, 2–6 μm at base, with brownish yellow, densely granulose contents in KOH, and occasionally with globose, hyaline extracellular encrustations and swollen at the basal septum. *Glebal trama* hyaline, of tightly packed, parallel to slightly interwoven hyphae diverging toward hymenium; hyphae cylindrical,
Fig. 1. Maximum likelihood (ML) phylogram (-ln = 57979.037507) based on RPB1, RPB2, and 28S ribosomal DNA sequences depicting phylogenetic relationships of the Boletaceae and new sequestrate Guyanese taxa (in red bold). ML bootstrap support values greater than 70 are shown above the nodes. Other sequestrate taxa are indicated in bold with solid black dots preceding their binomials. Previously identified clades with multiple species from the same higher taxon are collapsed into triangles for visual simplification as is a clade of outgroup taxa from several non-Boletaceae lineages of Boletales.
infrequently swollen at septa or branch points; cells 8–82 × 2–8 μm, hyaline in KOH, thin-walled, often heavily gelatinized and separating in mature specimens; contents not evident or sparsely guttulate-granulose; conductive hyphae frequent in subhymenial region, 2–7(–9) μm wide, golden-brown, opaque. **Hymenium** lining locules composed of a palisade of basidioles, basidia and cystidia arising from dichotomously branching subhymenial hyphae. **Basidia** abundant in younger specimens, increasingly rare to absent with age, subclavate to clavate, tapering evenly toward base, or rarely cylindrical, 23–50 μm long, 7–12 μm broad at apex, 6–9(–12) μm at centre, (3–)4–6 μm at base, thin-walled, hyaline in KOH and H₂O, unreactive in Melzer’s solution; contents not evident or opaque granulose-guttulate; guttules refractive, variably-sized, solitary to numerous; sterigmata two, three, or four per basidium, straight, even, 1.5–2 × 1–2 μm. **Basidiospores** statismosporic, smooth, subfusiform to fusiform, occasionally amygdaloid, bilaterally symmetrical in all views, (13–)14–18(–20) × 6–8 μm (mean = 16.0 ± 1.6 × 6.9 ± 0.6 μm; Qr = 1.88–2.83(–3.25), Qm = 2.34 ± 0.25; n = 120), initially light pinkish brown, more reddish brown at maturity in KOH and H₂O, inamyloid; sterigma detaching irregularly from basidium and leaving a pedicel (0.5–)1–3.5(–5) μm long at basidiospore base; wall 0.3–0.9 μm thick, nearly smooth under light microscopy, under SEM surface of short, irregular layers. **Cystidia** abundant in young specimens, less frequent with maturation, arising from lower subhymenium, not projecting above hymenial palisade, cylindrical, subulate, or subventricose, rarely cylindroclavate, (30–)34–72(–88) μm long, 5–9 μm broad at apex, 6–12 μm at centre, 4–6(–8) μm at base, thin-walled, faintly grey and highly refractive in KOH and H₂O, strongly dextrinoid in Melzer’s; contents initially granulose-guttulate, later a uniform, highly refractive cytoplasm, deliquescing into locules with advanced age. **Clamp connections** absent.

**Habit, habitat, and distribution:** Solitary or in small groups semi-emergent on mineral soil/humic layer interface on the forest floor, or hypogeous deeper within mineral soil, in forests on lateritic or white sand soils under *Aldina insignis*, *Dicymbe altsonii*, *D. corymbosa*, *D. jenmanii*, or *Pakaraimaea dipterocarpacea*; known from the Upper Potaro and Upper Mazaruni River Basins of Guyana.
New sequestrate Boletaceae from Guyana

Additional specimens examined: Guyana: Region 8 Potaro-Siparuni: Pakaraima Mountains, Upper Potaro River Basin, ~1.5 km southwest of base camp at 5°18′04.8″ N 59°54′40.4″ W, on Cathie’s Hill, in lateritic soil under Dorycnium, 12 June 2012, Aime 4891 (BRG 41211; PUL F2833; HSU G1118; GenBank accession number ITS: KR261060); 100 m south-east of base camp near Dicyomia plot JP5, in alluvial sand soil under D. corymbosa, 12 June 2015, Henkel 10077 (BRG 41221; HSU G1128). Region 7 Cuyuni-Mazaruni: Pakaraima Mountains, Upper Mazaruni River Basin, ~10 km west of Mt Ayanganna in vicinity of Pegaima savanna base camp at 5°26′21.3″ N 60°04′43.1″ W, vicinity of base camp. in white sand soils under P. dipterocarpacea and D. jenmanii, 25 Dec. 2010, Henkel 9540 (BRG 41212; HSU G1119); 27 Dec. 2010, Henkel 9555 (BRG 41213; HSU G1120); ~200 m south of base camp, in white sand soils under P. dipterocarpacea and D. jenmanii, 1 June 2012, Henkel 9661 (BRG 41214; HSU G1121); 2 km south-west of base camp in Pakaraimaean plot 2, in white sand soils under P. dipterocarpacea and D. jenmanii, 5 June 2012, Henkel 9689 (BRG 41215; HSU G1122).

Commentary: Jimtrappea guyanensis is recognized in the field by the white peridium, unchanging tissues, pink, loculate gleba, and short columella. Micromorphologically J. guyanensis is distinguished by the smooth, subfusciform, reddish brown basidiospores and prominent dextrinoid cystidia. Smooth basidiospores are relatively rare among sequestrate Boletaceae, and the dextrinoid cystidia of J. guyanensis are unprecedented among sequestrate Boletales with smooth basidiospores (e.g. Dodge 1931, Smith & Singer 1959, Peclet et al. 1989, Pacioni & Sharp 2000, Lumyong et al. 2003, Nouhra et al. 2005, Yang et al. 2006, Desjardins et al. 2008, Moreau et al. 2011, Moreau et al. 2013, Lebel et al. 2012, Orihara et al. 2012, Trappe et al. 2013, Hayward et al. 2014). In the phylogenetic analysis reported here, J. guyanensis was putatively related to the South-East Asian sequestrate Durianella echinulata, albeit without bootstrap support (Fig. 1). These two species are distinct morphologically as D. echinulata is characterized by highly ornamented spores, a blue colour change upon exposure, and a rough, warted peridium (Desjardins et al. 2008). Additionally, J. guyanensis is putatively related to a cluster of Tylopius species, including the type species of that genus (T. felleus), but without bootstrap support (Fig. 1).

Jimtrappea guyanensis is micromorphologically most similar to the tropical African monotypic sequestrate genus Mackintoshia, originally described as a member of Agaricales (Pacioni & Sharp 2000), but now known to belong to Boletales based on ITS and 28S rDNA data (Fig. 1; Nuhn et al. 2013, Tedersoo & Smith 2013). Mackintoshia persica is characterized by prominent cystidia, smooth basidiospores, and a putative symbiotic association with ECM Caesalpinioideae (Pacioni & Sharp 2000). The subfusciform basidiospores and dextrinoid cystidia of J. guyanensis contrast with the ellipsoid basidiospores and non-dextrinoid cystidia of M. persica (Castellano et al. 2000, Pacioni & Sharp 2000). Although there is no bootstrap support for the placement of either M. persica or J. guyanensis in the phylogenetic analysis, they were resolved in highly divergent clades and on relatively long branches, suggesting no close relationship (Fig. 1).

Basidiospores of the Asian and Australasian genus Rossbeeveera are nearly smooth except for broad longitudinal ridges that give them a slight to distinct polar angularity that is lacking in J. guyanensis (Lebel et al. 2012, Orihara et al. 2012b). Rossbeeveera species also lack cystidia and have basidiomata that turn blue with exposure (Lebel et al. 2012, Orihara et al. 2012b). Additionally, Rossbeeveera is phylogenetically distant from J. guyanensis (Fig. 1), resolving in a well-supported, previously recovered “leccinoid” clade with other sequestrate and non-sequestrate taxa (Nuhn et al. 2013, Wu et al. 2014). The fusoid, smooth, pedicellate basidiospores of J. guyanensis also resemble those of species of Hysterangium (Hysterangiales) which otherwise differ in having a dendroid columella, a dark greenish or brown gleba with gel-filled locules, and in lacking cystidia (Castellano et al. 1989).

Species in a few other temperate sequestrate genera of Boletales have large, fusoid, smooth basidiospores that could potentially be confused with those of J. guyanensis, but differ, in addition to lacking cystidia, in the following ways: Alpova species have gel-filled locules and are associated primarily with Alnus; Melanogaster species have a black gleba with gel-filled locules; Rhizopogon species have an olivaceous to dark brown gleba and are associated with Pinaceae; and Truncocolumella species have a greenish brown gleba, a dendroid columella, and are associated with Pinaceae hosts (Trappe et al. 2009).
**Castellanea** T.W. Henkel & M.E. Sm., **gen. nov.**
Mycobank MB812361
(Figs 4A, 5–6)

*Etymology:* The genus is named in honor of Dr. Michael A. Castellano, a world authority on sequestrate fungi.


*Type species:* *Castellanea pakaraimophila* T.W. Henkel & M.E. Sm. 2015.

---

**Castellanea pakaraimophila** T.W. Henkel & M.E. Sm., **sp. nov.**
MycoBank MB812362
(Figs 4A, 5–6)

*Etymology:* *Pakaraimaea* and *–philus* (Gk.) = loving; in reference to occurrence of the species as basidiomata and mycorrhizas with *Pakaraimaea dipterocarpacea*.

*Diagnosis:* Diffsers from other known sequestrate taxa by the combination of the grey-orange to orange-brown peridium, brown unchanging gleba, basidiospores that are often dextrinoid and released in tetrads, and absence of cystidia.

*Type:* Guyana: Region 7 Cuyuni-Mazaruni: Pakaraima Mountains, Upper Mazaruni River Basin, ~10 km west of Mt Ayanganna in vicinity of Pegaima savanna base camp at 5°26’21.3” N 60°04’43.1” W, northern vicinity of base camp, in white sand soil under *P. dipterocarpacea* and *D. jenmanii*, 22 Dec. 2010, Henkel 9514 (BRG 41216 – holotype; HSU G1116, NY 02460743 – isotypes). GenBank accession number ITS and 28S: KC155381.

---

**Fig. 5.** Basidiomata of *Castellanea pakaraimophila*. **A.** Dorsal view (left) and ventral views (middle, right) showing short stipe (holotype; Henkel 9514). **B.** Longitudinal section showing highly folded gleba, basally thickened peridium, and short stipe (Henkel 9670). **C.** Orangish brown peridium (Henkel 9670). Bars = 10 mm.

**Fig. 6.** Microscopic features of *Castellanea pakaraimophila* (holotype; Henkel 9514). **A.** Basidiospores. **B–C.** Four-sterigmate basidia with mature basidiospores. **D.** Glebal trama showing distinct mediostratum and strongly diverging lateral stratum. B–C = phase contrast. Bars = 10 µm.
New sequestrate Boletaceae from Guyana

**Description:** Basidiomata irregularly flattened-ovate, 7–12 mm tall, 12–16 mm broad, subfaint, softer with age; surface light greyish orange (5A5–5B5–5B6) to orange-brown (7C8–7D8–7E8) with occasional darker humic stains, unchanging with pressure, glazed macroscopically, under hand lens a dense repent mat of light orange hyphae, with age viscid to nearly glutinous; base subtended by a short, concolourous stipe, this 1.5 × 1.5 mm, with a single concolourous hyphal cord. *Peridium* in longitudinal section extremely thin over apical ¼ (< 0.25 mm), concolourous with the surface, over basal 1/4 thickening to 0.75 mm and there off-white, single-layered, separable. *Gleba* dark brown (6E7–6F7, 7E7–7F7) throughout, unchanging with exposure, of irregularly shaped locules with interior surfaces minutely brownish hispid under hand lens; locule walls transluscent-gelatinous; columnella arising from the thickened basal peridium, with a single narrow gelatinous vein extending to apex. Odour slightly of iodine; taste not obtained. Macromorphological reactions not obtained.

*Peridium* 25–190 μm thick, single-layered, of tightly interwoven, repent hyphae, yellowish brown in KOH and H₂O, becoming more parallel and hyaline toward gleba; individual hyphae 2–5 μm wide, thin-walled; terminal cells cylindrical to subcapitate, 19–36 × 2–3 μm. *Glebal trama* with a distinct mediostratum and lateral stratum; mediostratum hyaline in H₂O and KOH, 12.4–29.6 μm wide, of parallel, slightly interwoven hyphae; individual hyphae 2–8 μm wide; lateral stratum divergent at a right angle from mediostratum, hyaline in H₂O and KOH, grading imperceptibly to the densely interwoven subhymenium. *Hymenium* a palisade of tightly packed basidia and basidioles. *Basidia* faintly grey in H₂O and KOH, changing in shape with maturity; in developing basidiomata (e.g. *Henkel* 9670) subclavate, infrequently cylindro-clavate, rarely cylindrical, 36–54 μm long, 6.0–12.5 μm broad at apex, 5.0–11.5 μm at centre, 5.0–8.5 μm at base, thin-walled; sterigmata four, straight, 4–7.5 × 0.9–1.5 μm; in fully mature basidiomata (e.g. *Henkel* 9514) consistently clavate, 20–25 μm long, with four short (<1 μm), highly reduced sterigmata. *Basidiospores* statisomosphic, smooth, subfusiform, bilaterally symmetrical in all views, 12–18 × 5.5–8(–10) μm (mean = 14.7 ± 1.20 × 7.1 ± 0.91 μm; Qr = (1.5–)1.9–2.7, Qm = 2.1 ± 0.25; n = 61), light yellowish brown in H₂O and KOH, often with one dextrinoid guttule, with age viscid to bruising or exposure (Lebel et al. 2012, Orihara et al. 2012b).

Based on the basidiome colour and basidiospore shape, *C. pakaraimophila* species also undergo a blue or blackish colour change upon bruising or exposure (Lebel et al. 2012, Orihara et al. 2012b).

**Habit, habitat and distribution:** Solitary or in a small group partially emergent on mineral soil/humic layer interface on the forest floor under *P. dipterocarpacea*, or immersed in decaying wood humus at base of dead *P. dipterocarpacea*; known only from the type locality in the Upper Mazaruni River Basin of Guyana.

**Additional specimen examined.** **Guyana:** Region 7 Cuyuni-Mazaruni: Pakaraima Mountains, Upper Mazaruni River Basin, ~10 km west of Mt Ayanganna in vicinity of Pegaima savanna base camp at 5°26’21.3” N 60°04’43.1” W, 150 m northeast of base camp, in wood humus at base of dead *P. dipterocarpacea*, 3 June 2012, *Henkel* 9670 (BRG 41217; HSU G1123). GenBank accession number ITS: LC054831.

**Commentary:** *Castellanea pakaraimophila* is recognized in the field by the ovate basidiomata, orange-brown peridium, dark brown loculate gleba, short stipe, unchanging tissues upon exposure, and association with *P. dipterocarpacea*. Micromorphologically *C. pakaraimophila* is characterized by the smooth, yellowish brown, frequently dextrinoid basidiospores that abscise in tetrads, and well-defined mediostratum of the glebal trama. *Castellanea pakaraimophila* has been confirmed as an ECM symbiont of *P. dipterocarpacea* based on analysis of ITS rDNA sequences from ECM roots (Smith et al. 2013). In the phylogenetic analysis reported here, *C. pakaraimophila* is putatively related to a cluster of *Tylopilus* species, including the type species of the genus *T. felleus*, but without bootstrap support (Fig. 1).

*Castellanea pakaraimophila* is similar to *Mackintoshia persica* because both have dextrinoid or partially dextrinoid, light yellowish brown or ochraceous-yellow basidiospores (Castellano et al. 2000, Pacioni & Sharp 2000). However, *C. pakaraimophila* differs from *M. persica* in its subfusiform basidiospores released in tetrads and lack of cystidia (Pacioni & Sharp 2000). Additionally, the glena trama in *M. persica* ranges from 200–330 μm wide, is gelatinous, and lacks a distinct mediostratum. The protologue description of *M. persica* notes that basidium morphology changes with basidioma age in a manner similar to that seen in *C. pakaraimophila* (Pacioni & Sharp 2000). However, with *C. pakaraimophila* the basidia become smaller and more angular with age, whereas the initially clavate basidia of *M. persica* become long-utriiform to fusiform (Pacioni & Sharp 2000). The two species are also unrelated phylogenetically (Fig. 1).

Some *Rossbeevera* species can nominally resemble *C. pakaraimophila* because they have nearly smooth basidiospores and lack cystidia. However, the basidiospores of *Rossbeevera* are non-dextrinoid, individually abscised, slightly to distinctly longitudinally ridged, and barely angular to stellate in polar view. In contrast, the dextrinoid basidiospores of *C. pakaraimophila* lack angularity and are frequently abscised in tetrads. Basidiomata of *Rossbeevera* species also undergo a blue or blackish colour change upon bruising or exposure (Lebel et al. 2012, Orihara et al. 2012b).

Species in a few other temperate sequestrate genera of *Boletaceae* have large, fusoid, smooth basidiospores that could possibly resemble *C. pakaraimophila* because they have nearly smooth basidiospores and lack cystidia. However, the basidiospores of *Alpova* (Paxillaceae) and *Mycoamaranthus* (Boletaceae). However, *Alpova* species have smaller basidiospores, a pseudoparenchymatous peridium, abundant clamp connections, and are usually associated with *Alnus* (Dodge 1931, Nouhra et al. 2005, Moreau et al. 2011, Moreau et al. 2013, Hayward et al. 2014). Species of *Mycoamaranthus*, though similar in peridial micromorphology to *C. pakaraimophila*, have finely ornamented to spinulose basidiospores, a bright yellow peridium, and are currently only known from Africa, South-East Asia, and Australasia (Castellano et al. 2000, Lumyong et al. 2003). The fusoid, smooth, pedicellate basidiospores of *C. pakaraimophila* also resemble those of species of *Hysterangium* (*Hysterangiales*) which otherwise differ in having a dendroid columnella, a dark greenish or brown gleba, and in lacking cystidia (Castellano et al. 1989).

Species in a few other temperate sequestrate genera of *Boletales* have large, fusoid, smooth basidiospores that could...
potentially be confused with those of C. pakaraimophila, but differ, in addition to having non-dextrinoid basidiospores, in the following ways: Melanogaster species have a black gleba with gel-filled locules; Rhizopogon species have olivaceous to dark brown gleba colours and are associated with Pinaceae; and Truncocolumella species have greenish brown gleba colours, a dandroid columella, and are associated with Pinaceae (Trappe et al. 2009).

**Costatisporus** T.W. Henkel & M.E. Sm., *gen. nov.*
MycoBank MB812363
(Figs 4C, 7–8)

*Etymology:* *Costatus* (L. adj. A) = ribbed or ridged and – *sporus* (L. adj. A) = –spored; in reference to the distinctively ridged ornamentation of the basidiospores.

*Diagnosis:* Distinguished from other Boletaceae by a combination of the following characters: *Basidiomata* hypogeous to partially emergent, sequestre. *Peridium* greyish yellow, staining dark blue, glabrous to subtomentose, thin. Gleba brown, unchanging, sterile veins absent. *Basidiospores* statismosporic, subglobose to oblong, light brown, inamyloid, with costate ornamentation of longitudinal ridges pole to pole, these entire or discontinuous, pedicel infrequent. *Basidia* clavate. *Cystidia* and clamp connections absent.

*Type species:* *Costatisporus caerulescens* T.W. Henkel & M.E. Sm. 2015.

**Costatisporus cyanescens** T.W. Henkel & M.E. Sm., *sp. nov.*
MycoBank MB812364
(Figs 4C, 7–8)

*Etymology:* *Cyanescens* (L. adj. A) = becoming dark blue; referring to the dark blue auto-oxidation reaction of the bruised peridium.

*Diagnosis:* Easily differentiated from other sequestrate taxa by the off-white to greyish yellow peridium that stains dark blue, brown acolumellate gleba, strong chocolate nutty odour, and large basidiospores with costate ornamentation.

*Type:* **Guyana:** Region 8 Potaro-Siparuni: Pakaraima Mountains, Upper Potaro River Basin, ~15 km east of Mt Ayanganna, 2.5 km southeast of base camp at 5°18'04.8"N 59°54'40.4"W, in Lance plot 1, solitary on lateritic mineral soil/humic layer interface under *D. corymbosa*, 19 June 2009. *Henkel* 9061 (BRG 41218 – holotype; HSU G1117, NY 02460744 – isotypes). GenBank accession numbers ITS: KT447439; *RPB1*: LC053663; *RPB2*: LC053664.

*Description:* *Basidiomata* subglobose to ovate and slightly lobed, 12–26 mm tall, 12–33 mm broad; surface initially off-white to light to greyish yellow (4A3–4A4, 4B3) where unstained by soil, developing increasingly deep blue (23C8–23D8–23E8) stains slowly and progressively over 5–10 minutes where squeezed or bruised, firm, softer in areas, with small, possibly invertebrate mycophagist excavations, glabrous macroscopically, under a hand lens matted tomentose, with age gelatinizing in areas to dark brown and viscid; base subtended by one to several tan hypyal cords. *Peridium* in longitudinal section thin, 0.3–0.7 mm, appearing single-layered macroscopically, white initially, bluing slowly but intensely on exposure in younger specimens, separable. Gleba densely loculate with brown-hispid interior surfaces under hand lens, initially brown (7E8) to reddish brown (7F6–7F7–7F8, 8F8) throughout, with age gelatinizing and darker brown (9F4–9F5–9F6), with advanced age violet brown (10F5) over outer 1/6 with locule structure breaking down and blue stains evident on glebal trama under hand lens, acolumellate, with a greyish, gelatinous sterile basal pad 1 × 5 mm. *Odour* strong, variously described as chocolate-nutty, musky, soapy, putty-like, or of mushroom bullion; *taste* indistinctive, fungoid. *Macrochemical reactions:* KOH reddish brown on peridium and gleba; NH4OH negative on all surfaces.

*Peridium* 150–615 μm thick, two-layered; outer layer 50–415 μm thick, dark yellow to brownish, of tightly interwoven hyphae; individual hyphae with yellow cytoplasmic pigment in H2O and KOH, occasionally hyaline; intercalary cells 20–92.5 × 5–10 μm, thin-walled, frequently with spiraled to ring-like extracellular encrustations; inner layer 50–250 μm thick, hyaline, of loosely interwoven to parallel hyphae, these 3–8 μm wide, thin-walled. *Glebal trama* composed of mediostratum and lateral stratum; mediostratum hyaline in H2O and KOH, 25–45 μm wide, often splitting at locule junctions, of parallel to loosely interwoven hyphae, these hyaline in KOH and H2O or infrequently with pale yellow cytoplasmic pigments, 2–8 μm wide, thin-walled; lateral stratum moderately to strongly diverging, 10–49 μm thick; individual hyphae hyaline in H2O and KOH, 4–7 μm wide, thin-walled, grading into interwoven subhymenial hyphae, these hyaline to pale yellow, 5–9 μm wide, thin-walled. *Hymenium* a palisade of basidia and basidioles. *Basidia* subclavate to clavate, hyaline to faintly grey in H2O and KOH, 39.0–66.4 μm long, 7.5–12.2 μm broad at apex, 3–9 μm at the centre, 2.5–5.8 μm at the base, thin-walled; sterigma three or four, 4.5–5.5 × 1–2 μm. *Basidiospores* statismosporic, yellowish to light brown in H2O and KOH, inamyloid, with complex costate ornamentation of 5–10 longitudinal, somewhat spiraled main ridges running pole to pole; ridges entire or discontinuous and occasionally bifurcating, with numerous narrow, shallow, nearly perpendicular cross-ridges, subglobose to oblong with ornamentation included, 17–25 × (10–)13–20 μm (mean = 19.9 ± 14.1 × 15.9 ± 1.96 μm; *Qr* = 1.00–1.38(–2.30), *Qm* = 1.27 ± 0.20; *n* = 118), ovate to subfusiform and basally acuminate with ornamentation excluded, 14–21 × 8–11.5 μm (mean = 16.8 ± 1.32 × 10.0 ± 0.65 μm; *Qr* = 1.36–2.21, *Qm* = 1.69 ± 0.17; *n* = 100); pedicel infrequent, 0.5–4 × 1–2 μm.

*Cystidia* and clamp connections absent.

*Habit, habitat and distribution:* Solitary to scattered and hypogeous to partially emergent on mineral soil/humic layer interface on the forest floor, under *D. corymbosa* or *A. insignis*; known only from the type locality and a second site ~8 km distant in the Upper Potaro River Basin of Guyana.

*Additional specimens examined:* **Guyana:** Region 8 Potaro-Siparuni: Pakaraima Mountains, Upper Potaro River Basin, ~15 km east of
New sequestrate Boletaceae from Guyana

Mt Ayanganna, within 10 km radius of base camp at 5°18’04.8” N 59°54’40.4” W, ~8 km southeast of base camp on lateritic soil-leaf litter interface under A. insignis, 25 July 2009, Henkel 9067 (BRG 41219; HSU G1124). GenBank accession number ITS and 28S: LC053662; ~1 km southeast of base camp immersed hypogeous in decaying wood humus under D. corymbosa on lateritic soils, 11 June 2015, Henkel 10060 (BRG 41222; HSU G1125); GenBank accession number ITS: KT380011; 16 June 2015, Henkel 10100 (BRG 41223; HSU G1126); vicinity of base camp, under D. corymbosa, in Guyanagaster plot 40, 21 June 2015, Aime 5850 (BRG 41224; HSU G1127; PUL F2871).

Commentary: Costatisporus cyanescens is recognized in the field by the blue-bruising peridium with occasional mycophagist excavations, and dark brown to violet-brown, finely loculate, acolumellate gleba that gelatinizes with maturity. Micromorphologically, the basidiospore ornamentation of longitudinal main and lateral secondary

Fig. 7. Basidiomata of Costatisporus cyanescens. A. Unsectioned basidioma showing blue stains on bruised peridium (holotype; Henkel 9061). B. Longitudinal section showing mature dark brown gleba with gelatinization around margins (holotype; Henkel 9061). C. Longitudinal sections of three basidiomata showing dark blue peridial stains, mycophagist excavations (left) and glebal maturation (left to right) (Henkel 10100). Bars = 10 mm.
ridges is distinctive. The basidiospore ornamentation is remarkably similar to that in species of the epigeous bolete genus *Boletellus*, which has no known sequestrate members (Singer 1986, Mayor et al. 2008, Halling et al. 2015). The similar basidiospore ornamentations of *Costatisporus* and *Boletellus* are apparently coincidental, as *C. cyanescens* has no close phylogenetic relationship with *Boletellus*, which occurs in the Xerocomoideae clade (Fig. 1; Table 1).

The basidiospores of South-East Asian sequestrate *Rhodactina* (Boletaceae) species are longitudinally ridged but lack the intervening secondary ridges observed in *C. cyanescens*, are purple in water mounts, and dextrinoid (Pegler et al. 1989, Yang et al. 2006). Although no 28S, RPB1, or RPB2 sequences were available for the genus *Rhodactina*, comparison of the ITS1 sequence from *Rhodactina incarnata* with that of *C. cyanescens* did not indicate a close relationship. The tropical Asian genus *Durianella* has sequestrate basidiomata that undergo a deep blue colour change upon exposure, but also have a well-developed columella, fibrillose exoperidial warts, and echinulate basidiospores (Desjardin et al. 2008).

Species of the sequestrate genera *Rossbeevera* (East Asia, Australasia) and *Chamonixia* (mostly North Temperate) feature longitudinal ridging of the basidiospores and often undergo a blue, green, or blackish colour change upon bruising or exposure (Smith & Singer 1959, Lebel et al. 2012, Orihara et al. 2012b). Basidiospore ornamentation in *Rossbeevera* lacks intervening subridges and has 4–5 short, broad longitudinal ridges which contribute to their slight to stellate polar angularity (Lebel et al. 2012, Orihara et al. 2012b), contrasting with the 5–10 spiralled, acute ridges of *C. cyanescens* that impart a consistently stellate polar shape. Furthermore, *Rossbeevera* is phylogenetically distant from *C. cyanescens* (Fig. 1). The ridged basidiospore ornamentation of *Chamonixia* species superficially resembles that of *C. cyanescens*, but their longitudinal ridges are straight with rounded margins and lack intervening subridges (Smith & Singer 1959).

Although the longitudinally ridged basidiospore ornamentation of *C. cyanescens* bears some resemblance to that seen in *Gautieria* (*Gomphales*) and *Austrogautieria* (*Hysterangiales*), no species of these genera undergo a blue colour change upon bruising or exposure (Zeller & Dodge 1918, Stewart & Trappe 1985). Additionally, *Gautieria* species differ from *C. caerulescens* in having gently rounded basidiospore ridges that terminate before the poles, a persistent dendroid columella, and globose cells in the peridium (Dodge & Zeller 1934). While *Austrogautieria* species are aculmellate and have apically convergent basidiospore ridges with subacute margins, only one species, the Australian *A. manjipumana*, overlaps with *C. caerulescens* in the number of basidiospore ridges (5–10); all other species of *Austrogautieria* have 8–14 ridges (Stewart & Trappe 1985). *Austrogautieria manjipumana* differs from *C. cyanescens* in the smaller (14–20 × 8–13 µm), more ellipsoid basidiospores that lack intervening subridges (Stewart & Trappe 1985). Additionally, *Gautieria* and *Austrogautieria*, as members of the Phallomycetidae, are phylogenetically distant from *Boletales* (Giacini et al. 2006, Hosaka et al. 2006).

In the phylogenetic analysis presented here, *C. cyanescens* is well supported as sister to *Sutorius* (formerly the *Tyopilus eximius* complex), which is a genus of dark maroon to purple, epigeous bolete species occurring in the Americas, Australasia, and sympatrically with *C. cyanescens* in Guyana (Fulgenzi et al. 2007, Halling et al. 2012). The molecular-based analysis may reflect evolutionary reality, but *Costatisporus* and *Sutorius* are very different morphologically. *Sutorius* species have robust, pileate-stipitate basidiomata with exposed hymenophores that bruise cinnamon-brown, ballistosporic basidia, and smooth, fusiform basidiospores, among many other different micromorphological features (Fulgenzi et al. 2007, Halling et al. 2012). The *Costatisporus-Sutorius* clade indicated here is supported as sister to the recently characterized, mostly epigeous genus *Neoboletus* (Fig. 1) (Wu et al. 2014, 2015). While the sole sequestrate species of *Neoboletus*, *N. thibetanus*, stains blue upon exposure, it is easily distinguished from *C. cyanescens* by its notable stipe and bright yellow peridium. In the future it will be necessary to sequence additional loci from species within this emerging clade to shed light on the putatively strong relationships between *Sutorius, Neoboletus*, and *C. cyanescens*. 

---

**Fig. 8.** Microscopic features of *Costatisporus cyanescens* (holotype: Henkel 9061). A. Basidiospores. B. Three-sterigmate basidium with developing basidiospores. C. Four-sterigmate basidium. D. Peridium hyphae with ring-like external encrustations. B–D = phase contrast. Bars = 10 µm.
ACKNOWLEDGEMENTS

We thank the following funding sources: National Science Foundation (NSF) DEB-0918591 to T.W.H., NSF DEB-1354802 to M.E.S., NSF DEB-0732968 to M.C.A., and the National Geographic Society’s Committee for Research and Exploration to T.W.H. Additional funding for M.E.S. and K.O. was provided by the University of Florida’s Institute for Food and Agricultural Sciences (IFAS). Dillon Husbands functioned as Guayanese local counterpart and assisted with field collecting, descriptions, and specimen processing. Additional field assistance in Guyana was provided by Mei Lin Chin, Jessie Uehling, Christopher Andrew, Valentino Joseph, Peter Joseph, Francino Edmund, and Luciano Edmund. Jim Trappe provided useful discussions prior to description. Two reviewers provided valuable comments on an earlier version of the manuscript. Research permits were granted by the Guyana Environmental Protection Agency. This paper is number 210 in the Smithsonian Institution’s Biological Diversity of the Guiana Shield Program publication series.

REFERENCES


Ge ZW, Smith ME (2013) Phylogenetic analysis of rDNA sequences indicates that the sequestrate Amogaster vindiglebus is derived from within the agaricoid genus Lepiota (Agaricaeae). Mycological Progress 12: 151–155.


New sequestrate Boletaceae from Guyana

**Phytophthora boodjera** sp. nov., a damping-off pathogen in production nurseries and from urban and natural landscapes, with an update on the status of *P. alticola*

Agnes V. Simamora1, Mike J. C. Stukely2, Giles E. St.J. Hardy1, and Treena I. Burgess1

1Centre for Phytophthora Science and Management, School of Veterinary and Life Sciences, Murdoch University, Perth, WA, 6150, Australia; corresponding author e-mail: tburgess@murdoch.edu.au
2Science Division, Department of Parks and Wildlife, Locked Bag 104, Bentley Delivery Centre, WA 6983, Australia

Abstract: A new homothallic *Phytophthora* species, isolated in Western Australia (WA), is described as *Phytophthora boodjera* sp. nov. It produces persistent, papillate sporangia, oogonia with thick-walled oospores, and paragynous antheridia. Although morphologically similar to *P. arenaria*, phylogenetic analyses of the ITS, cox1, HSP90, j-α-tubulin and enolase gene regions revealed *P. boodjera* as a new species. In addition, *P. boodjera* has a higher optimal temperature for growth and a faster growth rate. *Phytophthora boodjera* has only recently been found in Western Australia and has mostly been isolated from dead and dying *Eucalyptus* seedlings in nurseries and from urban tree plantings, and occasionally from disturbed natural ecosystems. It is found in association with declining and dying *Agonis flexuosa*, *Banksia media*, *B. grandis*, *Corymbia calophylla*, *Eucalyptus* spp., and *Xanthorrhoea preissii*. The status of *P. alticola* was also reviewed. The loss of all isolates associated with the original description except one; discrepancies in both sequence data and morphology of the remaining isolate with that presented the original description, and inconclusive holotype material places the status of this species in doubt.

**Key words:**
- *Eucalyptus*
- multi-gene phylogeny
- Oomycota
- *Phytophthora arenaria*

**INTRODUCTION**

Numerous *Phytophthora* species have been associated with damping-off and seedling diseases in plant production nurseries worldwide (Hardy & Sivasithamparam 1988, Davison et al. 2006, Warfield et al. 2008, Moralejo et al. 2009, Goss et al. 2011, Lilja et al. 2011, Leonberger et al. 2013, Pérez-Sierra & Jung 2013, Prospero et al. 2013, Schoebel et al. 2014). *Phytophthora* species are dispersed via the roots of infected plants, soil from potted plants, growth media and water, and in some cases by aerial transmission. Transfer of plants and plant products by human activity and through globalisation in trading is now generally accepted as the main method of introduction of exotic pathogens and pests. The most high-risk pathway for the movement of *Phytophthora* is “plants for planting” (Brasier 2008, Liebhold et al. 2012, Scott et al. 2013). Plants infected at production nurseries can potentially distribute *Phytophthora* species to parks and reserves, amenity plantings, plantations, rehabilitation and biodiversity plantings, wildflower farms, retail nurseries, and gardens. Many *Phytophthora* species, such as *P. nicotianae*, *P. plurivora* (often reported as *P. citricola*), *P. cactorum* and *P. citrophthora*, tend to be the most commonly recovered from nurseries worldwide, strongly supporting their dissemination through the nursery trade. Because of the level of attention that has been given to this important topic, it is now rare for a new species to be detected in nurseries (Moralejo et al. 2009). Nevertheless the number of reports of *Phytophthora* species damaging to nursery trees, forests and natural ecosystems is increasing and this has significant implications for international plant biosecurity and plant health practice (Kroon et al. 2012).

The most significant new detection of the past 20 years is *Phytophthora ramorum* (Grünwald et al. 2012, Parke & Grünwald 2012). *Phytophthora ramorum* was first detected infecting *Viburnum* and *Rhododendron* in plant nurseries in Germany and The Netherlands in 1993 (Werres et al. 2001), and has subsequently been found in various nurseries all over Europe and North America. It has been recognized as an alien aggressive species in natural areas of the west coast of the USA where it causes sudden oak death, and in Cornwall in the UK (Rizzo et al. 2002, Brasier et al. 2004). Spread through the international nursery trade, *P. ramorum* poses a serious risk to plant biosecurity worldwide (Brasier 2008, Parke & Lucas 2008, Parke & Grünwald 2012).

In recent years, many new *Phytophthora* species have been described from natural ecosystems in Western Australia (WA) (Burgess et al. 2009, Scott et al. 2009, Rea et al. 2010, Jung et al. 2011a, b, Rea et al. 2011, Aghighi et al. 2012, Burgess et al. 2012, Crous et al. 2012, Hüberli et al. 2013). In 2011, a new damping-off disease was reported in WA nurseries growing *Eucalyptus* and other species for
restoration of agricultural land. ITS sequence data of the isolates did not match any known species, but were closely related to *P. alticola* and *P. arenaria* and were an exact match for a single WA isolate designated as “*P.* taxon arenaria-like” by Rea et al. (2011).

*Phytophthora arenaria* has been isolated primarily from Kwongan vegetation and mainly from *Banksia* species on the northern sandplains in south-west WA (Rea et al. 2011). *Phytophthora alticola* was first isolated and described by Maseko et al. 2007 from cold-tolerant *Eucalyptus* species (*E. dunnii*, *E. baijsenia*, and *E. macarthurii*) with collar and root rot in South African plantations at an altitude above 1150 m. The new taxon has been isolated in WA from dead and dying *Eucalyptus* seedlings in nurseries and from adult plants in the urban landscape, predominantly from eucalypts, and occasionally from *Banksia* species and *Corymbia calophylla* in natural ecosystems.

Further investigation of isolates thought to be *P. arenaria* in the Vegetation Health Service (VHS) collection of the WA Department of Parks and Wildlife (Burgess et al. 2009) and other recent collections from urban surveys (Barber et al. 2012) revealed two distinct groups of isolates. The first group were of *P. arenaria*, while the second appeared to be a new species related to *P. alticola* (Maseko et al. 2007). In the current study, the *P. alticola*/*P. arenaria* species complex was re-evaluated using a combination of morphology and a multi-gene phylogeny resulting in the recognition of a new species, described here as *P. boodjera* sp. nov., and an investigation into the status of *P. alticola*.

**MATERIALS AND METHODS**

**Isolates**

The majority of isolates used were obtained from the Vegetation Health Service (VHS) Collection, Department of Parks and Wildlife, Perth, Western Australia. All isolates were bailed from soil and root material using *Eucalyptus sieberi* cotyledons. The isolates were maintained in 90 mm Petri dishes on V8 agar (V8A, 0.1 L filtered V8 juice, 17 g agar, 0.1 g CaCO3, 0.9 L distilled water) and on 5 mm V8A discs stored in 20 mL sterile water in McCartney bottles at room temperature. The ex-type isolates of *P. alticola* were obtained from CBS (CBS-KNAW Fungal Biodiversity Centre, Utrecht). Sequence data from related species were obtained from GenBank (www.ncbi.nlm.nih.gov/genbank) the Phytophthora Database (PD; www.phytophthoradb.org), and q-bank (www.q-bank.eu). When all isolates in the CMW collection (Forestry and Agriculture Biotechnology Institute, University of Pretoria, SA) were evaluated and it was found that all isolates of *P. alticola* except CMW 19425 had perished, that isolate was re-numbered CMW 34279. All isolates used in this study are detailed in Table 1, and the status of all *P. alticola* isolates is given in Table 2.

**DNA isolation, amplification and sequencing**

The *Phytophthora* isolates were cultured on half-strength potato dextrose agar (PDA) (Becton Dickinson, Sparks, MD), 19.5 g PDA, 7.5 g agar and 1 L of distilled water) at 20 °C for 2 wk. Mycelium was collected by scraping from the agar surface with a sterile blade and placing in a 1.5 mL sterile Eppendorf® tube. It was frozen in liquid nitrogen and crushed to a fine powder, and genomic DNA was extracted following the method of Andjic et al. (2007). In all cases, the PCR reaction mixtures were as described previously (Andjic et al. 2007) but using the PCR conditions described in the original papers (cited below). The region spanning the internal transcribed spacer (ITS1-5.8S-ITS2) region of the ribosomal DNA was amplified using the primers DC6 (Cooke et al. 2000) and ITS-4 (White et al. 1990). The mitochondrial gene cox1 was amplified with primers FM77 and FM 84 (Martin & Tooley 2003). Heat shock protein 90 (HSP) was amplified with HSP90-F int and HSP90-R1 primers (Blair et al. 2008). β-tubulin (BT) was amplified with primers BTFA1 and BTR1, and enolase (ENO) was amplified with primers Enl Fy and Enl R1 according to Kroon et al. (2004).

All gene regions were sequenced in both directions with the primers used in amplification. The clean-up products and sequencing were accomplished as described previously (Sakalidis et al. 2011). All sequences derived in this study were added to GenBank, and the accession numbers are provided in Table 1.

**Phylogenetic analysis**

The data set consisted of sequences of *Phytophthora boodjera* sp. nov., *P. alticola* and *P. arenaria* isolates used in this study, and other closely related species in ITS clade 4 (Table 1) which were compiled and manually edited in Geneious v. R7 (http://www.geneious.com/) and Bayesian analysis conducted using a MrBayes (Ronquist et al. 2012) plugin within Geneious after determining the most appropriate substitution model with jModelTest-2.1.4 (Darriba et al. 2012). Alignment files and trees can be viewed on TreeBASE (http://www.treebase.org/).

**Culture characteristics**

Circular inoculum plugs (5 mm diam) were taken from the margin of 7 d-old cultures on V8A and placed in the centre of 90 mm Petri dishes prepared for growth stimulation. The plates were then moved to incubators fixed at 4, 10, 15, 20, 25, 30, 32.5, 35 and 37.5 °C. Plates were observed daily to ensure that the colonies did not reach the edge of the Petri dish; the radial growth rate was measured after 4–7 d, along two lines crossing the middle of the inoculum plug at right angles, and the mean growth rates (mm per day) were assessed. After 7 d, plates with no colony growth at 35 °C and 37.5 °C were returned to 20 °C for 7 d to check the isolate viability.

**Morphology**

Sporangia were produced by flooding 15 x 15 mm square agar discs, removed from the growing edge of 3–5-d-old
Phytophthora boodjera sp. nov., a damping-off pathogen

Colonies on V8A in 90 mm Petri dishes, with sterile water at 18–25 °C with their surfaces submerged, in natural daylight. This water was decanted and replaced twice (after 4 and 6 h). In the final change, 1 mL of non-sterile soil extract was also added and the Petri dishes were incubated overnight. The soil extract was made by suspending 100 g of pine (Pinus radiata) bark potting mixture in 1 L distilled water and incubating this on an orbital shaker for 24 h at 20 °C before filtering through Whatman no. 1 paper to remove soil particles. After 18–36 h, dimensions and characteristic features of 50 mature sporangia of each isolate, selected at random, were ascertained at 400x in a BX51 Olympus microscope.

Gametangia were produced by all isolates on V8A in the dark at 20 °C after 7 d. After 14 d, dimensions and characteristic features of 50 randomly-selected mature oogonia, oospores and antheridia were measured at 400x. The oospore wall index was calculated as the ratio between the volume of the oospore wall and the volume of the whole oospore (Dick 1990).

The preserved type materials of P. alticola available from the National Mycological Herbarium in Pretoria (PREM 59214, PREM 59215, PREM 59216, PREM 59217) were re-examined. The slides were rehydrated with 85 % lactic acid and observed with a Zeiss Axioskop 2 Plus compound microscope fitted with an Axiocam MRc camera. Dimensions were measured using Axiovision v. 4.8 software.

RESULTS

Phylogenetic analysis

CMW 19417 was designated as the type isolate of Phytophora alticola by Maseko et al. (2007), but no sequence data were provided for this isolate. A subsequent sequence of this same isolate, CBS 121937 available on q-bank, actually corresponds to P. palmivora (Fig. 1). CMW 19424 and CMW 19425 were originally designated as paratypes and ITS sequence data were provided for these isolates. All of these isolates were subsequently lost except CMW 19425 (= CBS 121939 = CMW 34279 = P19861). ITS sequence data for isolates presented with the original description, including CMW 19425 (DQ988196), differ by 3 bp from all recent sequences of CMW 34279, CBS 121939 and P19861 (Fig. 1). However, when resequenced CMW 19424 (= CBS 121938) was found to actually be an isolate of P. frigida (Fig 1). Based on ITS sequence data, the WA isolates investigated in this study cluster with either isolate CMW 34279 or with P. arenaria (Fig. 1).

BT sequences data was also provided in the original description (Maseko et al. 2007): all isolates assigned to P. alticola were identical, but differ by 2 bp from the new sequence of isolate CMW 34279 and by 4 bp from P. boodjera sp. nov. (figure available on request from the authors). The coxl sequence of isolate CMW 34279 from three separate databases is identical and clusters separately from isolates assigned to P. boodjera sp. nov. (figure available on request from the authors). Isolates of P. arenaria cluster together, although intraspecific sequence variation is observed. In the concatenated dataset (Fig. 2), isolate CMW 34279 clusters with isolates of P. boodjera sp. nov., although it differs by 8 bp across the five gene regions examined. If the isolate is duplicated it forms a strongly supported cluster on its own (data not shown). Isolates of P. arenaria also reside in a strongly supported clade, although intraspecific variation is observed (Fig. 2).

Status of Phytophthora alticola

In 2008, the World Phytophthora Collection (WPC; http://phytophthora.ucr.edu/default.html) was sent four isolates from the CMW collection, two isolates each of P. alticola and P. frigida. When the WPC sequenced them, they realised the identities were incorrect and informed the CMW collection (Table 2). Isolates of P. alticola and P. frigida were then checked in the CMW collection and it was discovered that all isolates of P. alticola had perished or were incorrectly identified, except for CMW19425 which was cleaned and renumbered CMW34279. This isolate was then sent to WPC where it was given the code P19861. Also in 2007, three isolates were sent to CBS; of these, the ex-holotype isolate CBS 121937 (= CMW 19417) is actually P. palmivora (the sequence associated with this isolate is available from q-bank), the ex-paratype isolate CBS 121938 (= CMW 19424) was not re-sequenced but is now determined as of P. frigida, leaving the same single isolate CBS 121939 (= CMW 34279) (Table 2).

At the start of this project, it was known that the ex-holotype isolate of P. alticola had perished, as indeed had all other isolates except an ex-paratype isolate CMW 19425 (= CMW 35429, = CBS 121939, = P19861). The ITS sequence of this isolate from all collections is identical, although there are a few bp different from the ITS sequence of the same isolate in the original description (Fig. 1). The sequence in the original description is short and the differences are at the end of the sequence and could have been erroneously labelled. Controversially, sequence data of other isolates in various collections designated as P. alticola match different species (Table 2).

It was originally considered that epitypification would be possible with the intention to designate CMW 34279 as the epitype. However, morphological examination of this isolate revealed that it differed from the original description: the sporangia are not caducous and chlamydospores are not produced (Table 3). Subsequent examination of the holotype and paratypes from PREM were inconclusive (Table 3). Each of the PREM types consisted of a semi-dried agar disc kept at 4 °C and a microscopic slide. The agar disks were all contaminated with bacteria and a dark hyphomycete, most of the mycelia had lysed, but a few aborted oospores were observed in PREM 59216 (= CMW 19424) and PREM 59217 (= CMW 19425). Some reproductive structures were present on the slides. Sporangia and chlamydospores were present for PREM 59214 (= CMW 19416) and PREM 59215 (= CMW 19417). The sporangia were predominantly ovoid, caducous and papillate, and produced in close sympodia (Table 3, Fig. 3). The dimensions of these sporangia match the original description of P. alticola (Maseko et al. 2007). However, in the original description the sporangia were described as borne on terminal or branched sporangiophores, while the slide associated with the holotype had sporangia borne...
Table 1. Identity, date and location of isolation, host information and GenBank accession numbers (where available) for Phytophthora spp. considered in this study.

<table>
<thead>
<tr>
<th>Species</th>
<th>Location</th>
<th>Isolation date</th>
<th>Host association</th>
<th>Isolate number&lt;sup&gt;2&lt;/sup&gt;</th>
<th>GenBank Accession No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. alicola&lt;sup&gt;1&lt;/sup&gt;</td>
<td>Midillovo, KwaZulu-Natal</td>
<td>2000-2004&lt;sup&gt;4&lt;/sup&gt;</td>
<td>Eucalyptus badjensis</td>
<td>CMW 19417</td>
<td>ITS: q-bank&lt;sup&gt;5&lt;/sup&gt; BT: DQ988197 ENO: DQ988236</td>
</tr>
<tr>
<td>ex-holotype</td>
<td>(KZN), South Africa</td>
<td></td>
<td></td>
<td>CBS 121937</td>
<td></td>
</tr>
<tr>
<td>P. alicola&lt;sup&gt;1&lt;/sup&gt;</td>
<td>Midillovo, KZN, South Africa</td>
<td>2000-2004</td>
<td>E. macarthurii</td>
<td>CMW 19424</td>
<td>ITS: DQ988196 ENO: DQ988235</td>
</tr>
<tr>
<td>ex-paratype</td>
<td>CBS 121938</td>
<td></td>
<td></td>
<td>CBS 121939</td>
<td></td>
</tr>
<tr>
<td>ex-paratype</td>
<td>CBS 121938</td>
<td></td>
<td></td>
<td>CBS 121938</td>
<td></td>
</tr>
<tr>
<td>P. alicola&lt;sup&gt;1&lt;/sup&gt;</td>
<td>unknown</td>
<td>05/2011</td>
<td>Unknown</td>
<td>P 16052</td>
<td>ITS: GU259141 ENO: HQ261245</td>
</tr>
<tr>
<td>Ravensthorpe, WA</td>
<td>08/2006</td>
<td>Banksia media</td>
<td>VHS 26631&lt;sup&gt;4&lt;/sup&gt;</td>
<td>VHS 26806&lt;sup&gt;4&lt;/sup&gt;</td>
<td>ITS: KJ396705 ENO: KJ396733 ENO: KJ396683</td>
</tr>
<tr>
<td>Kensington, Perth, WA</td>
<td>02/2012</td>
<td>Eucalyptus sp.</td>
<td>VHS 26806&lt;sup&gt;4&lt;/sup&gt;</td>
<td>VHS 27015&lt;sup&gt;1&lt;/sup&gt;</td>
<td>ITS: KJ396708 ENO: KJ396736 ENO: KJ396687</td>
</tr>
<tr>
<td>ex-holotype</td>
<td>Tincurren, WA</td>
<td>03/2012</td>
<td>Soil dump</td>
<td>VHS 27016&lt;sup&gt;1&lt;/sup&gt;</td>
<td>ITS: KJ396709 ENO: KJ396737 ENO: KJ396737</td>
</tr>
<tr>
<td>Tincurren, WA</td>
<td>04/2012 Eucalyptus sp.</td>
<td>VHS 27017&lt;sup&gt;1&lt;/sup&gt;</td>
<td>KJ3967244</td>
<td>KJ3967244</td>
<td>ITS: KJ396711 ENO: KJ396739 ENO: KJ396689</td>
</tr>
<tr>
<td>Tincurren, WA</td>
<td>04/2012 Eucalyptus sp.</td>
<td>VHS 27018&lt;sup&gt;1&lt;/sup&gt;</td>
<td>KJ3967245</td>
<td>KJ3967245</td>
<td>ITS: KJ396712 ENO: KJ396740 ENO: KJ396690</td>
</tr>
<tr>
<td>Tincurren, WA</td>
<td>04/2012 Eucalyptus sp.</td>
<td>VHS 27020&lt;sup&gt;1&lt;/sup&gt;</td>
<td>KJ3967248</td>
<td>KJ3967248</td>
<td>ITS: KJ396713 ENO: KJ396741 ENO: KJ396691</td>
</tr>
<tr>
<td>Tincurren, WA</td>
<td>04/2012 Eucalyptus sp.</td>
<td>VHS 27021&lt;sup&gt;1&lt;/sup&gt;</td>
<td>KJ3967249</td>
<td>KJ3967249</td>
<td>ITS: KJ396714 ENO: KJ396742 ENO: KJ396692</td>
</tr>
<tr>
<td>Tincurren, WA</td>
<td>04/2012 Eucalyptus sp.</td>
<td>VHS 27022&lt;sup&gt;1&lt;/sup&gt;</td>
<td>KJ3967250</td>
<td>KJ3967250</td>
<td>ITS: KJ396715 ENO: KJ396743 ENO: KJ396693</td>
</tr>
<tr>
<td>Tincurren, WA</td>
<td>04/2012 E. polybractea</td>
<td>VHS 27171&lt;sup&gt;1&lt;/sup&gt;</td>
<td>KJ3967241</td>
<td>KJ3967241</td>
<td>ITS: KJ396716 ENO: KJ396744 ENO: KJ396694</td>
</tr>
<tr>
<td>P. arenaria</td>
<td>Kailbarri, WA</td>
<td>06/1986</td>
<td>Kwongan heathland</td>
<td>DDS 1221&lt;sup&gt;4&lt;/sup&gt;</td>
<td>ITS: QE593266 ENO: KJ396724 ENO: KJ396752</td>
</tr>
<tr>
<td>ex-holotype</td>
<td>Eneabba, WA</td>
<td>02/2009</td>
<td>E. drummondii</td>
<td>CBS 127950&lt;sup&gt;4&lt;/sup&gt;</td>
<td>ITS: HQ013205 ENO: KJ396726 ENO: KJ396771</td>
</tr>
<tr>
<td>Bunbury, WA</td>
<td>02/2002</td>
<td>B. littoralis</td>
<td>VHS 10154</td>
<td>VHS 10154</td>
<td>ITS: Vue301118 ENO: KJ396729 ENO: KJ396745</td>
</tr>
<tr>
<td>Badgingarra, WA</td>
<td>04/2006</td>
<td>B. attenuata</td>
<td>VHS 15453&lt;sup&gt;4&lt;/sup&gt;</td>
<td>VHS 15453&lt;sup&gt;4&lt;/sup&gt;</td>
<td>ITS: Vue301118 ENO: KJ396729 ENO: KJ396745</td>
</tr>
</tbody>
</table>
Table 1. (Continued).

<table>
<thead>
<tr>
<th>Species</th>
<th>Location</th>
<th>Isolation date</th>
<th>Host association</th>
<th>Isolate number¹</th>
<th>GenBank Accession No.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ITS</td>
</tr>
<tr>
<td>Ph. boodjera sp. nov.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>BT</td>
</tr>
<tr>
<td>Badgingarra, WA</td>
<td>04/2006</td>
<td>B. attenuata</td>
<td>VHS 15489</td>
<td>HQ013216</td>
<td>KJ372292</td>
</tr>
<tr>
<td>Eneabba, WA</td>
<td>06/2008</td>
<td>B. attenuata</td>
<td>VHS 19931</td>
<td>HQ013217</td>
<td>KJ372293</td>
</tr>
<tr>
<td>Eneabba, WA</td>
<td>11/2008</td>
<td>B. attenuata</td>
<td>VHS 20537</td>
<td>KJ372253</td>
<td>KJ372299</td>
</tr>
<tr>
<td>Ellenbrook, Perth, WA</td>
<td>09/2011</td>
<td>Banksia sp.</td>
<td>VHS 25370</td>
<td>KJ372254</td>
<td>KJ372300</td>
</tr>
<tr>
<td>Dongara, WA</td>
<td>11/2012</td>
<td>Banksia sp.</td>
<td>VHS 28145</td>
<td>KJ372251</td>
<td>KJ372294</td>
</tr>
<tr>
<td>Muchea, WA</td>
<td>12/2012</td>
<td>X. preissi</td>
<td>VHS 28269</td>
<td>KJ372252</td>
<td>KJ372295</td>
</tr>
<tr>
<td>P. frigida</td>
<td>South Africa</td>
<td></td>
<td></td>
<td>P 16059</td>
<td>GU259147</td>
</tr>
<tr>
<td>P. palmivora</td>
<td>United States</td>
<td></td>
<td></td>
<td>P 0113</td>
<td>GU259121</td>
</tr>
<tr>
<td>P. heveae</td>
<td>United States</td>
<td></td>
<td></td>
<td>P 10167</td>
<td>GU259516</td>
</tr>
<tr>
<td>P. quercetorum</td>
<td>United States</td>
<td></td>
<td>MD 9.2</td>
<td></td>
<td>EU080796</td>
</tr>
<tr>
<td>P. castaneae</td>
<td>Japan</td>
<td></td>
<td>P 10187</td>
<td>FJ801304</td>
<td>EU080803</td>
</tr>
<tr>
<td>P. megakarya</td>
<td>Sao Tome and Principe</td>
<td></td>
<td>P 8516</td>
<td>PD²</td>
<td>EU079970</td>
</tr>
<tr>
<td>P. nicotianae</td>
<td>Australia</td>
<td></td>
<td>Nicotiana tabacum</td>
<td>332</td>
<td></td>
</tr>
<tr>
<td>P. cactorum</td>
<td>United States</td>
<td></td>
<td>Malus sylvestris</td>
<td>NY 568</td>
<td></td>
</tr>
<tr>
<td>P. plurivora</td>
<td>Germany</td>
<td></td>
<td>Quercus robur</td>
<td>CBS 124087</td>
<td></td>
</tr>
</tbody>
</table>

¹See Table 2 for explanation on the status of these isolates.
²Abbreviations of isolates in culture collections (where known): CBS = Centraalbureau voor Schimmelcultures, The Netherlands; IMI = CABIBioScience (formerly International Mycological Institute), UK; VHS = Vegetation Health Service Collection, Department of Parks and Wildlife, Perth, Australia; DDS = earlier prefix of VHS Collection; PAB = Paul Barber, in Murdoch University (MU) Culture Collection; TP = Trudy Paap, in Murdoch University (MU) Culture Collection; CMW = culture collection of Forestry and Agriculture Biotechnology Institute, University of Pretoria, South Africa; P = isolate codes from World Phytophthora Collection, University of California, Riverside.

³Designated as Phytophthora taxon arenaria-like by Rea et al. (2011).
⁴Isolates used in the morphological study.
⁵Sequence available on Phytophthora database (http://www.phytophthoradb.org/) or q-bank (http://www.q-bank.eu/).
⁶No specific dates provided by Maseko et al. (2004), just date range under ‘sampling and isolation’.
in close sympodia. These sporangia and their branching patterns resemble more those produced by *P. palmivora* rather than those of living isolate CMW 34279 (Table 3, Fig. 3). Oospores only were present in paratypes PREM 59216 (= CMW 19424) and PREM 59217 (= CMW 19425). The dimensions of these aplerotic oospores match the
Phytophthora boodjera sp. nov., a damping-off pathogen

In the original description (Maseko et al. 2007), no sequence data were provided for PREM 59214 (= CMW 19416) and PREM 59215 (= CMW 19417). When the ex-holotype isolate was submitted to CBS and sequenced for q-bank (CBS 121937) it was found to be an isolate of *P. palmivora* (Fig. 1, Table 2). Caducous, papillate sporangia and chlamydospores matching *P. palmivora* were observed in PREM 59214 (= CMW 19416) and PREM 59215 (= CMW 19417) (Fig. 3). When the ex-paratype isolate CMW 19424 was submitted to CBS it was found to be *P. frigida*, as were several isolates labelled as *P. alticola* that were sent to WPC (Fig. 1, Table 2). *Phytophthora frigida* has aplerotic oogonia with amphigynous antheridia, as observed for PREM 59216 (= CMW 19424) and PREM 59217 (= CMW 19425). Thus, we believe that while in the original description of *P. alticola* the sequence data provided was identical for all isolates, the actual morphological description is based on a set of isolates from more than one species; these are most probably *P. palmivora*, *P. frigida*, and a species represented by isolate CMW 34279. As there are no other living isolates linked to the original description available for examination and as no more isolates have been recovered in South Africa, despite extensive sampling, it is not possible to amend the description of *P. alticola* or to designate PREM 59217 (= CMW 19425, = CMW 35429) as an epitype. At this point in time the application of the name *P. alticola* is in doubt and will remain so until more isolates from similar hosts or locations can be made and this taxon will be referred to hereafter as *P. alticola nom. dub*.

Compared with the description of *P. alticola nom. dub.*, CMW 34279 has a higher optimum temperature for growth, faster growth rate, persistent sporangia, no chlamydospores and paragynous antheridia, and is very similar in morphology to isolates from Australia described here as *P. boodjera*.

---

**Fig. 2.** Bayesian inference tree based on concatenated sequence data from ITS, β-tubulin, HSP90, enolase and cox1 gene regions generated in MrBayes using the GTR +G substitution model showing relationship between *P. alticola* nom. dub. (green), *P. boodjera* sp. nov. (blue) and *P. arenaria* (red). The posterior probability is shown at the nodes. *Phytophthora castaneae* and *P. heveae* were used as outgroup taxa.
Phytophthora boodjera A.V. Simamora & T.I. Burgess, sp. nov.
MycoBank MB809223
(Figs 4–5)

Etymology: the species name is derived from the Noongar (local Aboriginal) name for earth, ground, or sand plain.

Type: Australia: Western Australia: Tincurrin, from nursery soil dump, Mar. 2012, collected by the Vegetation Health Service of the Department of Parks and Wildlife (MURU 470—holotype; cultures ex-type CBS 138637 = VHS 26806). ITS, ß-tubulin, HSP90, enolase and coxl sequence GenBank KJ372244, KJ372283, KJ396710, KJ396738 and KJ396688 respectively).

Diagnosis: P. boodjera is phylogenetically closely related to P. alticola nom. dub. but differs in having persistent sporangia, paragynous antheridia and no chlamydospores. P. boodjera is morphologically similar to P. arenaria but differs in having a higher lethal temperature and larger sporangia and oogonia.

Description (type): Papillate, persistent predominantly ovoid sporangia (52 %) but also limoniform (45 %) and distorted shapes (3 %). Sporangia averaged 34.7 ±1.16 x 27 ± 0.78 µm and ranged 15.2–62.3 x 14.6–42.5 µm. Homothallic; aplerotic oogonia averaged 28.9 ± 2.13 µm, ranging from 24.3–34 µm. Oospores averaging 26.3 ± 1.42 µm diam, range 20.9–29.4 µm. Growth rate at optimum of 25 °C was 11.2 mm/d. Colonies were appressed with no pattern and had regular smooth margins on CA, V8A, MEA and PDA.

Description (species): Sporangia papillate, persistent, abundantly produced in soil extract water on simple sporangiophores frequently with globose swellings close to the sporangial base (Fig. 4f). Although predominantly ovoid (64 %, Fig. 4a–g), various sporangial shapes were observed including limoniform (20 %, Fig. 4d right, 4h), peanut-shaped (10 %) and distorted shapes (6 %, Fig. 4i, j). Bipapillate (Fig. 4i) sporangia were also occasionally observed.
Sporangiophores often laterally attached to sporangia (Fig. 4c, k), and sometimes constricted (Fig. 4e); branched sporangiophores rare (Fig. 4d). Sporangia from 12 isolates averaged 39.2 ± 4.4 x 29.7 ± 3.4 µm (range 32.5–44.5 x 24.5–33.5 µm), exit pores narrow, 6 ± 1 µm, length:breadth ratio 1.27 ± 0.16 (Table 3). Chlamydospores absent.

Homothallic, readily producing oogonia (and sporangia) in single culture on CA and V8A. Oospores matured within 14 days.
to 21 d. Oogonia averaged $29.4 \pm 2.3$ µm diam with isolate means ranging from 24.6 to 33.4 µm (Table 3). Oospores aplerotic in all isolates, containing ooplasts when semi-mature to mature (Fig. 4s–v). Oospores averaged $25.5 \pm 1.9$ µm diam with isolate means ranging from 21.3 to 29.5 µm (Table 3). Oospore walls thick ($2.5 \pm 0.33$ µm) (Fig. 4s–v),
Phytophthora boodjera sp. nov., a damping-off pathogen

Table 2. Status of Phytophthora alticola isolates submitted to different culture collections.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Sequence</th>
<th>Notes on status of isolate</th>
</tr>
</thead>
<tbody>
<tr>
<td>CMW 19416</td>
<td>no sequence (OD)</td>
<td>Lost in CMW collection. Only papillate, caducous sporangia and chlamydospores observed from preserved slide associated with PREM 59214</td>
</tr>
<tr>
<td>PREM 59214-paratype</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CMW 19417</td>
<td>no sequence (OD)</td>
<td>Lost in CMW collection. Supposed corresponding isolate in CBS is actually P. palmivora and all sequence data on q-bank associated with this isolate is P. palmivora. Only papillate, caducous sporangia and chlamydospores observed from preserved slide associated with PREM 59215</td>
</tr>
<tr>
<td>PREM 59215-holotype</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CBS 121937</td>
<td>ITS, CO, YPT1, TEF (q-bank)</td>
<td>Living in CMW collection and renamed CMW 35429. ITS and BT of re-sequenced isolate differ from original description by 3 and 2 bp respectively. ITS and CO sequence on q-bank is identical to sequence of isolate CMW 35429 obtained in the current study. Only aplerotic oospores and amphigynous antheridia observed from preserved slide associated with PREM 59217</td>
</tr>
<tr>
<td>CMW 19419</td>
<td>ITS and BT (OD)</td>
<td>Lost in CMW collection</td>
</tr>
<tr>
<td>PD 01642</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CMW 19421</td>
<td>ITS and BT (OD)</td>
<td>Lost in CMW collection</td>
</tr>
<tr>
<td>PD 01641</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CMW 19422</td>
<td>ITS and BT (OD)</td>
<td>Lost in CMW collection</td>
</tr>
<tr>
<td>PD 01640</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CMW 19423</td>
<td>ITS and BT (OD)</td>
<td>Lost in CMW collection</td>
</tr>
<tr>
<td>PD 01639</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CMW 19424</td>
<td>ITS and BT (OD)</td>
<td>Lost in CMW collection. Sequence on q-bank of ITS and BT is from the original description. The ITS of isolate re-sequenced in this study corresponds to P. frigida. Only aplerotic oospores and amphigynous antheridia observed from preserved slide associated with PREM 59216</td>
</tr>
<tr>
<td>PREM 59216-paratype</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CBS 121938</td>
<td>ITS and BT (OD)</td>
<td>Lost in CMW collection</td>
</tr>
<tr>
<td>PD 01638</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CMW 19425</td>
<td>ITS and BT (OD)</td>
<td>Lost in CMW collection.</td>
</tr>
<tr>
<td>PREM 59217-paratype</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CBS 121939</td>
<td>ITS, CO, YPT1, TEF (q-bank)</td>
<td></td>
</tr>
<tr>
<td>PD 01637</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CMW 35429</td>
<td>ITS, cox1, ENO, HSP, BT ITS (GA)</td>
<td>Was sent to WPC as CMW 35429 as a replacement for P. alticola, and named WPC 16948. ITS sequence supplied by Gloria Abad is identical to that obtained in the current study for isolate CMW 35429</td>
</tr>
<tr>
<td>P16948</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PD 01914</td>
<td>cox2 and cox1 (PD)</td>
<td>Was sent to WPC as P. alticola isolate CMW 19424 but when sequenced it was identified as being an isolate of P. frigida</td>
</tr>
<tr>
<td>P16053</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PD 02043</td>
<td>cox2 and cox1 (PD)</td>
<td>Was sent to WPC as P. frigida isolate CMW 19433 and when sequenced it was identified as being an isolate of P. frigida</td>
</tr>
<tr>
<td>P16051</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PD 02044</td>
<td>cox2 and cox1 (PD)</td>
<td>Was sent to WPC as P. alticola isolate CMW 19425 but when sequenced it was identified as being an isolate of P. frigida</td>
</tr>
<tr>
<td>P16054</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PD 02775</td>
<td>cox1 (PD)</td>
<td>Was sent to WPC as P. frigida isolate CMW 20311 but when sequenced it was identified as being an isolate of P. alticola and thus cannot be linked to any isolate from CMW collection</td>
</tr>
<tr>
<td>P16052</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VHS 26631</td>
<td>ITS, cox1, ENO, HSP, BT</td>
<td>List in WPC as a neotype for P. alticola, but this is not recommended as the isolate is from a different host and a different country from the original description. In current study this is considered an isolate of P. boodjera.</td>
</tr>
<tr>
<td>P19861</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>


Oospore wall index 0.47 ± 0.05 μm (Table 3). Antheridia paragynous (Fig. 4r–v), averaging 10.4 ± 1.9 x 8.3 ± 1.5 μm. Hyphal swellings catenulate, some with radiating hyphae, formed rarely in non-sterile soil extract water.

Cultures: All isolates produced colonies that were appressed with no distinctive growth pattern and regular smooth margins on CA, V8A, MEA, and PDA (Fig. 5). Growth on MEA was sparser than on the other media. Optimum temperature for the growth on V8A 25–30 °C, where the average growth rate was 9.18 ± 0.56 mm/d (Fig. 6). The maximum temperature for growth was 35 °C (Table 3). Although no growth occurred at 37.5 °C, this temperature was not lethal since isolates resumed growth when subsequently incubated at 20 °C.

Additional specimens examined: Australia: Western Australia: Mt Claremont, Perth, from roots of dying Agonis flexuosa, May 2011, Paul Barber (PAB 11.56, private collection); Dalkeith, from roots of dying Eucalyptus marginata, May 2011 Paul Barber (PAB 11.67, private collection); Northam, from Corymbia calophylla, Sept. 2013,Trudy Paap (TP13.39, private collection). Ravensthorpe, from Banksia media, Aug. 2006, (VHS 16282); Kensington, Perth, WA, from Eucalyptus sp., Feb. 2012, (VHS 26631); Tincurnin, from Eucalyptus spp., Apr. 2012, (VHS 27016, VHS 27017, VHS 27018, VHS 27020, VHS 27021, VHS 27022); Tincurnin, from roots of E. polybractea, Apr. 2012, (VHS 27171); Stirling,
Table 3. Comparison of morphological characters and dimensions, and temperature-growth relations of Phytophthora palmivora, P. frigida, P. alticola (from original description, holotype and paratype material and living isolate CMW 19425 = CMW 34279), P. boodjera, and P. arenaria.

<table>
<thead>
<tr>
<th>Species and sources of data</th>
<th>P. palmivora (Erwin &amp; Ribero, 1995)</th>
<th>P. frigida (Maseko 2007)</th>
<th>P. alticola (holotype)</th>
<th>P. alticola (paratype)</th>
<th>CMW 34279</th>
<th>P. boodjera (this study)</th>
<th>P. arenaria (Rea 2011)</th>
<th>P. arenaria (this study)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No of isolates</td>
<td>10</td>
<td>10</td>
<td>12</td>
<td>10</td>
<td>9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sporangia (mm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LxB mean</td>
<td>45.3 x 29.8</td>
<td>33 x 37</td>
<td>31.1 ± 5.0 x</td>
<td>30.9 ± 4.5</td>
<td>36 x 28</td>
<td>38.9 ± 5.4 x</td>
<td>39.2 ± 4.4 x</td>
<td>31.8 ± 4.6 x 23.7 x</td>
</tr>
<tr>
<td>Range</td>
<td>40-60 x 25-35</td>
<td>24-40 x 20-33</td>
<td>27.7-45.7 x 23.0-29.4</td>
<td>20.4-60.7 x 19.0-38.9</td>
<td>30-45 x 20-35</td>
<td>20.4-60.7 x 19.0-38.9</td>
<td>15.2-64.5 x 13.9-42.5</td>
<td>20.2-63.0 x 12.5-35.0</td>
</tr>
<tr>
<td>Range of isolates means</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>32.6-44.6 x 24.7-33.3</td>
<td>9.9-30.7</td>
</tr>
<tr>
<td>L/B ratio</td>
<td>1.2-1.8</td>
<td>1.22</td>
<td>1.21 ± 0.12</td>
<td>1.4 (&lt;1.6)</td>
<td>1.4 ± 0.03</td>
<td>1.27 ± 0.16</td>
<td>1.27 ± 0.16</td>
<td>1.22 ± 0.20</td>
</tr>
<tr>
<td>Range of isolates means</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>1.19-1.35</td>
<td>1.2-1.5</td>
<td>1.08-1.65</td>
</tr>
<tr>
<td>Sporangial characteristics</td>
<td>Papillate</td>
<td>Papillate, rarely bipapillate</td>
<td>Papillate</td>
<td>Papillate, rarely bipapillate</td>
<td>Papillate, rarely bipapillate</td>
<td>Papillate, rarely bipapillate or bilobed</td>
<td>Papillate, rarely bi/tripapillate or bilobed</td>
<td>Papillate, rarely bipapillate or bilobed</td>
</tr>
<tr>
<td>Persistence</td>
<td>caducous</td>
<td>caducous</td>
<td>semi-caducous</td>
<td>caducous</td>
<td>persistent</td>
<td>persistent</td>
<td>persistent</td>
<td>persistent</td>
</tr>
<tr>
<td>Sporangiphores</td>
<td>Lax or close sympodia</td>
<td>Lax or close sympodia</td>
<td>simple</td>
<td>simple or branched sympodia</td>
<td>simple or branched sympodia</td>
<td>simple or branched sympodia with bulbous base, very often laterally attached</td>
<td>simple or branched sympodia with bulbous base, very often laterally attached</td>
<td>simple or branched sympodia with bulbous base, very often laterally attached</td>
</tr>
<tr>
<td>Sporangia shape</td>
<td>ellipsoid, ovoid spherical</td>
<td>ovoid, sometimes obpyriform</td>
<td>Usually ovoid to broad ovoid</td>
<td>usually ovoid or ellipsoid, sometimes obpyriform or peanut-shaped</td>
<td>ovoid 66 %, limoniform 14 %, peanut-shaped 8 %, obpyriform 6 %, distorted 6 %</td>
<td>ovoid 64 %, limoniform 20 %, peanut-shaped 10 %, distorted 6 %</td>
<td>usually ovoid, also obpyriform or distorted</td>
<td>ovoid 40 %, subglobose 20 %, globose 14 %, obpyriform 12 %, distorted 4 %</td>
</tr>
<tr>
<td>Proliferation</td>
<td>absent</td>
<td>absent</td>
<td>absent</td>
<td>absent</td>
<td>absent</td>
<td>absent</td>
<td>absent</td>
<td>absent</td>
</tr>
<tr>
<td>Exit pores (mm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Width</td>
<td>5-6</td>
<td>6</td>
<td>6.21 ± 0.53</td>
<td>6.09 ± 1.02</td>
<td>6.09 ± 1.02</td>
<td>6.0 ± 1.00</td>
<td>5.50 ± 0.95</td>
<td>3.88-7.10</td>
</tr>
<tr>
<td>Chlamydospores (mm)</td>
<td>32-42</td>
<td>24-26</td>
<td>42.6 ± 5.8</td>
<td>28 (20-35)</td>
<td>Some isolates</td>
<td>absent</td>
<td>absent</td>
<td>absent</td>
</tr>
<tr>
<td>Hyphal swellings</td>
<td>Spherical</td>
<td>Irregular</td>
<td>Catenate, some with radiating hyphae</td>
<td>Catenate, some with radiating hyphae</td>
<td>Catenate, globose to sub-globose, some with radiating hyphae</td>
<td>Catenate, globose to sub-globose, some with radiating hyphae</td>
<td>Catenate, globose to sub-globose, some with radiating hyphae</td>
<td>Catenate, globose to sub-globose, some with radiating hyphae</td>
</tr>
<tr>
<td>Mean diameter (mm)</td>
<td></td>
<td>14.7</td>
<td>15.2</td>
<td></td>
<td>na</td>
<td>12.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breeding system</td>
<td>Heterothallic</td>
<td>Heterothallic</td>
<td>Homothallic</td>
<td>Homothallic</td>
<td>Homothallic</td>
<td>Homothallic</td>
<td>Homothallic</td>
<td>Homothallic</td>
</tr>
<tr>
<td>Oogonia (mm)</td>
<td></td>
<td>38</td>
<td>26.2 ± 2.3</td>
<td>284</td>
<td>27.3 ± 1.9</td>
<td>29.4 ± 2.3</td>
<td>25.3 ± 2.2</td>
<td>26.6 ± 1.6</td>
</tr>
<tr>
<td>Mean diameter</td>
<td>22-34.8</td>
<td>24-48</td>
<td>24-37</td>
<td>20-35</td>
<td>22.03-31.07</td>
<td>24.3-33.9</td>
<td>19.6-34.3</td>
<td>20.5-29.6</td>
</tr>
<tr>
<td>Diameter range</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range of isolates means</td>
<td>na</td>
<td>na</td>
<td>24.6-33.4</td>
<td>24.3-28.1</td>
<td>23.6-28.8</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 3. (Continued).

<table>
<thead>
<tr>
<th>Species and sources of data</th>
<th>P. palmivora</th>
<th>P. frigida</th>
<th>P. alticola</th>
<th>P. alticola</th>
<th>P. alticola</th>
<th>CMW 34279&lt;sup&gt;1&lt;/sup&gt;</th>
<th>P. boodjera</th>
<th>P. arenaria</th>
<th>P. arenaria</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Erwin &amp; Ribero, 1995)</td>
<td>(Maseko 2007)</td>
<td>(paratype)&lt;sup&gt;2&lt;/sup&gt;</td>
<td>(Maseko 2007)</td>
<td>(this study)</td>
<td>(this study)</td>
<td>(Rea 2011)</td>
<td>(this study)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PREM 59215</td>
<td>PREM 59217</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Oospores (mm)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean diameter</td>
<td>22.8 ± 0.1</td>
<td>33</td>
<td>28.2 ± 2.1</td>
<td>30 (28.3 x 30.5)</td>
<td>24.9 ± 2.1</td>
<td>25.5 ± 1.9</td>
<td>22.3 ± 1.8</td>
<td>23.8 ± 1.6</td>
<td></td>
</tr>
<tr>
<td>Range of isolates means</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>21.3–29.5</td>
<td>21.4–23.9</td>
<td>21.5–25.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wall thickness</td>
<td>na</td>
<td>2.51 ± 0.4</td>
<td>2.47 ± 0.33</td>
<td>2.30 ± 0.34</td>
<td>2.30 ± 0.34</td>
<td>2.57 ± 0.22</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oospore wall index</td>
<td>na</td>
<td>0.54 ± 0.05</td>
<td>0.47 ± 0.05</td>
<td>0.50 ± 0.05</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Oogonial characteristics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aplerotic</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Markedly aplerotic, oospores with thick inner walls</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aplerotic oospores</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mature oogonia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>with a slightly wavy surface and golden-brown discoloration</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aplerotic oospores</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mature oogonia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>with a slightly wavy surface and golden-brown discoloration</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aplerotic oospores</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mature oogonia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>with a slightly wavy surface and golden-brown discoloration</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antheridia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amphigynous</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mainly amphigynous</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paragynous, often with finger-like projections</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paragynous</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paragynous, often with finger-like projections</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LxB mean (mm)</td>
<td>na</td>
<td>10.6 ± 2.3 x</td>
<td>10.4 ± 1.9 x</td>
<td>11.2 ± 1.7 x</td>
<td>10.0 ± 2.1 x</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LxB range (mm)</td>
<td>na</td>
<td>8.3 ± 1.4</td>
<td>8.3 ± 1.5</td>
<td>8.4 ± 1.3</td>
<td>7.5 ± 1.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Growth Characteristics</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Max temp (°C)</td>
<td>34</td>
<td>30 to &lt;35</td>
<td>30 to &lt;35</td>
<td>35</td>
<td>35</td>
<td>32.5</td>
<td>35</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Opt temp (°C)</td>
<td>27.5–30</td>
<td>25</td>
<td>25</td>
<td>20–25</td>
<td>25–30</td>
<td>30</td>
<td>25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Min temp (°C)</td>
<td>&gt;5&lt;10</td>
<td>11</td>
<td>&gt;10&lt;15</td>
<td>&gt;10&lt;15</td>
<td>&gt;10&lt;15</td>
<td>&gt;10&lt;15</td>
<td>15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lethal temp (°C)</td>
<td>na</td>
<td>&gt;37.5</td>
<td>&gt;37.5</td>
<td>na</td>
<td>&lt;37.5</td>
<td>na</td>
<td>&lt;37.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Growth rate at optimum (mm/day)</td>
<td>ca. 7.5 (CA),</td>
<td>ca. 4.5 (CA),</td>
<td>ca. 8.20 (V8A)</td>
<td>ca. 7 (V8A)</td>
<td>ca. 4.5 (V8A),</td>
<td>ca. 8 (V8A)</td>
<td>ca. 7 (V8A)</td>
<td>5.9–7.4 (CA)</td>
<td>8.65 (V8A)</td>
</tr>
<tr>
<td>Growth rate at 20°C (mm/day)</td>
<td>5 (V8A)</td>
<td>4.5 (V8A),</td>
<td>7.75 (V8A)</td>
<td>6.12 (V8A)</td>
<td>3.8–5.2 (CA)</td>
<td>5.96 (V8A)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Phytophthora boodjera is morphologically very similar to isolate CMW 34279 linked to *P. alticola* nom. dub.; all measurements overlap, although CMW 34279 produces on average smaller sporangia, oogonia and oospores (Table 3). Colony morphologies on malt extract agar also differ (Fig. 5), and *P. boodjera* has a higher optimal temperature for growth and grows faster at higher temperatures (Fig. 6). Isolates of *P. boodjera* differ from CMW 34279 by one fixed single nucleotide polymorphism (SNP) in the ENO gene region, two in HSP and two in BT; three fixed SNPs separate the species in the cox1 gene region.

*Phytophthora boodjera* is closely related to *P. arenaria*. Morphologically, these species are very similar producing abundant thick walled oospores and sporangia of similar shapes and sizes (Table 3). The most marked differences between these species are: (1) 37.5 °C is lethal to *P. arenaria* but not to *P. boodjera*; (2) sporangia as well as oogonia and oospores are smaller in *P. arenaria*; and (3) 34 % of sporangia of *P. arenaria* are globose to subglobose while this shape is rare in *P. boodjera* (Table 3).

**DISCUSSION**

*Phytophthora* isolates from plant production nurseries in Western Australia (WA) were identified as closely related to *P. alticola* nom. dub. based on ITS sequence data. These isolates were compared to the single remaining isolate of *P. alticola* nom. dub. from the original description (Maseko et al. 2007). Based on morphology and molecular data from four nuclear and one mitochondrial gene region, the isolates from WA were recognized as a new species and described as *P. boodjera*. *Phytophthora boodjera* has emerged as a pathogen in some WA plant production nurseries and is now regularly recovered also from urban environments. However, it has been recovered infrequently (VHS 16282 from Ravensthorpe, VHS 28352 from Gingin, and TP 13.39 from Northam) from natural ecosystems in WA, despite widespread sampling in the region (Burgess et al. 2009, Rea et al. 2011).

*Phytophthora alticola* nom. dub. was originally described from *Eucalyptus* plantations in South Africa and has never been recovered from sampling within natural ecosystems in that region (Nagel et al. 2013, Oh et al. 2013). This suggests that *P. alticola* has been introduced into South Africa. Morphological studies of the remaining isolate CMW 34279 revealed three major discrepancies with the original description: firstly, *P. alticola* nom. dub. was described as having caducous sporangia, and secondly, as producing chlamydospores; however, the remaining isolate CMW 34279 has persistent sporangia and produced no chlamydospores. Thirdly, *P. alticola* nom. dub. was described as producing mainly amphigynous and some paragynous antheridia; however, in the remaining isolate CMW 34279, only paragynous
antheridia were observed. Although the ex-holotype isolate CMW 19417 has been lost, re-examination of the holotype PREM 59215 revealed sporangia and chlamydospores matching the original description of *P. alticola nom. dub.* except that they were produced in close sympodia rather than simple or branched sympodia (Maseko *et al.* 2007). CMW 19417 was submitted to CBS and the sequence of this isolate reveals that it is *P. palmivora*. The dimensions and characteristics of sporangia and chlamydospores observed in the holotype match those of *P. palmivora*.

Discrepancies in sequence data were found between the original description of *P. alticola nom. dub.* and the remaining ex-paratype isolate CMW 19425 (= CMW 34279). Unfortunately only oospores can be observed on the paratype PREM 59217 (= CMW 19425), but even these differ from the original description in that all antheridia are amphigynous in the holotype material, but they are all paragynous for CMW 34279. Thus, after examining the holotype and paratype material and resequencing isolates submitted to CBS, we have concluded that the original description was based on a mix of species and, as no further isolates similar to CMW 34279 have been recovered in South Africa despite extensive sampling (Oh *et al.* 2013), the status of *P. alticola* is in doubt.

*Phytophthora arenaria* (Rea *et al.* 2011), the species most closely related to *P. boodjera* in Western Australia, has been recovered exclusively from natural Kwongan vegetation on the coastal sand plains of south-west WA, where it was mainly isolated from dead and dying *Banksia* species and from the rhizosphere soil associated with such plants. This species appears to be restricted to the Kwongan vegetation and to be adapted to this ecosystem, suggesting that *P. arenaria* is native to WA. *Phytophthora boodjera* has only recently been found in WA and has mostly been isolated from dead and dying eucalypt seedlings in plant production nurseries and from declining trees (predominantly *Myrtaceae*) in disturbed urban landscapes, and once from *Xanthorrhoea preissii*. It has been isolated from natural ecosystems on only three occasions (from *Banksia media*, *B. grandis*, and *Corymbia calophylla*) and currently we consider this to be an introduced species.

Recent outbreaks of the damping-off disease of young eucalypt seedlings, caused by *P. boodjera*, have raised new concerns about the risk of *Phytophthora* species in plant production nurseries in WA. The dispersal of *Phytophthora* from nurseries to field plantings in previously non-infested areas may result in serious threats to biodiversity in natural ecosystems in these areas.

**ACKNOWLEDGEMENTS**

Trudy Paap, Keith Parnell, and Paul Barber were all involved in the collection of isolates for this study. Mike Coffey, Gloria Abad, Seonju Marincowitz and Arthur de Cock have all provided valuable help and discussion in resolving the current status of *Phytophthora alticola*. Diane White is thanked for technical assistance. A.S. thanks DIKTI program from the Indonesian Government for a PhD Scholarship.

**REFERENCES**


Matsushimamyces, a new genus of keratinophilic fungi from soil in central India

Rahul Sharma¹, Rohit Sharma², and Pedro W. Crous³

¹Centre for Biodiversity Exploration and Conservation (CBEC), 15, Kundan Residency, 4th Mile Mandla Road, Tilhari, Jabalpur 482021, MP, India; corresponding author e-mail: rahulpremasharma@gmail.com
²Microbial Culture Collection, National Centre for Cell Science, Ganeshkined, Pune 411007, India
³CBS-KNAW Fungal Biodiversity Centre, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands

Abstract: During a collecting trip exploring new habitats and locations for keratinophilic fungi, soil samples were collected from Bohani village in the central Indian state of Madhya Pradesh. Following isolation using a hair baiting technique, one sample yielded an interesting hyphomycetous fungus. The fungus, which could easily be observed under a dissecting microscope, formed prominently large, dark brown to opaque, fusoid conidia on horse hair after 2 mo of incubation. Single-conidial colonies were cultivated on Sabouraud dextrose agar medium by direct transfer, using a fine needle under a dissecting microscope. Morphologically the fungus resembles genera such as Bahugada, Hadrosporum, Manoharacarioniella, Pithomyces, and Septosporiopsis, but differs with regard to its conidia and conidigenous cells. Based on its unique morphology, the present collection is described as a new genus, Matsushimamyces, with M. bohannensis as type species. Phylogenetic analysis of the LSU region placed Matsushimamyces in Latoraceae (Plasporales, Dothideomycetes). Furthermore, based on the morphological and phylogenetic similarity (ITS and LSU rDNA), a second species, Polyschema venustum, is also referred to Matsushimamyces.

INTRODUCTION

Tropical regions of the world harbour an enormous biodiversity, including fungi (Hawksworth 1998). India is a vast country full of diverse habitats that are rich in fungal diversity, a large portion of which remains unknown. The notion that non-hotspot regions in India also harbour a large number of unknown species is supported from recent discoveries, such as those of Arthrinium jatrophae, Auxarthronopsis bandhavgartensis, Chaetomium jatrophae, and Gymnoascus verruicosus (Sharma et al. 2013, 2014, Sharma & Singh 2013). Two biodiversity hotspots were identified in India, namely the north-eastern Himalayas and the Western Ghats (Myers et al. 2000). After this demarcation, there was a marked increase in the number of fungal diversity exploration studies in these two areas, resulting in the discovery of several new fungi. This did, however, result in a diversion of focus from other regions in India, from which novel fungi still await collection or isolation and description. The present communication is a report of one such study from a village in Central India.

MATERIAL AND METHODS

Isolates

Soil was collected in sealed polythene bags using a sterile spatula, and brought to the laboratory for processing. The soil sample was stored at room temperature until processed. A slight modification of Vanbreuseghem's hair baiting technique was employed for recovery of fungi from soil (Sharma & Rajak 2003). Stereo microscopic observations were made under a UNICON dissecting microscope (Unique Scientific Traders, Ambala, India). Single conidial colonies were obtained by removing the conidia with the help of a fine needle and plating them on Sabouraud dextrose agar medium (SDA; Hi-Media and Merck, Mumbai) and 2% potato dextrose agar (PDA; Merck, Mumbai). Fungal-colonised horse hair was mounted in lactophenol and visualised under an Olympus BX53 (Olympus Corporation, Japan) as well as under a Nikon Eclipse Ni microscope with attached DS100 camera. The ex-type culture(s) of the fungus were deposited in the Centre for Biodiversity Exploration and Conservation (CBEC), Jabalpur, Microbial Culture Collection (MCC) in Pune, and the Microbial Type Culture Collection (MTCC) in Chandigarh.

DNA isolation, phylogeny and analysis

Fungal genomic DNA was extracted following the CTAB method described by Voigt et al. (1999). The only modification was in the crushing step. The samples were crushed using a micro-pestle (GV-28541, Geovista, India) for 1–2 min. The concentrations of extracted DNAs were measured by a NanoDrop ND-1000 spectrophotometer (Thermo Scientific).
RESULTS

Isolates
Soil sample CBEC 001, which was collected from Bohani village, Madhya Pradesh, and subjected to horse hair baiting, yielded an unusual fungus. The fungus formed dark brown to black, fusoid conidia attached to beaded conidiophores with prominently swollen conidiogenous cells. The fungus was first observed after 2 mo of incubation, suggesting slow germination and growth. On SDA, the fungus was also slow growing (< 3 cm diam after 45 d).

DNA isolation, phylogeny and analysis
The partial LSU and ITS regions were sequenced for CBEC 001, and sequences were deposited in GenBank with accession no. KP765516 (ITS) and KR350633 (LSU). The ITS sequencing resulted in a 500 bp long sequence. The NCBI BLASTn result in the UNITE database also revealed hits with many uncultured sequences but with lesser identity. All these genera are also quite different morphologically and do not support inclusion of CBEC 001 in any of them.

TAXONOMY

Matsushimamyces Rahul Sharma, Rohit Sharma & Crous, gen. nov.
MycoBank MB810895

Etymology: Matsushimamyces; named in honour of the Japanese mycologist Takashi Matsushima (Kobe, Japan) whose fungal monographs remain a source of inspiration.

Diagnosis: Conidiophores solitary, erect, rarely branched, straight or flexuous, brown apically, smooth, beaded near conidium attachment points, constricted at the septa. Conidiogenous cells pale brown, coarsely verrucose, monoblastic, terminal. Conidia solitary, straight or curved, fusoid to broadly ellipsoidal, multi-septate, apical and basal cell thin-walled, subhyaline to brown, median cells dark brown to black, thick-walled, roughly to coarsely verrucose.

Type species: Matsushimamyces bohaniensis Rahul Sharma et al. 2015.

Description: Colonies effuse, black, shortly hairy, slow growing, reverse black. Mycelium partly superficial partly immersed in the artificial substratum, composed of a network of branched, septate, pale to medium brown hyphae, smooth to sparsely verrucose, thick-walled, stroma at times present. Conidiophores macronematous, mononematous, at times aggregated, erect, unbranched (or rarely branched), straight or flexuous, hyaline to brown apically, smooth, beaded near conidium attachment, constricted at septa, intermediate cells dark brown or blackish brown, cylindrical, doliform or lageniform. Conidiogenous cells pale brown, coarsely verrucose, monoblastic, integrated determinate, terminal. Conidia solitary, straight or curved, fusoid to broadly ellipsoidal, multi-septate, apical and basal cell thin-walled, subhyaline to brown, median cells dark brown to black, thick-walled, roughly to coarsely verrucose, basal cell conic or round, truncate or apiculate.

Notes: Matsushimamyces is unique based on the following combination of characters: the solitary, pigmented conidiophores that are beaded near the terminal monoblastic conidiogenous cell, and solitary, straight or curved, fusoid to broadly ellipsoidal, multi-septate conidia. The conidia are also subhyaline to brown, with dark brown to black, thick-walled, roughly to coarsely verrucose median cells, and apical and basal cells that are thin-walled, and pale brown.
Matsushimamyces bohaniensis  
Rahul Sharma, Rohit Sharma & Crous, sp. nov.
MycoBank MB810896

(Article)

Description
Rahul & Rohit Sharma
Apr. 2014,
001 collected from village garbage frequented by pigs, 16 growing on white horse hair (keratin bait) in soil CBECS Mycelium exudate in readymade SDA or PDA (Merck). red exudate in SDA (Hi-Media ingredients), but lacking any Pradesh, India.

Distribution
Baited with horse hair from soil.

Conidia verruculose, monoblastic, integrated determinate, terminal. pale brown, doliiform or laginiform. Conidiogenous cells intermediate cells dark brown or blackish brown, cylindrical, doliiform or laginiform. Conidiogenous cells pale brown, verruculose, monoblastic, integrated determinate, terminal. Conidia solitary, straight or curved, fusoid to broadly ellipsoid, 6–7-septate, 50–104 x 26–33 µm, apical and basal cell thin-walled, subhyaline to brown.

Type: India: Madhya Pradesh: Bohani (22.9047°N, 79.4587°E) near Gadarwara, Narsinghpur, black conidia growing on white horse hair (keratin bait) in soil CBECS 001 collected from village garbage frequented by pigs, 16 Apr. 2014, Rahul & Rohit Sharma (CBECH 001 – holotype; CBECC 001 = MTCC12245 – ex-type cultures).

Description: Colonies effuse, black, hairy, slow growing, reaching 2 cm diam on SDA after 45 d, reverse black, with red exudate in SDA (Hi-Media ingredients), but lacking any exudate in readymade SDA or PDA (Merck). Mycelium partly superficial partly immersed in artificial substratum, composed of a network of branched, septate, pale to medium brown hyphae, smooth to sparsely verruculose, hyphae 3 µm thick, stroma at times present. Conidiophores macronematous, mononematous, solitary to aggregated, rarely branched, straight or flexuous, hyaline to brown apically, smooth, beaded near conidium attachment, constricted at sepa, intermediate cells dark brown or blackish brown, cylindrical, doliiform or laginiform. Conidiogenous cells pale brown, verruculose, monoblastic, integrated determinate, terminal. Conidia solitary, straight or curved, fusoid to broadly ellipsoid, 6–7-septate, 50–104 x 26–33 µm, apical and basal cell thin-walled, subhyaline to brown, median cells dark brown to black, thick-walled, roughly to coarsely verruculose, basal cell conic or round, truncate or apiculate, apical cell round or nipple-shaped. On PDA after 40 d the conidia have big protuberances (warts), appearing abnormal with various irregular shapes.

Sexual morph: Not known.

Substratum: Baited with horse hair from soil.

Distribution: Known only from type locality, Bohani, Madhya Pradesh, India.

Matsushimamyces venustum (Castañeda et al.) Rahul Sharma, Rohit Sharma & Crous, comb. nov.
MycoBank MB814040
Basionym: Polyschema venustum R.F. Castañeda et al., Mycotaxon 57: 452 (1996); as “venusta”.

Description and illustrations: Castañeda et al. (1996).

Substratum: On decaying leaves of unidentified tree.

Distribution: Known only from type locality Jaruco, Habana province, Cuba.

Notes: Morphologically Polyschema venustum (MUCL 39001 = CBS 140212 – ex-type cultures) resembles Matsushimamyces bohaniensis in having fusiform conidia which are quite distinct from the conidial shapes of other species of Polyschema s. str. It differs from M. bohaniensis in faster growing colonies (10–15 mm in 7 days); non-beaded conidiophores and smaller conidia (30–60 x 13–20) with more septa (5–10). The new combination Matsushimamyces venustum is therefore made for P. venustum based on its conidial morphology and DNA phylogeny.

DISCUSSION

Matsushimamyces bohaniensis somewhat resembles species of the genus Manoharachariella (based on M. lignicola). Manoharachariella is distinct, however, in that it has dictyospores that frequently have a basal appendage, lacks a beaded conidiophore, and does not have a swollen apical cell. Another morphologically similar genus is Pitthomyces. However, Pitthomyces has ellipsoidal dictyospores and phragmospores, as well as solitary, brown, rhexolytic, sympodial denticles. Matsushimamyces differs from Bahugada in that the latter has dictyospores formed on micronematous conidiophores, with a prominently pale brown basal cell. Hadrosporium forms a stroma with aggregated conidiophores, and has conidia that are uniformly brown except for their end cells. Another genus that needed to be considered was Septosporiopsis, as species in that genus also have dictyophragmospores, but the conidia in that case also have an equatorial appendage, and the conidiogenous cells proliferate percurrently. The conidia of Matsushimamyces superficially also resemble the dictyochlamydospores of Chlamydotubefila huaikangplaensis (Tubeufiaceae; Bonmee et al. 2011), but differ in that the conidia lack vertical septa, and the new genus clusters in Latoruaceae not Tubeufiaceae. Matsushimamyces bohaniensis closely resembles Polyschema venustum, but both species are morphologically and phylogenetically quite distinct from the type species of Polyschema, P. terricola (Castañeda-Ruiz et al. 2000).

In spite of various ongoing studies, sequence databases still poorly reflect the diversity that exists in this group of fungi (Zhang et al. 2012, Boonmee et al. 2014). A neighbouring phylogenetic tree constructed (Fig. 3) using the LSU sequences of Matsushimamyces revealed that the genus belonged to a monophyletic lineage with Latorua and Polyschema species in Latoruaceae (Pleosporales). Matsushimamyces is separate from other families of suborder Massarinae, and forms a distinct clade of Latoruaceae (with 99 % bootstrap support) with six other members, four species of Polyschema forming a subclade and two species of Latorua (Fig. 3). The same topology was also achieved.
Fig. 1. Mittusimyces bohaniensis (CBEC001 – holotype)  

A. Soil collection site in village Bohani.  

B. Close-up of colony front on SDA after 55 d showing irregular margin and carbon black colour.  

C. Relative size of colony on a 90 mm plate showing partly submerged and partly emerged nature.  

D. Reverse of colony after 55 d.  

E. Diagrammatic representation of the developing conidia on surface of primary culture (actual slide preparation seen in Fig 2C, X100).  

F. Diagrammatic representation of developing conidia on horse hair (CBEC001 – holotype). Bars: E–F = 18 µm.
Fig. 2. *Matsushimamyces bohaniensis* (CBEC 001–holotype) **A.** Conidia on aggregated conidiophores. **B.** Fungus growing and sporulating on white horse hair. **C.** Numerous developing conidia on SDA surface. **D.** Conidium showing light coloured apical and basal cell including swollen spore mother cell/conidiogenous cell. **E.** Detached and attached conidium from conidiophore. **F.** Developing conidia at a very early stage of development. **G.** Single conidium with attached conidiophore indicating that it is not rheolytic dehiscence, also visible in F where conidiophore is still intact. **H.** Broken spore (asexual) due to external pressure exerted on cover slip showing characteristic fragmentation reminiscent of a phragmospore. Bars: A–B = 100 µm; C = 150 µm; D–H = 20 µm.
Fig. 3. A neighbour-joining phylogenetic tree constructed using 28S rDNA sequences of members of different families of Massarineae (order Pleosporales) along with Tubeufiales and Patellariales showing phylogenetic positioning of the new genus. Hysterium angustatum was used as outgroup. The analysis involved 54 nucleotide sequences and there were 575 positions in the final datasets.
by running maximum likelihood and parsimony analyses (trees not included). In these analyses, Matsushimamyces was most closely related to Latorua (Crous et al. 2015) and Polyschema, whose species are distinguished on the basis of their conidial morphology (Castañeda-Ruiz et al. 2000). The genus Matsushimamyces differs from other genera in this complex in conidial morphology, with one apical hyaline and basal cell, hyaline to brown, beaded conidiophores, monoblastic conidiogenous cells, and typically opaque, fusoid, verruculose conidia.

ACKNOWLEDGEMENTS

We thank Narendra D Sharma for valuable discussions and constructive criticisms that helped improve the manuscript. We also thank Johannes Z Groenewald for providing sequences of Polyschema venustum (MUCL 39001), and Cony Decock (MUCL) for making this strain available for comparison. In addition, we thank Madhu Swamy (Veterinary University, Jabalpur) for allowing us to use her Nikon microscope. Financial support for this study was provided by Centre for Biodiversity Exploration and Conservation (CBEC), Jabalpur (society registration certificate no. 04/1401/16738/14) and the Department of Biotechnology (DBT), Government of India, for funding the Microbial Culture Collection (MCC), Pune (grant letter no. BT/PR10054/NDB/52/94/2007).

REFERENCES


Phylogeny of *Hirsutella* species (*Ophiocordycipitaceae*) from the USA: remedying the paucity of *Hirsutella* sequence data

D. Rabern Simmons1*, Ryan M. Kepler2, Stephen A. Rehner2, and Eleanor Groden3

1University of Florida, Institute of Food and Agricultural Sciences, School of Forest Resources and Conservation, PO Box 110410, Gainesville, FL 32611-0410, USA; corresponding author e-mail: rabernsimmons@ufl.edu
2USDA Agricultural Research Service, Systematic Mycology and Microbiology Laboratory, Beltsville, MD 20705, USA
3University of Maine, School of Biology & Ecology, 5722 Deering Hall, Orono, ME 04473-5722, USA

**Abstract:** *Hirsutella* (*Ophiocordycipitaceae: Hypocreales*) is a genus of insect, mite, and nematode pathogens with an asexual morph, which generally produce a mucilaginous cluster of one or several conidia on phialides that are basally subulate and taper to a fine neck. The generic name *Hirsutella* has been proposed for suppression in favour of *Ophiocordycips* as a consequence of the ending of dual nomenclature for different morphs of pleomorphic fungi in 2011. Though the generic name is well established, geographically dispersed, and speciose, exceptionally few sequences are available in online databases. We examined 46 isolates of 23 *Hirsutella* species from the USA, curated by the USDA-ARS Collection of Entomopathogenic Fungal Cultures (ARSEF Culture Collection), that previously had not been molecularly characterized and produced a phylogeny of these organisms; we included previously published *Hirsutella* and *Ophiocordycips* taxa. In producing the largest phylogeny of *Hirsutella* isolates so far, we provide: (1) context for discussing previously-hypothesized relationships; (2) evidence for revisions as taxonomic transitions move forward; and (3) available molecular data to be incorporated into further evolutionary studies of *Ophiocordycipitaceae*.

**Key words:** 18S rDNA biocontrol entomopathogenic fungi *Hypocreales* pleomorphic fungi *rpb1* tef1

**Article info:** Submitted: 3 July 2015; Accepted: 28 September 2015; Published: 26 October 2015.

**INTRODUCTION**

*Hirsutella* (Patouillard 1892) contains the names of over 70 species of asexually-reproducing pathogens of insects, mites, and nematodes that are distributed mainly within *Ophiocordycipitaceae* (Kepler et al. 2013, Quandt et al. 2014), though the genus is usually considered to be associated with the genus *Ophiocordycips* typified by a sexual morph (Sung et al. 2007). *Hirsutella* species are distinguished from other asexually typed genera by the basally swollen or subulate phialides that taper to an apex, where a mucilaginous packet of one or several conidia forms. Since being described, the morphological concept of the genus has expanded to include species with traits either varying or lacking from a general suite of characters (e.g. phialides without basal inflation, conidia unbounded by a mucilaginous sheath, polyphialidic conidigenous cells), thereby making morphological inferences of interspecific relationships confusing, if not impossible.

*Hirsutella* is one of the many fungal taxa typified by asexual morphs affected by the ending of dual nomenclature for pleomorphic fungi in 2011 (McNeill et al. 2012). Quandt et al. (2014) argued that *Hirsutella* should be suppressed in favour of *Ophiocordycips* because of a lack of type material for clarification and the larger task of new combinations to be made if *Hirsutella* were selected. Also, the desire to preserve the term ‘cordycips’ within the genus to reflect the cultural and economic importance of *O. sinensis* (Sung et al. 2007) was expressed. Indeed, the adoption of *Ophiocordycips* for a new species only known from a *Hirsutella* morph already has been put to practice. Simmons et al. (2015) described *Ophiocordycips myricarum* from asexually reproducing “*Hirsutella*” cultures, isolated from European fire ant (*Myrmica rubra*) populations in Maine, for which the sexual morph was not observed. The suppression of the generic name, however, is not to imply a lack of knowledge or importance of these fungi, but instead should act to propel research into the phylogeny of these organisms so that they may be incorporated into the larger evolutionary history and impending taxonomic revisions of the family *Ophiocordycipitaceae*.

Hodge (1998) produced the only monograph of *Hirsutella*, and was among the first to produce a molecular phylogeny of this genus to determine interspecific relationships that could not be deduced through morphological comparisons. Hodge examined 13 isolates and determined that *Hirsutella* was monophyletic with three *Harposporium* isolates under parsimony analyses of nuclear ITS rDNA sequences. Hodge (1998: 210) stated, however, that “. . . analyses that include more data from different areas of the genome and more taxon sampling are in order before nomenclatural changes can be suggested.” Indeed, Chaverri et al. (2005) described *Podocrella peltata* with supporting data from the 28S rDNA region from a *Harposporium* sp. (ARSEF 5410), which Hodge (1998) had found belonged within the *Hirsutella* monophyletic
group. Quandt et al. (2014) concurred with the synonymy of Harposporium and Podocrella, but placed these taxa in a clade disparate from Ophiocordycipitaceae, and thus Hirsutella s. str.; the authors also argued for the suppression of the generic name Podocrella in favour of Harposporium, citing the nomenclatural precedence of Harposporium (i.e. priority of publication), and the shared conidia morphology within the clade.

Since the study of Hodge (1998), little phylogenetic work has been conducted with Hirsutella, except for limited discussion in descriptions of new species (Seifert & Boulay 2004, Evans et al. 2010, Ciancio et al. 2013, Simmons et al. 2015). The lack of available molecular data, however, has made the task of species designation difficult and potentially misleading, in that by limiting the taxa to which an investigator compares a new species risks falsely inflating the genus with a species that has already been described, but for which molecular data have not been generated.

To populate online databases with sequence data from a well-curated and accessible fungal collection, we examined isolates named as belonging to Hirsutella in the USDA-ARS Collection of Entomopathogenic Fungal Cultures (ARSEF Culture Collection). We produced a phylogeny of these taxa based on three genetic loci, and we assembled morphological data of these species, or specific isolates from the literature. We discuss the relationships of these species hypothesized in Hodge’s monograph and other studies, provide evidence for revisions as taxonomic transitions move forward under the new rules (McNeill et al. 2012), and make available molecular data to facilitate the use of these fungi and their associated data in further evolutionary studies of Hirsutella, Ophiocordycipitaceae, and Ophiocordycipitaceae.

MATERIALS AND METHODS

DNA extraction, PCR amplification and sequencing

Cultures of ARSEF isolates were provided by Richard A. Humber (ARSEF Culture Collection, Ithaca, NY), and tissues were lyophilized at the USDA-ARS Systematic Mycology and Microbiology Laboratory (Beltsville, MD), before being sent to the University of Maine (Orono, ME) for DNA extraction. Genomic DNA was extracted from 47 ARSEF Hirsutella isolates (Table 1) with MO BIO UltraClean® Microbial DNA isolation kits (MO BIO Laboratories, Carlsbad, CA) following the manufacturer’s protocol, with further disruption of the tissue with a Mini-BeadBeater 16 (Biospec Products, Bartlesville, OK) for 3 min. Stock extracts were diluted before amplifications to decrease inhibitory contaminants from the extraction kit spin filters.

Amplifications were conducted in Eppendorf Mastercyler® gradient (Eppendorf Nother America, Westbury, NY) or Bio-Rad T100 (Bio-Rad Laboratories, Hercules, CA) thermal cyclers. Final concentrations of PCR reagent solutions in 25 µL were: (1) 1× Promega GoTaq PCR buffer (Promega, Madison, WI); (2) 1.5 mM MgCl2; (3) 0.2 mM dNTPs; (4) 0.5 µM of each primer; (5) 1 U GoTaq DNA polymerase; and (6) 0.02–0.2 ng extracted DNA. PCR conditions for tef1, rp1, 18S rDNA, and 28S rDNA were as by Johnson et al. (2009) and for nuclear ITS rDNA as by Simmons et al. (2015).

Primer sequences were obtained from previous works or designed in Geneious (Geneious version 7.1.8). Primer combinations used for amplifications were: (1) 983F and 2218R (Carbone & Kohn 1999, Rehner & Buckley 2005) for tef1; (2) RPBI-313F (5’-TYGGRATTGCTTGCAYAAY-3’) and RPBI-1149R (5’-RCGGTTCACCTAAGTGTC-3’) or RPBIcW (5’-CCNGCDATNTCRTTRTCCATRA-3’), which was derived from RPBI1cR (Castlebury et al. 2004, for rp1; (3) NS1 and NS4 (White et al. 1990) for 18S rDNA; (4) LR0R and LRS (Vilgalys & Hester 1990, Rehner & Samuels 1994) for 28S rDNA; and (5) ITS1F or ITSS and ITS4 (White et al. 1990, Gardes & Brun 1993) for nuclear ITS rDNA. Amplified products were visualized and gel extracted as by Simmons (2011) and sequenced by the University of Maine Sequencing Facility. All products were sequenced with the same primers by which they were amplified, except some tef1 sequences, which were additionally sequenced with primers 1577F and 1567R (Rehner & Buckley 2005).

Phylogenetic analyses

Chromatograms were manually inspected for ambiguous nucleotides, assembled, and aligned with tef1, rp1, and 18S rDNA sequences of Ophiocordycipitaceae (Table 1) in Geneious (Geneious version 7.1.8). PartitionFinder v1.1.3 (Lanfear et al. 2012) recommended that the super-matrix of the three loci alignments be divided into seven partitions for phylogenetic consideration: one partition for the 18S rDNA alignment and for each of the three codon positions in both protein encoding genes tef1 and rp1. The Akaike information criterion (AIC) in jModeltest 0.1.1 (Guindon & Gascuel 2003, Posada 2008) was used to select the nucleotide substitution model for each partition. Maximum likelihood (ML) phylogenetic analyses were conducted in GARLII 2.01 (Zwickl 2006) with the recommended partition parameters to determine the best tree topology (Fig. 1) and bootstrap support values from 500 search replicates, which were summarized in SumTrees (Sukumaran & Holder 2010). Bayesian posterior probabilities (BPP) were estimated with the same partition parameters in an analysis conducted in MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003), in which two runs of four chains each were executed simultaneously for 5 000 000 generations, with sampling every 500 generations. SumTrees was used to compute BPP from a summary of 7501 trees retained after a burn-in of the first 2500 trees collected.

RESULTS

The super-matrix phylogenetic analyses included tef1, rp1, and 18S rDNA sequences from 47 ARSEF isolates referred to Hirsutella, 46 of which were represented by novel sequence data from these genetic loci (Table 1). One isolate, Hirsutella gigantea (ARSEF 30), had been investigated previously for a phylogeny with tef1 sequence data (Simmons et al. 2015), but additional data for rp1 from this culture are provided in the present study. We excluded alignments of nuclear ITS and 28S rDNA regions for combined phylogenetic analyses, because they generated incongruent tree topologies, but we report these sequences for further molecular identification of ARSEF cultures. The super-matrix of tef1, rp1, and 18S rDNA was deposited in TreeBASE submission 17863.
Phylogeny of *Hirsutella* (Ophiocordycipitaceae) species

The best ML tree from GARLI analysis (Fig. 1) grouped *Hirsutella* and *Ophiocordycips* taxa monophyletic to *Purpureocillium* (*Nomuraea atypicola*) and *Drechmeria* (*Cordyceps gunnii*) outgroup taxa, which are also classified within *Ophiocordycipitaceae* (Quandt et al. 2014). We recognize six distinct groups to facilitate discussion below, though support values for these groups vary. These six groups include *H. citiformis*, *H. thompsonii*, *H. nodulosa*, *H. sinensis*, and the *Hirsutella* ant pathogen clade.

Fig. 1. Best ML tree from GARLI analysis of combined tef1, rpb1, and 18S rDNA datasets, with taxa represented by sequences obtained in this study in bold. Culture designations of *Hirsutella* species and *Ophiocordycips mymicarum* indicated as: I, ex-isotype; N, ex-neotype; P, ex-paratype; T, ex-type. Values at nodes represent ML bootstrap percentages ≥70% from a summary of 500 replicates, and branches in bold represent BPP ≥ 95%.

The best ML tree from GARLI analysis (Fig. 1) grouped *Hirsutella* and *Ophiocordycips* taxa monophyletic to *Purpureocillium* (*Nomuraea atypicola*) and *Drechmeria* (*Cordyceps gunnii*) outgroup taxa, which are also classified within *Ophiocordycipitaceae* (Quandt et al. 2014). We recognize six distinct groups to facilitate discussion below, though support values for these groups vary. These six groups include *H. citiformis*, *H. thompsonii*, *H. nodulosa*, *H. sinensis*, and the *Hirsutella* ant pathogen clade.
### Table 1. Species and culture information of Ophiocordycipitaceae used for phylogenetic analyses, and sequences of ARSEF *Hirsutella* cultures additionally obtained in this study, with typification of relevant taxa. Sequences obtained in this study in **bold**.

<table>
<thead>
<tr>
<th>Species</th>
<th>Isolate no.</th>
<th>Host</th>
<th>GenBank accession no.</th>
<th>18S rDNA</th>
<th>ITS rDNA</th>
<th>28S rDNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hirsutella cf. haptospora</td>
<td>ARSEF 2228</td>
<td>Diptera: Itonidae</td>
<td>KM652001</td>
<td>KM652075</td>
<td>KM652166</td>
<td>KM652118</td>
</tr>
<tr>
<td>Hirsutella citriformis</td>
<td>ARSEF 490</td>
<td>Hemiptera: Delphacidae</td>
<td>KM651987</td>
<td>KM652151</td>
<td>KM652103</td>
<td>KM652104</td>
</tr>
<tr>
<td>Hirsutella citriformis</td>
<td>ARSEF 591</td>
<td>Hemiptera: Delphacidae</td>
<td>KM651988</td>
<td>KM652152</td>
<td>KM652104</td>
<td>KM652104</td>
</tr>
<tr>
<td>Hirsutella citriformis</td>
<td>ARSEF 1035</td>
<td>Hemiptera: Cixiidae</td>
<td>KM651989</td>
<td>KM652064</td>
<td>KM652153</td>
<td>KM652105</td>
</tr>
<tr>
<td>Hirsutella citriformis</td>
<td>ARSEF 1446</td>
<td>Hemiptera: Cixiidae</td>
<td>KM651990</td>
<td>KM652065</td>
<td>KM652154</td>
<td>KM652106</td>
</tr>
<tr>
<td>Hirsutella citriformis</td>
<td>ARSEF 2598</td>
<td>Hemiptera: Psyllidae</td>
<td>KM651991</td>
<td>KM652155</td>
<td>KM652107</td>
<td>KM652107</td>
</tr>
<tr>
<td>Hirsutella citriformis</td>
<td>ARSEF 9180</td>
<td>Hemiptera: Psyllidae</td>
<td>KM651992</td>
<td>KM652156</td>
<td>KM652108</td>
<td>KM652108</td>
</tr>
<tr>
<td>Hirsutella cryptosclerotium</td>
<td>ARSEF 4517</td>
<td>Hemiptera: Pseudococcidae</td>
<td>KM651993</td>
<td>KM652066</td>
<td>KM652157</td>
<td>KM652109</td>
</tr>
<tr>
<td>Hirsutella fusiformis</td>
<td>ARSEF 5474</td>
<td>Coleoptera: Curculionidae</td>
<td>KM651994</td>
<td>KM652067</td>
<td>KM652110</td>
<td>KM652110</td>
</tr>
<tr>
<td>Hirsutella gigantea</td>
<td>ARSEF 30</td>
<td>Hymenoptera: Pamphiliidae</td>
<td>JX566980</td>
<td>KM652034</td>
<td>KM652034</td>
<td>KM652034</td>
</tr>
<tr>
<td>Hirsutella guyana</td>
<td>ARSEF 878</td>
<td>Hemiptera: Cicadellidae</td>
<td>KM651995</td>
<td>KM652068</td>
<td>KM652158</td>
<td>KM652111</td>
</tr>
<tr>
<td>Hirsutella haptospora</td>
<td>ARSEF 2226</td>
<td>Acari: Uropodina</td>
<td>KM651996</td>
<td>KM652069</td>
<td>KM652160</td>
<td>KM652112</td>
</tr>
<tr>
<td>Hirsutella illustris</td>
<td>ARSEF 5539</td>
<td>Hemiptera: Aphididae</td>
<td>KM651997</td>
<td>KM652070</td>
<td>KM652161</td>
<td>KM652113</td>
</tr>
<tr>
<td>Hirsutella kirchneri</td>
<td>ARSEF 5551</td>
<td>Acari: Eriophyidae</td>
<td>KM651998</td>
<td>KM652071</td>
<td>KM652162</td>
<td>KM652114</td>
</tr>
<tr>
<td>Hirsutella liboensis</td>
<td>ARSEF 9603</td>
<td>Lepidoptera: Cossidae</td>
<td>KM651999</td>
<td>KM652072</td>
<td>KM652163</td>
<td>KM652115</td>
</tr>
<tr>
<td>Hirsutella necatrix</td>
<td>ARSEF 5549</td>
<td>Acari</td>
<td>KM652000</td>
<td>KM652073</td>
<td>KM652164</td>
<td>KM652116</td>
</tr>
<tr>
<td>Hirsutella nodulosa</td>
<td>ARSEF 5473</td>
<td>Lepidoptera: Pyralidae</td>
<td>KM652001</td>
<td>KM652074</td>
<td>KM652165</td>
<td>KM652117</td>
</tr>
<tr>
<td>Hirsutella radiata</td>
<td>ARSEF 1369</td>
<td>Diptera</td>
<td>KM652002</td>
<td>KM652076</td>
<td>KM652119</td>
<td>KM652119</td>
</tr>
<tr>
<td>Hirsutella repens nom. inval.</td>
<td>ARSEF 2348</td>
<td>Hemiptera: Delphacidae</td>
<td>KM652003</td>
<td>KM652177</td>
<td>KM652167</td>
<td>KM652120</td>
</tr>
<tr>
<td>Hirsutella rhossiliensis</td>
<td>ARSEF 2931</td>
<td>Tylenchida: Heteroderidae</td>
<td>KM652004</td>
<td>KM652168</td>
<td>KM652121</td>
<td>KM652121</td>
</tr>
<tr>
<td>Hirsutella rhossiliensis</td>
<td>ARSEF 3207</td>
<td>Tylenchida: Criconematidae</td>
<td>KM652005</td>
<td>KM652169</td>
<td>KM652122</td>
<td>KM652122</td>
</tr>
<tr>
<td>Hirsutella rhossiliensis</td>
<td>ARSEF 3477</td>
<td>Tylenchida: Criconematidae</td>
<td>KM652006</td>
<td>KM652170</td>
<td>KM652123</td>
<td>KM652123</td>
</tr>
<tr>
<td>Hirsutella “salmaensis”</td>
<td>ARSEF 996</td>
<td>Lepidoptera: Pyralidae</td>
<td>KM652007</td>
<td>KM652171</td>
<td>KM652124</td>
<td>KM652124</td>
</tr>
<tr>
<td>Hirsutella sinensis</td>
<td>ARSEF 6282</td>
<td>Lepidoptera: Eriuchidae</td>
<td>KM652008</td>
<td>KM652172</td>
<td>KM652125</td>
<td>KM652125</td>
</tr>
<tr>
<td>Hirsutella sp. ARSEF 7578</td>
<td>ARSEF 7578</td>
<td>Hymenoptera: Formicidae</td>
<td>JX566981</td>
<td>KM652173</td>
<td>KM652126</td>
<td>KM652126</td>
</tr>
<tr>
<td>Hirsutella sp. ARSEF 8378</td>
<td>ARSEF 8378</td>
<td>Hemiptera: Cixiidae</td>
<td>KM652010</td>
<td>KM652174</td>
<td>KM652127</td>
<td>KM652127</td>
</tr>
<tr>
<td>Hirsutella sp. NHJ</td>
<td>NHJ 12525</td>
<td>Hemiptera</td>
<td>EF469063</td>
<td>EF469092</td>
<td>EF469125</td>
<td>EF469125</td>
</tr>
<tr>
<td>Hirsutella sp. OSC</td>
<td>OSC 128575</td>
<td>Hemiptera</td>
<td>EF469064</td>
<td>EF469093</td>
<td>EF469126</td>
<td>EF469126</td>
</tr>
<tr>
<td>Hirsutella stilbelliformis var. myrmicarum</td>
<td>IMI 396397</td>
<td>Hymenoptera: Formicidae</td>
<td>GQ866964</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hirsutella strigosa</td>
<td>ARSEF 2044</td>
<td>Hemiptera: Delphacidae</td>
<td>KM652011</td>
<td></td>
<td>KM652128</td>
<td>KM652128</td>
</tr>
<tr>
<td>Hirsutella strigosa</td>
<td>ARSEF 2197</td>
<td>Hemiptera: Cicadellidae</td>
<td>KM652012</td>
<td></td>
<td>KM652129</td>
<td>KM652129</td>
</tr>
</tbody>
</table>
Table 1. (Continued).

<table>
<thead>
<tr>
<th>Species</th>
<th>Isolate no.</th>
<th>Host</th>
<th>GenBank accession no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hirsutella subramanianii var. myrmicarum</td>
<td>IMI 396400</td>
<td>Hymenoptera: Formicidae</td>
<td>ITs rDNA1 28s rDNA1</td>
</tr>
<tr>
<td>Hirsutella subulata</td>
<td>ARSEF 2227</td>
<td>Lepidoptera: Microlepidoptera</td>
<td>EU797598</td>
</tr>
<tr>
<td>Hirsutella thompsonii</td>
<td>ARSEF 241</td>
<td>Acari: Eriophyidae</td>
<td>KM652015</td>
</tr>
<tr>
<td>Hirsutella thompsonii</td>
<td>ARSEF 253</td>
<td>Acari: Eriophyidae</td>
<td>KM652016</td>
</tr>
<tr>
<td>Hirsutella thompsonii</td>
<td>ARSEF 25</td>
<td>Acari: Eriophyidae</td>
<td>KM652017</td>
</tr>
<tr>
<td>Hirsutella thompsonii</td>
<td>ARSEF 256</td>
<td>Acari: Eriophyidae</td>
<td>KM652018</td>
</tr>
<tr>
<td>Hirsutella thompsonii</td>
<td>ARSEF 257</td>
<td>Acari: Eriophyidae</td>
<td>KM652019</td>
</tr>
<tr>
<td>Hirsutella thompsonii</td>
<td>ARSEF 258</td>
<td>Acari: Eriophyidae</td>
<td>KM652020</td>
</tr>
<tr>
<td>Hirsutella thompsonii</td>
<td>ARSEF 259</td>
<td>Acari: Eriophyidae</td>
<td>KM652021</td>
</tr>
<tr>
<td>Hirsutella thompsonii</td>
<td>ARSEF 414</td>
<td>Acari: Eriophyidae</td>
<td>KM652022</td>
</tr>
<tr>
<td>Hirsutella thompsonii</td>
<td>ARSEF 2012</td>
<td>Acari: Eriophyidae</td>
<td>KM652023</td>
</tr>
<tr>
<td>Hirsutella thompsonii</td>
<td>ARSEF 2464</td>
<td>Acari: Eriophyidae</td>
<td>KM652024</td>
</tr>
<tr>
<td>Hirsutella thompsonii</td>
<td>ARSEF 2800</td>
<td>Acari</td>
<td>KM652025</td>
</tr>
<tr>
<td>Hirsutella thompsonii</td>
<td>ARSEF 3323</td>
<td>Acari: Tenuipalpidae</td>
<td>KM652026</td>
</tr>
<tr>
<td>Hirsutella thompsonii</td>
<td>ARSEF 3482</td>
<td>Acari: Eriophyidae</td>
<td>KM652027</td>
</tr>
<tr>
<td>Hirsutella thompsonii</td>
<td>ARSEF 9457</td>
<td>Acari: Tetranychidae</td>
<td>KM652028</td>
</tr>
<tr>
<td>Hirsutella thompsonii “var. synnematosa”</td>
<td>ARSEF 1947</td>
<td>Acari: Tarsonemidae</td>
<td>KM652029</td>
</tr>
<tr>
<td>Hirsutella thompsonii “var. synnematosa”</td>
<td>ARSEF 2459</td>
<td>Acari: Eriophyidae</td>
<td>KM652030</td>
</tr>
<tr>
<td>Hirsutella thompsonii “var. synnematosa”</td>
<td>ARSEF 5412</td>
<td>Acari: Tetranychidae</td>
<td>KM652031</td>
</tr>
<tr>
<td>Hirsutella thompsonii var. thompsonii</td>
<td>ARSEF 137</td>
<td>Acari: Eriophyidae</td>
<td>KM652032</td>
</tr>
<tr>
<td>Hirsutella thompsonii var. vinacea</td>
<td>ARSEF 254</td>
<td>Acari: Eriophyidae</td>
<td>KM652033</td>
</tr>
<tr>
<td>Hirsutella versicolor</td>
<td>ARSEF 1037</td>
<td>Hemiptera: Membracidae</td>
<td>KM652034</td>
</tr>
<tr>
<td>Ophiocordyceps acicularis</td>
<td>OSC 110987</td>
<td>Coleoptera</td>
<td>KM652035</td>
</tr>
<tr>
<td>Ophiocordyceps cf. acicularis</td>
<td>OSC 128580</td>
<td>Coleoptera</td>
<td>KM652036</td>
</tr>
<tr>
<td>Ophiocordyceps agriotidis</td>
<td>ARSEF 5692</td>
<td>Arthropoda</td>
<td>KM652037</td>
</tr>
<tr>
<td>Ophiocordyceps aphodii</td>
<td>ARSEF 5498</td>
<td>Coleoptera: Scarabaeidae</td>
<td>KM652038</td>
</tr>
<tr>
<td>Ophiocordyceps brunneipunctata</td>
<td>OSC 128576</td>
<td>Coleoptera: Elateridae</td>
<td>KM652039</td>
</tr>
<tr>
<td>Ophiocordyceps davata</td>
<td>NBR 106961</td>
<td>Coleoptera: Elateridae</td>
<td>KM652040</td>
</tr>
<tr>
<td>Ophiocordyceps communis</td>
<td>NHU 12581</td>
<td>Isoptera</td>
<td>KM652041</td>
</tr>
<tr>
<td>Ophiocordyceps communis</td>
<td>NHU 12582</td>
<td>Isoptera</td>
<td>KM652042</td>
</tr>
<tr>
<td>Ophiocordyceps elongata</td>
<td>OSC 110989</td>
<td>Lepidoptera</td>
<td>KM652043</td>
</tr>
<tr>
<td>Ophiocordyceps entomorrhiza</td>
<td>KEW 53484</td>
<td>Coleoptera</td>
<td>KM652044</td>
</tr>
<tr>
<td>Ophiocordyceps gracilis</td>
<td>EFCC 8572</td>
<td>Lepidoptera</td>
<td>KM652045</td>
</tr>
<tr>
<td>Species</td>
<td>Isolate no.</td>
<td>Host</td>
<td>tef1</td>
</tr>
<tr>
<td>---------------------------------</td>
<td>-------------</td>
<td>-----------------------</td>
<td>----------</td>
</tr>
<tr>
<td>Ophiocordyceps heteropoda</td>
<td>EFCC 10125</td>
<td>Hemiptera</td>
<td>EF468752</td>
</tr>
<tr>
<td>Ophiocordyceps irangiensis</td>
<td>OSC 128578</td>
<td>Hymenoptera: Formicidae</td>
<td>DQ522345</td>
</tr>
<tr>
<td>Ophiocordyceps irangiensis</td>
<td>OSC 128579</td>
<td>Hymenoptera: Formicidae</td>
<td>EF469060</td>
</tr>
<tr>
<td>Ophiocordyceps kniphofioides</td>
<td></td>
<td>Hymenoptera: Formicidae</td>
<td>KC610739</td>
</tr>
<tr>
<td>Ophiocordyceps konnoana</td>
<td>EFCC 7295</td>
<td>Coleoptera</td>
<td></td>
</tr>
<tr>
<td>Ophiocordyceps konnoana</td>
<td>EFCC 7315</td>
<td>Coleoptera</td>
<td>EF468753</td>
</tr>
<tr>
<td>Ophiocordyceps longissima</td>
<td>EFCC 6814</td>
<td>Hemiptera: Cicadidae</td>
<td>EF468757</td>
</tr>
<tr>
<td>Ophiocordyceps melolonthae</td>
<td>OSC 110993</td>
<td>Coleoptera: Scarabaeidae</td>
<td>DQ522331</td>
</tr>
<tr>
<td>Ophiocordyceps myrmicarum</td>
<td>ARSEF 11864</td>
<td>Hymenoptera: Formicidae</td>
<td>JX566973</td>
</tr>
<tr>
<td>Ophiocordyceps nigrella</td>
<td>EFCC 9247</td>
<td>Lepidoptera</td>
<td>EF468758</td>
</tr>
<tr>
<td>Ophiocordyceps nutans</td>
<td>OSC 110994</td>
<td>Hemiptera: Pentatomidae</td>
<td>DQ522333</td>
</tr>
<tr>
<td>Ophiocordyceps puninosa</td>
<td>NHJ 12994</td>
<td>Hemiptera</td>
<td>EU369024</td>
</tr>
<tr>
<td>Ophiocordyceps pulvinata</td>
<td>TNS F30044</td>
<td>Hymenoptera: Formicidae</td>
<td>GU904209</td>
</tr>
<tr>
<td>Ophiocordyceps ravenelli</td>
<td>OSC 110995</td>
<td>Coleoptera</td>
<td>DQ522334</td>
</tr>
<tr>
<td>Ophiocordyceps rhizoidea</td>
<td>NHJ 12522</td>
<td>Isoptera</td>
<td>EF468764</td>
</tr>
<tr>
<td>Ophiocordyceps sinensis</td>
<td>EFCC 7287</td>
<td>Lepidoptera</td>
<td>EF468767</td>
</tr>
<tr>
<td>Ophiocordyceps sobolifera</td>
<td>KEW 78842</td>
<td>Hemiptera: Cicadidae</td>
<td></td>
</tr>
<tr>
<td>Ophiocordyceps sp. OSC</td>
<td>OSC 110997</td>
<td>Hymenoptera: Formicidae</td>
<td>EF468774</td>
</tr>
<tr>
<td>Ophiocordyceps stylophora</td>
<td>OSC 111000</td>
<td>Coleoptera: Elateridae</td>
<td>DQ522337</td>
</tr>
<tr>
<td>Ophiocordyceps unilateralis</td>
<td>OSC 128574</td>
<td>Hymenoptera: Formicidae</td>
<td>DQ522339</td>
</tr>
<tr>
<td>Ophiocordyceps variabilis</td>
<td>ARSEF 5365</td>
<td>Diptera: Xylophagidae</td>
<td>DQ522340</td>
</tr>
</tbody>
</table>

Outgroup:

<table>
<thead>
<tr>
<th>Species</th>
<th>Isolate no.</th>
<th>Host</th>
<th>GenBank accession no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cordyceps gunnii</td>
<td>OSC 76404</td>
<td>Lepidoptera</td>
<td>AY489616</td>
</tr>
<tr>
<td>Nomuraea atypicola</td>
<td>CBS 744.73</td>
<td>Arachnida</td>
<td>EF468786</td>
</tr>
</tbody>
</table>

1Indicates ex-isotype culture.
2Indicates ex-neotype culture.
3Indicated ex-paratype culture.
4Indicates ex-holotype culture.
5Sequences were not used for phylogenetic analyses.
6Ophiocordyceps myrmicarum is noted, because it is only known from its asexual life stage.
DISCUSSION

Morphology
Morphological characters of mitosporic reproductive structures (presence of synnemata; phialide appearance; conidia shape and condition) traditionally have been used as a basis for species description and hypothesized relatedness within *Hirsutella*. However, our data support Hodge’s (1998) contention that the morphological features of *Hirsutella* are insufficient for determining interspecific relationships. Her subsequent phylogeny of the genus from nuclear ITS rDNA sequences (~600 bp each), including those obtained from eleven ARSEF isolates, seven of which are in this study, supported the monophyly of *Hirsutella*, so long as it includes three isolates of *Harpseudium*, two of which would produce synanamorphs in culture studies. Based on our molecular phylogeny (Fig. 1), few trends in host taxa or morphological characters appear phylogenetically informative (Table 2). Some host based groupings can be inferred: the majority of the isolates from ant hosts cluster in one lineage, and species in the *H. nodulosa* clade primarily are associated with Lepidoptera hosts. However, other isolates originating from Lepidoptera are distributed within other clades, and isolates from Hemiptera hosts are distributed throughout all clades except one (*H. nodulosa*). Additional sampling of species from additional geographic regions or rare *Hirsutella* taxa, however, may expand these clades and make more relevant traits apparent.

*Hirsutella thompsonii* clade
*Hirsutella thompsonii* is the most widely studied of the *Hirsutella* species, being an important biocontrol agent for mite pests in agriculture. Isolates considered in this study, all originating from *Acarí* hosts, form a clade including the morphologically similar species *H. necatrix* (Minter et al. 1983), another mite pathogen, and *H. cryptosclerotium* (Fernández-García et al. 1990), which originated from a different host taxon in *Hemiptera*. The monophyletic, core clade of *H. thompsonii* is composed of *Hirsutella thompsonii* var. *thompsonii* (ARSEF 137 – ex-neotype culture), most other *H. thompsonii* cultures, and *H. thompsonii* var. *vinacea* (ARSEF 254 – ex-holotype culture). Isolates ARSEF 1947 and 2459, which are included in the core clade, may form synnemata in culture (Humber et al. 2014), but they are not monophyletic with *H. thompsonii* var. *synnematoso* (ARSEF 5412), which is in a polyclony with *H. necatrix* and the core clade. Bayesian posterior probability support values deteriorate in the core clade because of differences in the placement of ARSEF 9457 by our analyses; Bayesian phylogenetic reconstruction removed ARSEF 9457 from the core clade and placed it in the polyclony alongside *H. necatrix* (ARSEF 5549 – ex-isotype culture) and *H. thompsonii* var. *synnematoso*. Additionally, ARSEF 258 and 2800, also considered *H. thompsonii*, lie outside of the core clade, grouping with *H. cryptosclerotium* (ARSEF 4517 – ex-holotype culture). In our phylogeny isolates of *Ophiocordyceps communis* are sister to the taxa described above, most likely because of long-branch attraction instead of true phylogenetic relatedness; regardless, Sung et al. (2007) describe the asexual morph of *O. communis* to be transitional between *Hymenostilbe* and *Hirsutella*.

Hodge (1998: 154) considered *H. thompsonii* to be “...the core of a group of morphologically related species including *H. necatrix*, *H. gregis*, *H. cryptosclerotium*, *H. tydeicola*, and *H. sphaerospora*, pathogens of mites or mealybugs.” She examined the relationship of two *H. thompsonii* isolates, which are within our core clade, and *H. cryptosclerotium*, and she found *H. cryptosclerotium* to be sister to *H. thompsonii* with weak bootstrap support (67 %) in a nuclear ITS rDNA phylogeny. *Hirsutella cryptosclerotium* is distinguished by the production of pigmented sclerotia, but similar chlamydospore structures have been noted in other species, including *H. thompsonii* (Fernández-García et al. 1990). Hodge (1998) believed *Hirsutella necatrix* was more difficult to distinguish from *H. thompsonii*, except that the phialides were often formed in a verticillate arrangement and conidia were occasionally more ellipsoid.

Hodge (1998) posited that *H. kirchneri* and *H. gregis* (Minter et al. 1983), the latter of which we did not examine in this study, are variants from the same host and collection location. Similarly, Balazy et al. (2008) produced phylogenies of nuclear ITS rDNA sequences that closely allied *H. gregis* and *H. kirchneri* isolates. A BLAST query of the nuclear ITS rDNA region generated from *H. kirchneri* (ARSEF 5551, GenBank KM652161) is 100 % and 99 % similar to the *H. gregis* and *H. kirchneri* isolates, respectively, as reported by Balazy et al. (2008). The imperfect match of the two *H. kirchneri* sequences is explained by two ambiguous sites in the sequence determined by Balazy et al. (2008) that are apparent in our ARSEF 5551 sequence. *Hirsutella kirchneri* and *H. thompsonii* are vastly disparate in our phylogeny, so the relationship of *H. gregis* to *H. thompsonii* within a morphologically defined ‘core clade’ as suggested by Hodge (1998) is not supported by our phylogeny.

*Hirsutella tydeicola* (Samson & McCoy 1982) is known only from the type collections, but Hodge (1998) considered *H. kirchneri* and *H. gregis* similar to this isolate in appearance aside from slight variations in conidia shape, suggesting that *H. tydeicola* may be more closely related to *H. kirchneri* in our phylogeny and therefore disparate from *H. thompsonii*. These morphological characters, however, may also prove misleading, and it could be that *H. tydeicola* resides in an unexpected location within the phylogeny; only a molecular characterization of this taxon will determine its phylogenetic position. Similarly, *Hirsutella sphaerospora* (Evans & Samson 1982), which we did not examine, was theorized to be closely related to *H. cryptosclerotium* (Fernández-García et al. 1990, Hodge 1998), which also exhibits polyphialidic conidigenous cells. The morphological association of *H. sphaerospora* to both *H. cryptosclerotium* and *H. thompsonii* may provide additional support for this species’ placement within a ‘core clade’, but its true position is questionable.

*Hirsutella guyana* clade
The *Hirsutella guyana* clade is represented by three *Hirsutella* isolates characterized in our study. The ex-type culture of *H. haptopsora* (Balazy & Wiśniewski 1986), which is considered in our phylogeny (ARSEF 2226), was isolated from mites in *Formica* nests from Poland. Hodge (1998) stated that this species was closely allied to *H. rhossiliensis* (Minter & Brady 1980), but our phylogeny supports the placement of
Table 2. Morphology of *Hirsutella* species included in Fig. 1. Characters from Hodge (1998) unless specified.

<table>
<thead>
<tr>
<th>Species</th>
<th>Conidioma form</th>
<th>Phialides</th>
<th>Conidia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Clade</td>
<td>Mononematous</td>
<td>Synnematous</td>
</tr>
<tr>
<td><em>Hirsutella citriformis</em></td>
<td><em>H. citriformis</em></td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td><em>H. cryptosclerotium</em></td>
<td><em>H. thompsonii</em></td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td><em>H. fusiformis</em></td>
<td><em>H. citriformis</em></td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td><em>H. gigantea</em></td>
<td><em>H. citriformis</em></td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td><em>H. guyana</em></td>
<td><em>H. guyana</em></td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td><em>H. haptoospora</em></td>
<td><em>H. guyana</em></td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td><em>H. illustris</em></td>
<td><em>H. sinensis</em></td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td><em>H. kirchneri</em></td>
<td><em>H. sinensis</em></td>
<td>Yes</td>
<td>In culture</td>
</tr>
<tr>
<td><em>H. lecaniiicola</em></td>
<td><em>H. sinensis</em></td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td><em>H. liboensis</em></td>
<td><em>H. nodulosa</em></td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td><em>H. necatrix</em></td>
<td><em>H. thompsonii</em></td>
<td>Yes</td>
<td>In culture</td>
</tr>
<tr>
<td><em>H. nodulosa</em></td>
<td><em>H. nodulosa</em></td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td><em>H. radiata</em></td>
<td><em>H. citriformis</em></td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td><em>H. rossilliensis</em></td>
<td><em>H. sinensis</em></td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td><em>H. satsumaensis</em></td>
<td><em>H. nodulosa</em></td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td><em>H. sinensis</em></td>
<td><em>H. sinensis</em></td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td><em>H. stilbelliformis</em></td>
<td><em>H. nodulosa</em></td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td><em>H. strigosa</em></td>
<td><em>H. sinensis</em></td>
<td>Yes</td>
<td>Rarely</td>
</tr>
<tr>
<td><em>H. subramaniiani</em></td>
<td><em>H. nodulosa</em></td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td><em>H. subulata</em></td>
<td><em>H. nodulosa</em></td>
<td>In culture</td>
<td>Yes</td>
</tr>
<tr>
<td><em>H. thompsonii</em></td>
<td><em>H. thompsonii</em></td>
<td>Yes</td>
<td>Some varieties</td>
</tr>
</tbody>
</table>
Phylogeny of *Hirsutella* (Ophiocordycipitaceae) species

Table 2. (Continued).

<table>
<thead>
<tr>
<th>Conidia form</th>
<th>Phialides</th>
<th>Condidioma form</th>
<th>Clade</th>
<th>Species</th>
<th>Host</th>
<th>Mucous sheath</th>
<th>Sclerotia</th>
<th>Host Morphology</th>
<th>Verruculose</th>
<th>Host</th>
<th>Leafhoppers</th>
<th>Myrmica</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mononematous</td>
<td>Phialides</td>
<td>Mononematous</td>
<td>H. versicolor</td>
<td>H. guyana</td>
<td>No</td>
<td>Ovoid</td>
<td>No</td>
<td>No</td>
<td>Ovoid</td>
<td>Yes</td>
<td>No</td>
<td>Leafhoppers</td>
</tr>
<tr>
<td>Synnematous</td>
<td></td>
<td>Synnematous</td>
<td></td>
<td></td>
<td>Leafhoppers</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Synnematous</td>
<td></td>
<td></td>
<td>Myrmica</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Four *Hirsutella* isolates clustered in the *Hirsutella nodulosa* clade. *Hirsutella nodulosa* (Petch 1926) and *H. satumaensis* (Aoki et al. 1957) are monophyletic with little variation in our phylogeny, which is similar to the findings of Hodge (1998) from a nuclear ITS rDNA dataset. *Hirsutella nodulosa* was described from a lepidopteran in Sri Lanka, and ARSEF 5473 is from a lepidopteran in Michigan. Minter & Brady (1980) examined the type material and a culture from spider mites, and they suggested that the defining nodules described by Petch (1926) were conidia adhering to their mucilaginous sheath to hyphae, a condition that Hodge (1998) found to be a common occurrence in other *Hirsutella* species. Minter & Brady (1980) also documented helical twisting at the apex of phialides and warts on phialides and hyphae, features not originally described by Petch (1926). Other species also may exhibit warted phialides (e.g., *H. rhossiliensis*, Minter & Brady 1980) though not to the degree seen in *H. nodulosa*. *Hirsutella brownorum* (Minter & Brady 1980) and *H. liboensis* (Zou et al., 2010), the latter of which groups with *H. nodulosa* in our phylogeny, also possess phialides with apical helical twists. The determination of the phylogenetic position of *H. brownorum*, and additional taxa with apical helical twists (Zou et al., 2010), however, would be necessary before concluding that this trait evolved only once.

*Hirsutella satumaensis* was described from case-making clothes moths and silkworms, and ARSEF 996 is derived from one of Aoki’s cultures of this species (Aoki et al. 1957). Hodge (1998), however, saw no evidence for this isolate to be defined as *H. satumaensis*, reported no other isolates or type material, and believed ARSEF 996 is derived from one of Aoki’s cultures of this species (Aoki et al. 1957). Hodge (1998) noted the morphological similarity of *H. satumaensis* to *Hirsutella* nodulosa, but other species also may exhibit warted phialides (e.g., *H. rhossiliensis*, Minter & Brady 1980) though not to the degree seen in *H. nodulosa*. *Hirsutella brownorum* (Minter & Brady 1980) and *H. liboensis* (Zou et al., 2010), the latter of which groups with *H. nodulosa* in our phylogeny, also possess phialides with apical helical twists. The determination of the phylogenetic position of *H. brownorum*, and additional taxa with apical helical twists (Zou et al., 2010), however, would be necessary before concluding that this trait evolved only once.

*Hirsutella sinensis* clade

The *Hirsutella sinensis* clade includes isolates originating from a variety of taxa including nematodes, mites, and both hemi- (*Hemiptera*) and holometabolist
(Coleoptera, Lepidoptera) insect hosts. Our data supports the evidence of Liu et al. (2001) that H. sinensis (Liu et al. 1989) is the anamorph of Cordyceps sinensis (Sung et al. 2007). Liu et al. (2001, as C. sinensis) used nuclear ITS rDNA sequences and morphological examination of ascospore microcyclic conidia of C. sinensis (Saccardo 1878) to argue that H. sinensis was the genuine asexual morph of C. sinensis, for which several names based on asexual morphs had been suggested.

Hirsutella strigosa (Petch 1939) forms a monophyletic group with H. repens (nom. inval.; Humber et al. 2014), for which no morphological data is published, and H. kirchneri, with which it shares some morphological features. Petch (1939) found H. strigosa with H. versicolor, and our phylogeny clearly differentiates these taxa. Hodge (1998) notes a distinguishing feature of this species is its exceptionally long and verruculose phialides, similar to H. nodulosa, but not apically twisted as in that species. Furthermore, Hodge noted that H. illustris and H. rhossiliensis, also within our H. sinensis clade, similarly share large phialides. H. kirchneri and H. lecaniicola (Petch 1933), however, do not share this characteristic.

Hirsutella citriformis clade
Hirsutella citriformis (Speare 1920) is a widely distributed species infecting the Asian citrus psyllid Diaphorina citri (e.g. Subandiyah et al. 2000, Hall et al. 2012, Pérez-González et al. 2015) and is represented in our phylogeny by isolates collected in the Republic of Indonesia, Republic of the Philippines, and the USA. This species is monophyletic, indicating reliable morphological identification by different investigators, but Pérez-González et al. (2015) argued that morphologies of phialides and conidia of isolates vary from those of the original description and warn that new isolates could show increasing variability in these structures.

Other isolates in this clade originate from a diversity of insect taxa. Hirsutella fusiformis (Speare 1920) is poorly known from the type description and illustrations; Hodge (1998) examined the putative isolate of H. fusiformis we investigated in this study and found that it would not sporulate on tested media. In our phylogeny, H. fusiformis was associated with H. radiata (Petch 1935). Hodge (1998) postulated that H. guignardii (Samson et al. 1984) could be a synonym of H. radiata, based on the similarity of synnemata branching, though more delicate in the latter species. It is possible, based on the short branch lengths in our phylogeny comparable to other taxonomically identical taxa, that this putative H. fusiformis is instead one of these species but has degenerated in storage. Additionally in this clade, Simmons et al. (2015) previously confirmed the relationship of Hirsutella gigantea (Petch 1937) and Ophiocordyceps elongata (Sung et al. 2007) based on tef1 data, and our wider phylogeny including an rpb1 sequence obtained from this isolate provides further support for the association.

Hirsutella ant pathogen clade
Our phylogeny places all Hirsutella isolates from Formicidae in a monophyletic clade. This clade, however, excludes isolates of Ophiocordyceps irangiensis, which, while also derived from Formicidae hosts, produce a typical Hymenostilbe asexual morph (Sung et al. 2007). Simmons et al. (2015) noted similar phylogenetic relationships of these taxa in their ML analysis of tef1, rpb1, and 18S rDNA, but a Bayesian phylogeny of those same loci grouped O. irangiensis with isolates of O. communis, which produces a transitionary Hirsutella/Hymenostilbe asexual morph (Sung et al. 2007).

An ongoing investigation
Hodge (1998) produced the only monographic work on Hirsutella that incorporated genetic sequences for an initial phylogenetic understanding of the genus. Our examination of ARSEF Hirsutella isolates from the USA sought to increase the sampling of these fungi in molecular databases, but a monographic revision of this genus would be incomplete if it did not consider the entirety of Ophiocordyceps, with which Hirsutella species are being synonymized. Indeed, more rigorous morphological examinations of the Hirsutella isolates in our molecular phylogeny are necessary before taxonomic revisions (combinations, typifications, etc.) can take place, but our molecular phylogeny provides an evolutionary context for morphological features and physiology and a measure of species’ similarity that will facilitate the next steps in this process. Additionally, phylogenetic investigations into international or rare Hirsutella species and isolates, which we were unable to examine in this study, will likely lead to a deeper understanding of the evolution, taxonomy, and physiology of these fungi.

ACKNOWLEDGEMENTS
We thank Richard A Humber for access to the ARSEF Culture Collection and his expert advice; Patty Singer and Dave Cox of the DNA Sequencing Facility at the University of Maine for their services. We also thank Joyce E Longcore for participation in taxonomic and phylogenetic discussions, and Jerry R Longcore for critical consultation in preparing the manuscript. This project was supported by the Agricultural and Food Research Initiative Competitive Grant No. 2013-67012-21115 from the USDA National Institute of Food and Agriculture, and the Maine Agriculture and Forestry Experiment Station at the University of Maine. This is MAFES Publication No. 3438.

REFERENCES
Phylogeny of Hirsutella (Ophiocordycipitaceae) species


Petch T (1926) Entomogenous fungi. Additions and corrections, II. Transactions of the British Mycological Society 11: 258–266.


New 1F1N Species Combinations in Ophiocordycipitaceae (Hypocreales)

Joseph W. Spatafora1, C. Alisha Quandt2, Ryan M. Kepler3, Gi-Ho Sung4, Bhushan Shrestha5, Nigel L. Hywel-Jones6, and J. Jennifer Luangsarad2

1Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331, USA; corresponding author e-mail: joseph.spatafora@oregonstate.edu
2Department of Ecology and Evolutionary Biology, The University of Michigan, Ann Arbor, MI 48109, USA
3USDA-ARS, Systematic Mycology and Microbiology Laboratory, Beltsville, MD 20705, USA
4Institutes for Bio-Medical Convergence and Integrative Medicine, International St Mary’s Hospital and College of Medicine, Catholic Kwandong University, Inchon 404-843, Korea
5Institute of Life Science and Biotechnology, Sungkyunkwan University, Suwon 440-746, Korea
6Milton Biotech Co. Ltd, Innovation Cluster 2, Tower D,141 Moo. 9, Khlong Nueng, Khlong Luang, Phathum Thani 12120, Thailand

Abstract: Based on the taxonomic and nomenclatural recommendations of Quandt et al. (2014) new species combinations are made for Ophiocordycipitaceae. These new combinations are compliant with recent changes in the International Code of Nomenclature for algae, fungi, and plants (ICN) and the abolition of the dual system of nomenclature for fungi. These changes include 10 new combinations into Drechmeria, four new combinations into Harposporium, 23 new combinations and 15 synonymies in Ophiocordyceps, and one new combination into Purpureocillium.

Key words: Cordyceps, Drechmeria, Harposporium, nomenclature, Ophiocordyceps, Purpureocillium

INTRODUCTION

Kirk et al. (2013) listed 11 genera of Ophiocordycipitaceae for protection as a result of changes in Art. 59 of the International Code of Nomenclature for algae, fungi, and plants (ICN; McNeill et al. 2012); these include Chaunopycnis, Drechmeria, Harposporium, Hirsutella, Hymenostilbe, Ophiocordyceps, Parasariya, Podocrella, Polycephalomyces, Sorospora and Tolypocladium. Informed by multigene phylogenetic analyses, Quandt et al. (2014) refined this list and proposed a generic classification for Ophiocordycipitaceae comprising six monophyletic genera, including Drechmeria, Harposporium, Ophiocordyceps, Polycephalomyces, Purpureocillium and Tolypocladium. This system was used as part of a natural classification of Sordariomycetes (Maharachchikumbura et al. 2015) and new species combinations were made for Polycephalomyces (Kepler et al. 2013) and Tolypocladium (Quandt et al. 2014), but the species composition of the remaining four genera remained ambiguous. Here we introduce necessary species combinations into the genera Drechmeria, Harposporium, Ophiocordyceps and Purpureocillium. These combinations are supported by the reference multigene phylogeny presented in Quandt et al. (2014: fig 1) and previous phylogenetic analyses (e.g., Sung et al. 2007, Luangsarad et al. 2011).


Commentary: Drechmeria includes the sexually reproductive species Cordyceps gunnii, which parasitizes lepidopteran larvae (Hepialidae) buried in soil; it has a known distribution in Australia and New Zealand (Berkeley 1848, Dingley 1953). Drechmeria also includes species classified in the asexually typified genus Haptocillium, which like Drechmeria includes a number of nematode pathogenic fungi. The nematode pathogen ecology of the asexual morphs associated with this clade suggests that nematode associations may also be a frequent trophic mode for sexually reproductive species. Ten new combinations are made here, resulting in a total of twelve names of accepted species in the genus.

Drechmeria bactrospora (Drechsler) Spatafora & Kepler, comb. nov.

MycoBank MB814713

Basionym: Acrostalagmus bactrosporus Drechsler, Phytopathology 31: 782 (1941).


© 2015 International Mycological Association

You are free to share - to copy, distribute and transmit the work, under the following conditions:

Attribution: You must attribute the work in the manner specified by the author or licensor (but not in any way that suggests that they endorse you or your use of the work).

Non-commercial: You may not use this work for commercial purposes.

No derivative works: You may not alter, transform, or build upon this work.

For any reuse or distribution, you must make clear to others the license terms of this work, which can be found at http://creativecommons.org/licenses/by-nc-nd/3.0/legalcode. Any of the above conditions can be waived if you get permission from the copyright holder. Nothing in this license impairs or restricts the author’s moral rights.

**Drechmeria balanoides** (Drechsler) Spatafora & Kepler, *comb. nov.*
MycoBank MB814714


**Drechmeria campanulata** (Glockling) Spatafora & Kepler, *comb. nov.*
MycoBank MB814715


**Drechmeria glocklingiae** (Zare & W. Gams) Spatafora & Kepler, *comb. nov.*
MycoBank MB814716


**Drechmeria gunnii** (Berk.) Spatafora, Kepler & Quandt, *comb. nov.*
MycoBank MB814717


**Drechmeria obovata** (Drechsler) Spatafora & Kepler, *comb. nov.*
MycoBank MB814718


**Drechmeria rhabdospora** (Zare & W. Gams) Spatafora & Kepler, *comb. nov.*
MycoBank MB814719


**Drechmeria sinensis** (K.Q. Zhang et al.) Spatafora & Kepler, *comb. nov.*
MycoBank MB814720


**Drechmeria sphaerospora** (Goodey) Spatafora & Kepler, *comb. nov.*
MycoBank MB814721


**Drechmeria zeospora** (Goodey) Spatafora & Kepler, *comb. nov.*
MycoBank MB814722


Commentary: *Harposporium* includes the sexually reproductive species of *Podocrella*, which typically parasitize coleopteran larvae buried in wood (Chaverri et al. 2005). The taxonomic history of these fungi has been unstable. All species classified in *Podocrella*, *Wakefieldiomyces*, and *Ophiocordyceps* are pathogens of nematodes suggesting that nematode associations may be a frequent trophic mode for sexually reproductive species, as well. Four new combinations are made here, resulting in a total of 37 names of accepted species in the genus.

**Harposporium fuscum** (Chaverri & K.T. Hodge) Spatafora & Kepler, *comb. nov.*
MycoBank MB814723


**Harposporium harposporiferum** (Samuels) Spatafora & Kepler, *comb. nov.*
MycoBank MB814724


**Harposporium peltatum** (Wakef.) Spatafora & Kepler, *comb. nov.*
MycoBank MB814725


Harpoperonory poronoides (Seaver) Spatafora & Kepler, comb. nov.
MycoBank MB814726
Basionym: Podocrella poronoides Seaver, Mycologia 20: 57 (1928).


Commentary: New combinations are made for species that were recently demonstrated to be members of Ophiocordyceps including Cordyceps annulata, Stilbella buquetii and Tilachlidiopsis nigra (Quandt et al. 2014), as well as species classified in Hymenostilbe, Paraisaria, Podonectria and Syngliocladium, which were demonstrated to have a phylogenetic affinity with the genus (Hodge et al. 1998, Sung et al. 2007, Quandt et al. 2014). All anticipated species recombinations are made here, resulting in a total of 223 names of accepted species in the genus.

Ophiocordyceps acridiora (H.C. Evans & P.A. Shah) B. Shrestha, G.H. Sung & Spatafora, comb. nov.
MycoBank MB814728

Ophiocordyceps acridiora var. madagascariensis (H.C. Evans & P.A. Shah) B. Shrestha, G.H. Sung & Spatafora, comb. nov.
MycoBank MB814729

Ophiocordyceps annulata (Kobayasi & Shimizu) Spatafora, Kepler & Quandt, comb. nov.
MycoBank MB814730

Ophiocordyceps aphidis (Petch) B. Shrestha, G.H. Sung & Spatafora, comb. nov.
MycoBank MB814731


Ophiocordyceps aranevarum (Petch) B. Shrestha, G.H. Sung & Spatafora, comb. nov.
MycoBank MB814732

Ophiocordyceps australiensis (Mains) B. Shrestha, G.H. Sung & Spatafora, comb. nov.
MycoBank MB814733

MycoBank MB8431869

Ophiocordyceps buquetii (Mont. & C.P. Robin) Spatafora, Kepler & Quandt, comb. nov.
MycoBank MB814734
Stilbum formicarum Cooke & Massee, Grevillea 18: 8 (1889).
Isaria buquetii (Mont. & C.P. Robin) Lloyd, Mycol. Writ. 7: 1119 (1922).

Ophiocordyceps campanoti (Mains) B. Shrestha, G.H. Sung & Spatafora, comb. nov.
MycoBank MB814735
Basionym: Hymenostilbe campanoti Mains, Mycologia 42: 586 (1950); as "camponoti".

Ophiocordyceps cicadellidicola (Kobayasi & Shimizu) Spatafora, Kepler & Quandt, comb. nov.
MycoBank MB814736

Ophiocordyceps citrina (Kobayasi & Shimizu) Spatafora, Kepler & Quandt, comb. nov.
MycoBank MB814737
MycoBank MB251478
Basionym: Sphaeria clavulata Schwein., Trans. Am. Phil. Soc. 4: 188 (1834) [*1832*].
Synonym: Xylaria clavulata (Schwein.) Berk. & M.A. Curtis, J. Linn. Soc., Bot. 10: 380 (1869) [*1868*].
Hymenostilbe lecaniicola (Jaap) Mains, Mycologia 42: 582 (1950).

Ophiocordyceps cleoni (Wize) B. Shrestha, G.H. Sung & Spatafora, comb. nov.
MycoBank MB814738


Basionym: Cordyceps dipterigena Berk. & Broome, J. Linn. Soc., Bot. 14: 111 (1875) [*1873*].

Cordyceps entomorrhiza (Dicks.) Fr., Summa Veg. Scand. 2: 567 (1849).


Ophiocordyceps furcata (Aung et al.) B. Shrestha, G.H. Sung & Spatafora, comb. nov.
MycoBank MB814739

Ophiocordyceps ghanensis (Samson & H.C. Evans) B. Shrestha, G.H. Sung & Spatafora, comb. nov.
MycoBank MB814740

Cordyceps entomorrhiza var. gracilis (Grev.) Cooke, Grevellea 12: 102 (1884).


Ophiocordyceps ichneumonophila (van Vooren & Audibert) B. Shrestha, G.H. Sung & Spatafora, comb. nov.
MycoBank MB814741

Ophiocordyceps intricata (Petch) B. Shrestha, G.H. Sung & Spatafora, comb. nov.
MycoBank MB814742


Ophiocordyceps siamensis (Hywel-Jones, Mongkolamsirit & Luangsa-ard, comb. nov. MycoBank MB814747

Cordyceps sobolifera (Hill ex Watson) Berk. & Broome, J. Linn. Soc., Bot. 14: 110 (1875) ["1873].

Ophiocordyceps spiculata (B. Huang et al.) B. Shrestha, G.H. Sung & Spatafora, comb. nov. MycoBank MB814748

Ophiocordyceps tetanopsis (K.T. Hodge et al.) B. Shrestha, G.H. Sung & Spatafora, comb. nov. MycoBank MB814749

Ophiocordyceps verrucosa (Mains) B. Shrestha, G.H. Sung & Spatafora, comb. nov. MycoBank MB814750


Commentary: Purpureocillium includes the sexually reproducing species Cordyceps cylindrica Petch 1937 which parasitizes trapdoor spiders. This species is linked to the asexual morph Nomuraea atypica, also a spider pathogen. Purpureocillium also includes P. takamizusunense, and Ban et al. 2015 is an asexual morph that has been linked to the sexual morph Cordyceps ryogamimontana Kobayasi 1963 (Ban et al. 2015). Nomuraea Maubl. 1903 included green-spored species, which were classified in Metarhizium (Kepler et al. 2014); all purple- to lilac-spored species are classified here in Purpureocillium. One new combination is made here, resulting in a total of twelve names of accepted species in the genus.

Purpureocillium atypicolum (Petch) Spatafora, Hywel-Jones & Luangsa-ard, comb. nov. MycoBank MB814727
CONCLUSIONS

We present species combinations for Ophiocordycipitaceae that are consistent with the abolition of Art. 59 of the ICN (McNeill et al. 2012) and the recommendations of Quandt et al. (2014). Six genera are currently recognized within the family and all include at least one species formerly classified in Cordyceps sensu Kobayasi (1941) and Mains (1958) and multiple species classified in several asexually typified genera. The principles of monophyly and priority were enforced in all cases and resulted in a total of 38 new combinations and 15 synonymies. We have purposefully reserved the treatment of Hirsutella for a subsequent publication; it is a large and complex genus and the genus name has been applied to a broad and heterogenous group of fungi across several families of Hypocreales (Sung et al. 2007). Many species currently classified in Hirsutella will be ultimately transferred to Ophiocordycipitaceae, but many will also be accommodated in other genera and more detailed taxonomic and phylogenetic analyses are required.

ACKNOWLEDGEMENTS

G H Sung and B Shrestha acknowledge the financial support of the Cooperative Research Program for Agricultural and Technology Development (PJ009241) of Rural Development Administration, Korea. N L H-J would like to thank Morakot Tanticharoen for her continued support of the BIOTEC insect-fungus programme over many years. J W S acknowledges financial support (DEB-1258162) from the National Science Foundation. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the National Science Foundation.

REFERENCES

Bringing *Laboulbeniales* into the 21st century: enhanced techniques for extraction and PCR amplification of DNA from minute ectoparasitic fungi

Danny Haelewaters¹,², Michał Gorczak³, Walter P. Pfliegl⁴,⁵,⁶, András Tartally⁷, Marta Tischer³, Marta Wrzosek³, Donald H. Pfister¹,²

¹Department of Organismic and Evolutionary Biology, Harvard University, 22 Divinity Avenue, Cambridge, MA 02138, USA; corresponding author e-mail: dhaelewaters@fas.harvard.edu
²Farlow Reference Library and Herbarium of Cryptogamic Botany, Harvard University, 22 Divinity Avenue, Cambridge, MA 02138, USA
³Department of Molecular Phylogenetics and Evolution, University of Warsaw, Aleje Ujazdowskie 4, Warsaw, Poland
⁴Department of Genetics and Applied Microbiology, University of Debrecen, Egyetem tér 1, 4032 Debrecen, Hungary
⁵Department of Biotechnology and Microbiology, University of Debrecen, Egyetem tér 1, 4032 Debrecen, Hungary
⁶Postdoctoral Fellowship Programme of the Hungarian Academy of Sciences (MTA), Hungary
⁷Department of Evolutionary Zoology and Human Biology, University of Debrecen, Egyetem tér 1, 4032 Debrecen, Hungary

**Abstract:** *Laboulbeniales* is one of the most peculiar orders of Ascomycota. These fungi are characterized by an ectoparasitic life-style on arthropods, determinate growth, lack of an asexual stage, high species richness, and intractability to culture. The order *Laboulbeniales*, sister to *Pyxidiophorales*, has only recently been assigned a separate class, the *Laboulbeniomycetes*, based on very few ribosomal DNA sequences. So far, DNA isolations and PCR amplifications have proven difficult. Here, we provide details of isolation techniques and the application of commercially available kits that enable efficient and reliable genetic analyses of these fungi. We provide 43 newly generated *Laboulbeniales* ribosomal DNA sequences, among which are the first published sequences for species in the genera *Gloeandromyces*, *Herpomyces*, *Laboulbia*, *Monoicomyces*, and *Polyandromyces*. DNA extractions were possible using from 1 to 30 thalli from hosts preserved in ethanol (70–100%). In two cases, we successfully isolated DNA from thalli on dried insect collections. *Laboulbeniales* molecular systematics could be substantially enhanced through these improved methods by allowing more complete sampling of both taxa and gene regions.

**Key words:** Ascomycota, DNA isolation, insect collections, *Laboulbeniales*-specific primers, ribosomal DNA, unculturable DNA

**Article info:** Submitted: 14 July 2015; Accepted: 29 October 2015; Published: 4 November 2015.

**INTRODUCTION**

*Laboulbeniales* are obligate ectoparasitic Ascomycota on arthropods. Over 2100 species in 140 genera are described, but many more species await discovery (Weir & Hammond 1997, Haelewaters & Yaakop 2014). *Laboulbeniales* differ from most other non-yeast Ascomycota in that they do not form hyphae but instead form discrete microscopic and multicellular thalli. Their only form of reproduction is sexual, during which they generate sticky ascospores that are usually transmitted directly from infected to uninfected hosts during mating or other contact (De Kesel 1996a). They are moderately to highly host specific; most species are associated with a particular host species (but see, e.g. De Kesel & Haelewaters 2014). It was experimentally shown that this specificity is driven by several factors: the characteristics of the integument and living conditions of the arthropod host, as well as the nature and availability of nutrients in the habitat chosen by the host (De Kesel 1996b). Study of these fungi also needs some expertise in entomology. Correct identification of a host often facilitates identification of its associated fungi, but since fortuitous infections of hosts occur, it is best to identify these fungi based on their morphology or DNA sequence comparisons. Host-parasite lists are available for some countries (Scheloske 1969, Huldén 1983, Majewski 1994, De Kesel 1998, Santamaría 1998, 2003) and regions (Santamaría et al. 1991). Useful advice about general methodology and identification of *Laboulbeniales* can be found in Thaxter (1896), Scheloske (1969), Benjamin (1971), Majewski (1994), and Santamaría (1998).

It was only recently that the order *Laboulbeniales* was recognized as a well-supported lineage in Ascomycota, as the class *Laboulbeniomycetes* that includes both *Laboulbeniales* and *Pyxidiophorales* (Weir & Blackwell 2001a). This phylogenetic determination was based on four (partial) SSU ribosomal DNA (rDNA) sequences (*Pyxidiophora* sp.1, *Stigmatomyces linnorhophae*, *Hesperomyces coccinelloides*, and *Zodiomyces vorticellarius*). Weir & Blackwell’s (2001a) phylogeny suggested a close relationship with *Sordariomycetes*. High bootstrap support for this hypothesis
was later achieved by Schoch et al. (2009) based on a six-gene phylogeny. The order Laboulbeniales was represented in that dataset by only SSU and LSU sequences for two species (Hesperomyces virescens and Stigmatomyces protudens).

Molecular studies of Laboulbeniales have proven difficult for several reasons. The thallus are microscopic, on average 200–300 µm in length. Among the smallest species known are Rickia euxesti (total length 40–68 µm), R. lenoiiri (45–67 µm), and Siemasskzoa annae (47–54 µm) (Thaxter 1896, 1926, Majewski 1994, Santamaria & Espadaler 2015). At the other end of the size spectrum are Zodiomyces vorticellarius (to 2.75 mm) and Laboulbenia kunckelii (2–4 mm) (Giard 1892, Sugiyama & Phanichapol 1984, Haelewaters unpubl.). For study and extraction of DNA, thalli need to be removed from their host, which requires micro-manipulation techniques and specific tools. Hosts may bear only a few thalli but certain hosts carry multiple, often position-specific species (e.g. Chitonomyces spp., De Kesel & Haelewaters 2012, Goldmann & Weir 2012; Hesperomyces coleomegillae and H. palustris, Goldmann et al. 2013). Many species are heavily pigmented with melanin in their cell walls, providing rigidity (Weir & Beakes 1996). This pigment interferes with PCR amplification by binding to the DNA polymerase (Eckhart et al. 2000). Thalli are relatively long-lasting and their form is such that they absorb impacts and friction during their entire existence on the hosts’ integument. These tough and resilient cells are difficult to break. Because Laboulbeniales have not been grown in culture to more than a few cells, obtaining DNA from cultured material has been impossible. Only Whisler (1968) was partly successful in this with Stigmatomyces ceratophorus, obtaining 20-celled thalli onto sterile fly wings on brain-heart infusion agar, but perithecia were not produced.

Laboulbeniales are a remarkable clade for their: (1) obligate biotrophy; (2) strictly determinate growth, with development from a two-celled ascospore to a thallus of up to several thousand cells; (3) bilateral symmetry; and (4) loss of germ tubes, hyphae, and conidia. Despite these special features, the order and the class were not included in studies dealing with “major lineages in fungi” (Prieto & Wedin 2013) or the subphylum “Pezizomycotina” (to 2.75 mm) and Laboulbenia kunckelii (2–4 mm) (Giard 1892, Sugiyama & Phanichapol 1984, Haelewaters unpubl.). For study and extraction of DNA, thalli need to be removed from their host, which requires micro-manipulation techniques and specific tools. Hosts may bear only a few thalli but certain hosts carry multiple, often position-specific species (e.g. Chitonomyces spp., De Kesel & Haelewaters 2012, Goldmann & Weir 2012; Hesperomyces coleomegillae and H. palustris, Goldmann et al. 2013). Many species are heavily pigmented with melanin in their cell walls, providing rigidity (Weir & Beakes 1996). This pigment interferes with PCR amplification by binding to the DNA polymerase (Eckhart et al. 2000). Thalli are relatively long-lasting and their form is such that they absorb impacts and friction during their entire existence on the hosts’ integument. These tough and resilient cells are difficult to break. Because Laboulbeniales have not been grown in culture to more than a few cells, obtaining DNA from cultured material has been impossible. Only Whisler (1968) was partly successful in this with Stigmatomyces ceratophorus, obtaining 20-celled thalli onto sterile fly wings on brain-heart infusion agar, but perithecia were not produced.

Laboulbeniales are a remarkable clade for their: (1) obligate biotrophy; (2) strictly determinate growth, with development from a two-celled ascospore to a thallus of up to several thousand cells; (3) bilateral symmetry; and (4) loss of germ tubes, hyphae, and conidia. Despite these special features, the order and the class were not included in studies dealing with “major lineages in Ascomycota” (Prieto & Wedin 2013) or the subphylum Pezizomycotina, to which they belong (Spatafora et al. 2006).

Extraction of DNA using a variety of methods and protocols have given poor results or failed. These include prolonged boiling of thalli (Henson 1992), microwave treatment (Goodwin & Lee 1993), immersion in liquid nitrogen (Haugland et al. 1999), and direct addition of entire thalli to PCR master mix (Haelewaters 2011). Also, the use of commercial kits (Puregene Kit A, DNeasy Plant Mini Kit, Qiagen; Haelewaters 2011) has so far proven unsuccessful. The first successful published extraction protocol involved transferring thalli to double distilled (dd) H₂O, air drying, and manually crushing thalli between microscope slides (Weir & Blackwell 2000). The success rate for this protocol was 25 %. Weir & Blackwell (2001) developed an improved technique in which thalli were manually crushed on a microscope slide and picked up with a micropipette facilitated by the use of a bed of dry ice, a modification from previous endeavors based on Conger & Fairchild (1953) and Lee & Taylor (1990). The technique from Weir & Blackwell (2001b) was successful only when hosts were preserved in 95 % ethanol for not more than six months. Thalli taken from dried insect specimens have not been available for molecular phylogenetic analyses because extractions have been unsuccessful with this type of material (Weir & Blackwell 2001b). This technical difficulty limits both the taxonomical and geographical diversity of species that can be included in phylogenetic studies (e.g. Thaxter 1899, 1900, 1901a, 1901b, 1902, 1905, Weir & Hammond 1997, Haelewaters et al. 2014, 2015a, 2015b).

Owing to the difficulties in DNA isolation and amplification of phylogenetically informative genes, the molecular phylogenetic relationships within this group have been understudied. Weir & Hughes (2002) constructed a partial SSU rDNA phylogeny of ten species of Laboulbeniales, representing three subfamilies (Ceratomyctoideae, Laboulbenioideae, Peyriteschielloideae). A combined dataset of the partial SSU and ITS rDNA regions was used to study the phenomenon of position specificity in 13 species of Chitonomyces on Laccophilus maculosus (Coleoptera: Dytiscidae; Goldmann & Weir 2012). Goldmann et al. (2013) described two position specific species of Hesperomyces on Coleomegilla maculata (Coleoptera: Coccinellidae), again based on partial SSU+ITS rDNA. All these studies used the extraction methodology of Weir & Blackwell (2001b).

We tested more generalized techniques that could be adapted to sample the thalli of Laboulbeniales.

MATERIAL AND METHODS

Collection

Insects were collected around the world by ourselves or collaborators using standard entomological methods (sticky traps, light trap, entomological net, and hand collecting) or obtained from the pet store (Blatta lateralis). Insects were killed in 70–100 % ethanol, ethyl acetate vapors, or simply by freezing. Screening for Laboulbeniales was done using a dissecting microscope at 50x.

Morphological studies

Individual thalli were removed from the host using an entomological pin (self-made, sometimes flattened) or the tip of a scalpel. Slide mounts followed techniques for permanent microscope slides (Benjamin 1971, Haelewaters et al. 2015b). Identification of Laboulbeniales followed Thaxter (1908, 1931), Majewski (1994), and De Kesel (2011). Voucher slides are deposited at BP (Botanical Department, Hungarian Natural History Museum), FH (Farlow Herbarium, Harvard University), and WA (Faculty of Biology, University of Warsaw). Herbarium acronyms are according to Thiers 2015.

DNA extraction protocols

Between one and 30 thalli were removed from each host specimen. In this study we wanted to test the efficacy of different commercial and noncommercial DNA extraction protocols. The following were used: (1) QiAamp DNA Micro
DNA extraction and amplification in Laboulbeniales

(1) QIAamp DNA Micro Kit: DNA was isolated from two to sixteen thalli for each extraction, following the manufacturer’s instructions. Some extracts received pre-treatment with liquid nitrogen or two cycles of heating to 95 °C and freezing on liquid nitrogen.

(2) Modified Extract-N-Amp Plant PCR Kit: The manufacturer’s instructions were followed but with 20 µL of Extraction Solution (EX) and 60 µL of Dilution Solution. One to 20 thalli were removed from the host with the help of a tiny drop of Hoyer’s medium (30 g arabic gum, 200 g chloral hydrate, 16 mL glycerol, 50 mL ddH2O) or glycerine at the very end of a micropip and then added to EX-filled 0.5 µL tubes. When hosts were preserved in dried collections, 16–30 thalli were used. Again, the pre-treatment described above was applied for some extracts.

(3) Heat-extraction protocol: This method was adapted from a protocol for single-spore extractions and subsequent PCR reactions (Ferreira & Glass 1996, based on Goodwin from a protocol for single-spore extractions and subsequent applied for some extracts. Again, the pre-treatment described above was applied for some extracts.

(4) ISOLATE II Plant DNA Kit: Up to twenty thalli were removed from the host and transferred to 1.5 mL Eppendorf tubes with 20–50 µL 95 % ethanol. Alternatively, in the case of Hesperomyces virescens, 20–30 thalli of Rickia wasmannii or a ~5 mm portion of a heavily infected Blatta lateralis antenna with Herpomyces stylopyguae thalli was removed, placed in 0.5 mL PCR tubes, and microwave-treated (750 W for 5 min). Then 50 µL ddH2O was added to the individual tubes, and the thalli (or antennal parts) were manually crushed using a sterile pipette tip under a dissecting microscope. Some loss of material did occur by capillary action, but it was minimal. The PCR tubes were incubated at -20 °C for 10 min. Strong pressure was applied to the ice inside the PCR tubes to further break apart thalli using a sterile pipette tip.

(5) ISOLATE II Plant DNA Kit: Up to twenty thalli were removed from the host and transferred to 1.5 mL Eppendorf tubes with 20–50 µL 95 % ethanol. Alternatively, in the case of Hesperomyces ectobiae on Blattella germanica, a piece of an antenna was isolated and transferred altogether. The 1.5 mL tubes were vacuum-dried at room temperature. Thalli were subsequently crushed in liquid nitrogen, using a sterile pipette with melted-closed tip. CTAB-based isolation buffer (PA1, ISOLATE II Plant DNA Kit) was added to the tubes and incubated in liquid nitrogen for 3 min, followed by incubation in a heat block set at 65–90 °C for 3 min. This cycle of freezing/heating was repeated twice. Further steps were performed following the ISOLATE II Plant DNA Kit manufacturer’s protocol.

PCR amplification and DNA sequencing

Three gene loci were amplified: partial rDNA SSU (ca 1100 bp), rDNA ITS (including 5.8S and ITS2; ca 500 bp), and partial rDNA LSU (ca 1300 bp). PCR amplification was performed using both previously published and newly designed primers (Table 1). Laboulbeniales-specific primers were designed for the SSU region based on existing sequences in GenBank (Table 1). PCR reactions were performed according to the protocols listed in the respective reference for mentioned primers, or, in the case of the Extract-N-Amp Plant PCR Kit, according to the suggested protocol in the manufacturer’s instructions. When PCR reactions did not produce clear bands during gel electrophoresis, conditions were optimized to include a two-step (60 °C, 55 °C) “touch-down” annealing phase (Sohrabi et al. 2010). In some cases, a semi-nested “touch-down” PCR was performed, using the product of the first, unsuccessful PCR reaction (e.g. PCR 1 using primers LR0R and LR5, semi-nested PCR using the product of PCR 1 with primers LR0R and LR3).

Products that showed clear bands on agarose gel were cleaned with Qiagen PCR Purification Kit (Qiagen, Stanford, CA) or ExtractMe DNA Gel-out kit (Blirt, Gdański, Poland) and subsequently sequenced. We prepared 10 µL sequencing reactions containing the same primer pairs and 1 µL of purified PCR product. The sequencing reactions were performed using the Big Dye® Terminat v3.1 Cycle Sequencing Kit (Life Technologies, Carlsbad, CA).

Sequences were trimmed, edited and assembled in Sequencer 4.10.1 (Gene Codes Corporation, Ann Arbor, MI). We performed BLAST searches on all of our sequences at http://ncbi.nlm.nih.gov/blast/Blast.cgi for similar sequences. For genera not yet represented in GenBank we compared sequences with our personal database, which is accessible at the Harvard University Herbaria internal server.

RESULTS

Our study shows that some simple, general DNA extraction protocols work. The commercial kits we tested are widely available.

Table 2 shows the success rates of the individual protocols, per genus extracted. Extractions using the QIAamp DNA Micro Kit yielded the lowest rates of success among the tested protocols, with seemingly no effect of pre-treatment. The overall success rate was 22 % (n = 27 extractions total), for Hesperomyces virescens extractions the success rate was 35 % (n = 17). Overall success of the Extract-N-Amp Plant PCR Kit was 64 % (n = 66), with 92 % success for Herpomyces spp. (n = 13) and 66 % for H. virescens (n = 35). For the third, heat-extraction protocol the success rate was 83 % for Herpomyces ectobiae (n = 6) and 100 % for H. virescens (n = 3). The ISOLATE II Plant DNA Kit gave an overall success rate of 59 % (n = 34), with a 100 % success rate for H. ectobiae (n = 5) and 86 % for H. virescens (n = 7). Interestingly, extracting DNA of Laboulbenia species was only successful 20 % of the time with the Extract-N-Amp Plant PCR Kit and 10 % with the ISOLATE II Plant DNA Kit. Four extraction attempts of Laboulbenia species with the QIAamp DNA Micro Kit were unsuccessful.

We generated 43 sequences (SSU, ITS, and/or LSU rDNA) for 18 isolates of the following species: Gloeandromyces nycteribiidarum, Hesperomyces chaetophilus, H. ectobiae, H. periplanetae, H. stylopyguae, Hesperomyces virescens, Laboulbenia diopsidis, Monoicomyces invisibilis, Polyanomomyces eptasemalis, Rhachomyces philonthinus, Rickia wasmannii, and Zodiomyces verruculans (Table 3). Rhachomyces philonthinus was removed from a specimen...
of Philonthus that had been collected by Tomasz Majewski in August 2004. The host specimen was preserved for 11 years in 70 % ethanol.

We were able to extract DNA from thalli of Hesperomyces virescens from dried insect specimens (with the Extract-N-Amp Plant PCR Kit); on Cycloneda sanguinea sanguinea from Guatemala collected in May 2013, and on Harmonia axyridis from Massachusetts collected in August 2006 (details in Haelewaters et al. 2015b). Extractions were performed from H. paranensis on a dried Archimandrita tessellata (Blattodea: Blaberidae) collected in 2001 [deposited at the Harvard Museum of Comparative Zoology] and from Rodaucea sp. on a dried Cholevinae sp. (Coleoptera: Leiodidae) collected in 1991 [part of the collection of Invertebrate Zoology at the American Museum of Natural History], but no bands were noted on the agarose gel after PCR.

### DISCUSSION

#### Micromanipulation practices

Laboulbeniales are more problematic to work with than many other groups of fungi. One of the main difficulties is their small size, which requires sterile micromanipulation with precise micropin handling.

It is preferable to separate thalli from the host’s body, but minute thalli of Rickia, Herpomyces or Siemaszkoa are hard to detach. Using whole infected body parts in an extraction makes the procedure faster and easier. Most of the primers used in this study do not amplify the host insect’s DNA, however, amplification of insect DNA by some primers may happen (as with LR0R/LR7 and the sets of SSU primers used in Wrzosek 2000). Prominent appendages, such as those in many species of Laboulbenia or Rhachomyces, pose another difficulty; debris is often observed to stick to the appendages and is very hard to impossible to wash away. In this case contamination with fungal propagules may be inevitable. Laboulbeniales-specific primers will serve to reduce the chance of amplifying non-target DNA. Another

<table>
<thead>
<tr>
<th>Locus</th>
<th>Primer name</th>
<th>Sequence</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>SSU rDNA</td>
<td>NS1</td>
<td>forward</td>
<td>GTAGTCATATGCTTGTCCT</td>
</tr>
<tr>
<td>SSU rDNA</td>
<td>NS2</td>
<td>reverse</td>
<td>GGCTGCTGGCACACAGTGTGC</td>
</tr>
<tr>
<td>SSU rDNA</td>
<td>NS4</td>
<td>reverse</td>
<td>CTTCCGCTCAATTTTAAGTTCCT</td>
</tr>
<tr>
<td>SSU rDNA</td>
<td>SL344</td>
<td>forward</td>
<td>GGTGCAAGGCTGAAACTTA</td>
</tr>
<tr>
<td>SSU rDNA</td>
<td>NS6</td>
<td>reverse</td>
<td>GCATCACAGACCTGTATTGCCT</td>
</tr>
<tr>
<td>SSU rDNA</td>
<td>SL122</td>
<td>forward</td>
<td>AGGGCGCGCAAATACCAAT</td>
</tr>
<tr>
<td>SSU rDNA</td>
<td>SR4</td>
<td>reverse</td>
<td>AAACCAACAAAATAGAA</td>
</tr>
<tr>
<td>SSU rDNA</td>
<td>NSL1</td>
<td>forward</td>
<td>GTAGTGTCCTCACATGTTCCT</td>
</tr>
<tr>
<td>SSU rDNA</td>
<td>NSL2</td>
<td>reverse</td>
<td>AATCyAGAATTTCCATCTGAC</td>
</tr>
<tr>
<td>SSU rDNA</td>
<td>L</td>
<td>forward</td>
<td>AACCGTGTTGATCCTGCCAGT</td>
</tr>
<tr>
<td>SSU rDNA</td>
<td>402</td>
<td>forward</td>
<td>GCTACCAACATCCAAGGAAAG</td>
</tr>
<tr>
<td>SSU rDNA</td>
<td>416</td>
<td>reverse</td>
<td>ATTTGCGCGCCTGCTGCTTCC</td>
</tr>
<tr>
<td>SSU rDNA</td>
<td>895</td>
<td>forward</td>
<td>GTCAAGGAGTAAATTTCTGAGT</td>
</tr>
<tr>
<td>SSU rDNA</td>
<td>898</td>
<td>reverse</td>
<td>TAAATCCAAGAATTTACCTCT</td>
</tr>
<tr>
<td>SSU rDNA</td>
<td>1144</td>
<td>forward</td>
<td>GCCCTCGGCTTATTTGACTCAACA</td>
</tr>
<tr>
<td>SSU rDNA</td>
<td>1308</td>
<td>reverse</td>
<td>CTTCCTGCTTACGGAATTAACC</td>
</tr>
<tr>
<td>SSU rDNA</td>
<td>R</td>
<td>reverse</td>
<td>TGATCCTCTTGAGGTTACCATCG</td>
</tr>
<tr>
<td>ITS rDNA</td>
<td>ITS1f</td>
<td>forward</td>
<td>CTTGTCATTAGAGAGAGTAA</td>
</tr>
<tr>
<td>ITS rDNA</td>
<td>ITS4</td>
<td>reverse</td>
<td>TCCTCCGCTTATGATGAT</td>
</tr>
<tr>
<td>ITS rDNA</td>
<td>ITS4_kyo1</td>
<td>reverse</td>
<td>TCCTCGCCTTACGGGTATGC</td>
</tr>
<tr>
<td>ITS rDNA</td>
<td>ITS5</td>
<td>forward</td>
<td>GGAAGTAAAGTCTGAACGAG</td>
</tr>
<tr>
<td>ITS rDNA</td>
<td>ITS2</td>
<td>reverse</td>
<td>GCTGCCTCTTGCTCACGAGT</td>
</tr>
<tr>
<td>LSU rDNA</td>
<td>LR0R</td>
<td>forward</td>
<td>ACCCGCTGAACCTTGAAC</td>
</tr>
<tr>
<td>LSU rDNA</td>
<td>LR1R</td>
<td>forward</td>
<td>AGGAAAGAAACCAACC</td>
</tr>
<tr>
<td>LSU rDNA</td>
<td>LIC24R</td>
<td>forward</td>
<td>GAAACCAACAGGATGGT</td>
</tr>
<tr>
<td>LSU rDNA</td>
<td>LR3</td>
<td>reverse</td>
<td>GGTGCCTGTTTCAAGAC</td>
</tr>
<tr>
<td>LSU rDNA</td>
<td>LR5</td>
<td>reverse</td>
<td>ATCCTGAGGGAACCTT</td>
</tr>
<tr>
<td>LSU rDNA</td>
<td>LR7</td>
<td>reverse</td>
<td>TACTACCCACAGATCT</td>
</tr>
<tr>
<td>LSU rDNA</td>
<td>NL1</td>
<td>forward</td>
<td>GCATATCAATAAGCAGGAGAAG</td>
</tr>
<tr>
<td>LSU rDNA</td>
<td>NL4</td>
<td>reverse</td>
<td>GGTCGCTGTACGAGACGG</td>
</tr>
</tbody>
</table>
DNA extraction and amplification in *Laboulbeniales*

One of the most important concerns regarding successful molecular research is the method employed for preservation of material. The most effective option for extraction of *Laboulbeniales* DNA involves using freshly collected material preferably stored in ≥ 95 % ethanol. These two factors certainly contribute to most of our DNA isolation positive results. Storage in ≥ 95 % ethanol generally provides good DNA preservation for a prolonged period of time. Our DNA extraction protocols enabled us to amplify DNA and generate sequences from *Laboulbeniales* material that was on average 1–2 years old (one specimen was 11 years old), which is a novel development. Conditions that consistently yielded good results included: freshly collected specimens of larger species of *Laboulbeniales*, which provide ample DNA concentration even from a single thallus (e.g. *Zodiomyces vorticellarius*), and mature ascospore-containing thalli, which provide a higher concentration of DNA compared to immature or old thalli (always without ascospores). Many entomological practices involve preservation methods that interfere with successful DNA extraction of either the host or its associated fungi: most insect specimens are pinned in museum collections or preserved on 70 % ethanol. For morphological study of *Laboulbeniales*, researchers are able to make use of the many excellent systematic insect collections in natural history museums around the world. Such collections of dried pinned insects give relatively easily access to data (e.g. Weir & Hammond 1997, Haeflewaters et al. 2014). However, to date, extracting DNA from dried specimens has resulted in a 100 % failure rate (Weir & Blackwell 2001b). We present sequences obtained from two

<table>
<thead>
<tr>
<th>Table 2. Success rates per DNA extraction protocol used in this study, for all tested genera. <em>Laboulbeniales</em> from dried host insects were only extracted using the Extract-N-Amp Plant PCR Kit.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>QIAamp DNA Micro Kit</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Aphanandromyces</td>
</tr>
<tr>
<td>Chitonomyces</td>
</tr>
<tr>
<td>Gloeandromyces</td>
</tr>
<tr>
<td>Haplomyces</td>
</tr>
<tr>
<td>Herpomyces</td>
</tr>
<tr>
<td>Herpomyces (dried)</td>
</tr>
<tr>
<td>Hesperomyces</td>
</tr>
<tr>
<td>Hesperomyces (dried)</td>
</tr>
<tr>
<td>Laboulbenia</td>
</tr>
<tr>
<td>Monoicomyces</td>
</tr>
<tr>
<td>Polyanthomycycs</td>
</tr>
<tr>
<td>Rickia</td>
</tr>
<tr>
<td>Rodauceae (dried)</td>
</tr>
<tr>
<td><strong>Heat-extraction protocol</strong></td>
</tr>
<tr>
<td>Aphanandromyces</td>
</tr>
<tr>
<td>Chitonomyces</td>
</tr>
<tr>
<td>Gloeandromyces</td>
</tr>
<tr>
<td>Haplomyces</td>
</tr>
<tr>
<td>Laboulbenia</td>
</tr>
<tr>
<td>Monoicomyces</td>
</tr>
<tr>
<td>Polyanthomycycs</td>
</tr>
<tr>
<td>Rickia</td>
</tr>
<tr>
<td>Rodauceae (dried)</td>
</tr>
</tbody>
</table>
collections of *H. virescens* from dried ladybirds (DH167e and DH486c) collected in 2013 and 2006, respectively. Often thalli acquired from dried hosts are in poor condition and both identification based on morphological characters and DNA extraction may be a challenge.

Many insects in entomological collections are preserved in 70 % ethanol. This decreases the DNA quality of the insect and its associates – especially after an extended period of storage (e.g. A'Hara et al. 1998). Some studies have generated short segments of mitochondrial DNA (< 300 bp) from material in 70 % ethanol (e.g. Colgan et al. 2002). For phylogenetic studies, however, longer segments are needed, and these need to be acquired from non-degraded DNA. 

Non-degraded DNA is also required for PCR amplification of low copy-number nuclear genes commonly used in modern fungal phylogenies (e.g. Hibbett et al. 2007, Hansen et al. 2013, Wang et al. 2014). If 70 % ethanol was used to preserve insect hosts, it comes as no surprise that the DNA of *Laboulbeniales* harvested from them is adversely affected.

When working with *Laboulbeniales* from dried collections, another challenge is that information about the habitat or methods of collection and preservation is typically sparse. The extraction of DNA from insects can be drastically affected by using certain media (such as killing agents in pitfall traps) that degrade DNA. Some commonly used materials such as ethylene glycol or formalin have been linked to considerable DNA degradation (e.g. Dillon et al. 1996, Stoeckle et al. 2010).

**Negative results**

Our negative results can be explained based on protocols employed and/or the nature of the fungi that were under investigation. The 100 % failure rate of the QIAamp DNA
<table>
<thead>
<tr>
<th>Year of collection</th>
<th>Preservation</th>
<th>Number of thalli used</th>
<th>Extraction protocol</th>
<th>SSU</th>
<th>ITS</th>
<th>LSU</th>
</tr>
</thead>
<tbody>
<tr>
<td>2014</td>
<td>95 % EtOH</td>
<td>12 thalli</td>
<td>Extract-N-Amp (with glycerine)</td>
<td>KT800008</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2014</td>
<td>95 % EtOH</td>
<td>10 female thalli</td>
<td>Extract-N-Amp (with glycerine)</td>
<td>KT800023</td>
<td>KT800039</td>
<td>KT800009</td>
</tr>
<tr>
<td>2014</td>
<td>95 % EtOH</td>
<td>piece of antenna with ± 20 adult thalli</td>
<td>ISOLATE II Plant DNA Kit without freeze/thaw</td>
<td>KT800024</td>
<td>KT800040</td>
<td></td>
</tr>
<tr>
<td>2014</td>
<td>95 % EtOH</td>
<td>11 female thalli</td>
<td>Extract-N-Amp (with glycerine)</td>
<td>KT800025</td>
<td>KT800041</td>
<td>KT800010</td>
</tr>
<tr>
<td>2014</td>
<td>80 % EtOH</td>
<td>piece of antenna with ± 30 adult thalli</td>
<td>Heat-extraction</td>
<td>KT800026</td>
<td>KT800042</td>
<td>KT800011</td>
</tr>
<tr>
<td>2013</td>
<td>dried</td>
<td>18 adult thalli</td>
<td>Extract-N-Amp</td>
<td>KT800027</td>
<td>KT800012</td>
<td></td>
</tr>
<tr>
<td>2014</td>
<td>95 % EtOH</td>
<td>10 adult thalli</td>
<td>QiAmp DNA Micro Kit</td>
<td>KT800028</td>
<td>KT800043</td>
<td>KT800013</td>
</tr>
<tr>
<td>2006</td>
<td>dried</td>
<td>16 adult thalli</td>
<td>Extract-N-Amp (with glycerine)</td>
<td>KT800029</td>
<td>KT800044</td>
<td>KT800014</td>
</tr>
<tr>
<td>2013</td>
<td>95 % EtOH</td>
<td>2 adult thalli</td>
<td>Extract-N-Amp</td>
<td>KT800045</td>
<td>KT800015</td>
<td></td>
</tr>
<tr>
<td>2014</td>
<td>95 % EtOH</td>
<td>15 adult thalli</td>
<td>Extract-N-Amp (with Hoyer’s medium)</td>
<td>KT800030</td>
<td>KT800046</td>
<td>KT800016</td>
</tr>
<tr>
<td>2014</td>
<td>80 % EtOH</td>
<td>9 adult thalli</td>
<td>Heat-extraction</td>
<td>KT800031</td>
<td>KT800047</td>
<td>KT800017</td>
</tr>
<tr>
<td>2015</td>
<td>95 % EtOH</td>
<td>1 adult thallus</td>
<td>ISOLATE II Plant DNA Kit</td>
<td>KT800032</td>
<td>KT800048</td>
<td>KT800018</td>
</tr>
<tr>
<td>2013</td>
<td>100 % EtOH</td>
<td>12 adult thalli</td>
<td>Extract-N-Amp</td>
<td>KT800033</td>
<td>KT800049</td>
<td>KT800019</td>
</tr>
<tr>
<td>2015</td>
<td>95 % EtOH</td>
<td>1 adult thallus</td>
<td>ISOLATE II Plant DNA Kit</td>
<td>KT800034</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2009</td>
<td>95 % EtOH</td>
<td>7 female and 2 male thalli</td>
<td>Extract-N-Amp</td>
<td>KT800035</td>
<td></td>
<td>KT800020</td>
</tr>
<tr>
<td>2004</td>
<td>70 % EtOH</td>
<td>± 15 adult thalli</td>
<td>ISOLATE II Plant DNA Kit</td>
<td>KT800036</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2014</td>
<td>80 % EtOH</td>
<td>30 adult thalli</td>
<td>Heat-extraction</td>
<td>KT800037</td>
<td>KT800050</td>
<td>KT800021</td>
</tr>
<tr>
<td>2015</td>
<td>95 % EtOH</td>
<td>1 adult thallus</td>
<td>ISOLATE II Plant DNA Kit</td>
<td>KT800038</td>
<td></td>
<td>KT800022</td>
</tr>
</tbody>
</table>

Micro Kit for *Chitonomyces*, *Haplomyces*, and *Laboulbenia* is largely due to the fact that no pre-treatments were carried out for these extracts. However, for one *Laboulbenia* extraction using this protocol a pre-treatment was done involving two cycles of heating to 95 °C and freezing on liquid nitrogen. Then why was this extraction unsuccessful? *Laboulbenia* species are generally heavily melanized, and the melanin pigment seems to hinder PCR amplification reactions (Eckhart et al. 2000). Also in the Extract-N-Amp Plant PCR Kit and the ISOLATE II Plant DNA Kit the success of extracting DNA and subsequent PCR amplification of *Laboulbenia* species is considerably lower compared to other genera. This observation shows that variables other than isolation techniques, such as the presence of pigments, are important to the success of DNA extraction and amplification. The 0 % success rate of *Haplomyces* using both the QiAamp DNA Micro Kit and the Extract-N-Amp Plant PCR Kit probably is due to the combination of two factors: (1) the extract received no pre-treatment; and (2) host insects were collected and preserved (for four to five years) in 70 % ethanol. The relatively low success rate with *Rickia*, with the heat-extraction protocol, may be explained by the fact that these small but very rigid thalli are difficult to break during the treatments that were applied; visual inspection after performing the entire protocol shows many intact thalli. Thus, the amount of DNA available for the Taq polymerase during PCR was limited, despite the high number of thalli (20–30) per reaction.

We can only hint at the low success rate of extractions from dried material. The extraction of *Rodacea* sp. received no pre-treatment and the thalli were removed from a cholevinea specimen collected in 1991. It might have been too old for successful DNA extraction. The same may be true...
for the unsuccessful attempts to extract DNA of *Herpomycetes paranensis* from a pinned specimen of *Archimidrita tessallata* from 2001.

**CONCLUSIONS**

Even with fresh thalli available, successful extraction of DNA has been one of the greatest obstacles in applying molecular methods to research on *Laboulbeniales*. Their minute size, the difficulty in fracturing thalli to release DNA, and the fact that (to date) they remain resistant to isolation into culture makes molecular protocols applied to *Laboulbeniales* difficult. This is the reason "laboulbeniologists" need: (1) colleagues (entomologists) or museums to provide high-quality, properly prepared samples; and (2) DNA isolation protocols that focus heavily on deep homogenization of the material. Microwave heating, submersion in liquid nitrogen, freeze/thaw cycles, and simple yet effective crushing with pipette tips are all means of destroying the tough cell walls without damaging the DNA.

As stated in previous studies, both the SSU and ITS portions of rDNA are suited for molecular phylogenetics of the *Laboulbeniales* and universal fungal primers for these regions work well for most of the species (Weir & Blackwell 2001b, Goldmann & Weir 2012, Weir & Hughes 2002, Goldmann *et al*. 2013)). We have found that LSU sequences are also easily to obtain. Designing specific primers often facilitates the work. Well-designed primers specific for *Laboulbeniales* may perform better and their specificity helps to avoid contamination. As the number of genes being used in fungal phylogenetic studies increases it will be important that these new genes/regions/markers be explored in the *Laboulbeniales* as well.

We hope that sharing our experience with various techniques for extraction and PCR amplification of *Laboulbeniales* DNA will have a positive effect on present and future molecular biology research of *Laboulbeniomycetes* – the only class among the *Ascomycota* without a reliable multi-genе phylogeny.

**ACKNOWLEDGEMENTS**

DH acknowledges funding from the Harvard University Graduate School of Arts and Sciences, the American Museum of Natural History (Theodore Roosevelt Memorial Grant), and the National Park Service. AT was supported by the ‘AntLab’ Marie Curie Career Integration Grant within the 7th European Community Framework Programme and by a ‘Bolyai János’ scholarship of the Hungarian Academy of Sciences (MTA). This manuscript would not have come together without the various contributions of the many researchers, collaborators, and friends. For collecting and/or identifying host specimens used in this study: Elizabeth Brooks Thompson, Jasmin C. Camacho, Ted E. Cottrell, André De Kesel, Dimitri Forero, Thereza de A. Garbelotto, Louis S. Hesler, Heidi Hopkins, Walter Rossi, Jessica J. Rykken, Simon Tragust, Piotr Tykarski, Tristan W. Wang, and Richard S. Zack. For advice and helpful discussions: Rosanne A. Healy, Katherine F. LoBuglio, and Feng Xu. For assisting in the molecular lab: Harvard College undergraduates Hamidah Mahmud, Julie Park, and Tristan W. Wang. For general support: André De Kesel, István Pócsi, Matthias Sipiczki, and Tomasz Majewski. For reviewing earlier drafts of the manuscript: Pedro W. Crous, André De Kesel, David L. Hawksworth, Rosanne A. Healy, Monica Hughes, Tomasz Majewski, and Walter Rossi. DH would like to specifically thank Lee H. Herman at the American Museum of Natural History and Brian D. Farrell and Philip Perkins at the Harvard Museum of Comparative Zoology for curatorial support.

**REFERENCES**


Cercosporoid fungi (*Mycosphaerellaceae*) 4. Species on dicots (*Acanthaceae* to *Amaranthaceae*)

Uwe Braun¹, Pedro W. Crous², and Chiharu Nakashima³

¹Martin-Luther-Universität, Institut für Biologie, Bereich Geobotanik und Botanischer Garten, Herbarium, Neuwerk 21, 06099 Halle (Saale), Germany; corresponding author e-mail: uwe.braun@botanik.uni-halle.de
²CBS-KNAW, Fungal Biodiversity Centre, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands
³Graduate School of Bioresources, Mie University, 1577 Kurima-machiya, Tsu, Mie 514-8507, Japan

Abstract: The present paper continues a series of comprehensive taxonomic treatments of cercosporoid fungi (formerly *Cercospora s. lat.*), belonging to the *Mycosphaerellaceae* (*Ascomycota*). The fourth contribution of this series initiates treatments of cercosporoid fungi on dicots and comprises species occurring on hosts belonging to the families *Acanthaceae*, *Actinidiaceae*, *Adoxaceae*, *Aizoaceae*, *Altingiaceae*, and *Amaranthaceae*. The species are described and illustrated in alphabetical order under the particular cercosporoid genera, supplemented by keys to the species concerned. A detailed introduction, a survey of currently recognised cercosporoid genera, a key to the genera concerned, and a discussion of taxonomically relevant characters were published in the first part of this series. The following taxonomic novelties are introduced: *Cercospora blepharidicola* nom. nov., *C. celosigena* sp. nov., *C. justiciae-adhatodae* sp. nov., *C. justicigena* nom. nov., *C. sambucicolana* nom. nov., *C. thunbergiigena* nom. nov., *Cercospora pseudacyantheris* comb. nov., *Pseudocercospora cyathulanae* comb. nov., *P. depaeoidae* comb. nov., *P. varia* var. *viburni-sargentii* var. nov., *P. viburnicola* sp. nov., *P. viburni-erosi* sp. nov., and *P. viburni-nudi* sp. nov.

Article info: Submitted: 26 September 2015; Accepted: 29 October 2015; Published: 10 November 2015.

INTRODUCTION

True cercosporoid fungi belong to *Mycosphaerellaceae* (*Capnodiales*, *Ascomycota*) and comprise a very large group of plant pathogenic, leaf-spotting, economically relevant species that cause diseases on a wide range of hosts, including numerous cultivated plants. In spite of the enormous relevance of this fungal group, there is no modern comprehensive treatment of *Cercospora* and allied genera, and the only monograph published by Chupp (1954) is seriously outdated. Therefore, a monographic series with treatments of cercosporoid fungi based on host families was initiated (Braun et al. 2013) with the aim of working towards a comprehensive monograph of this generic complex. So far three contributions have been published: part one dealing with cercosporoid fungi on other fungi (mycophylic taxa), on ferns as well as gymnosperms (Braun et al. 2013); part two dedicated to species on monocots, excluding true grasses (Braun et al. 2014); and part three with a treatment of cercosporoids on hosts of *Poaceae* (Braun et al. 2015). General chapters with generic descriptions and keys to accepted genera are included in the first part. The present contribution is the first part devoted to cercosporoid fungi on dicots, encompassing species on hosts of the families *Acanthaceae*, *Actinidiaceae*, *Adoxaceae*, *Aizoaceae*, *Altingiaceae*, and *Amaranthaceae*. The structure of part 4 follows the principles circumscribed in part 1 (Braun et al. 2013).

MATERIALS AND METHODS

The present work is a compilation based on our papers and unpublished data, as well as the global literature. Details of methods are given in the papers cited under references. As far as new examinations are concerned, fungal structures have been examined by standard methods of light microscopy, using an Olympus BX50 microscope, with distilled water and lactic acid as media, but without any staining. If possible, measurements of 30 conidia and other structures have been made at a magnification of ×1000. All illustrations have been prepared by UB. The following abbreviations are used: author names follow Brummit & Powell (1992), journals Bridson (2004a, b), and exsiccatae http://www.botanischestaatssammlung.de/DatabaseClient/IndExs/index.jsp (IndExs – Index of Exsiccatae). Taxonomy and nomenclature of plant families, genera and species are based on the “Angiosperm Phylogeny Website” (http://www.mobot.org/mobot/research/apweb/), Tropicos database (http://www.tropicos.org/), and The Plant List (http://www.theplantlist.org).

© 2015 International Mycological Association

You are free to share - to copy, distribute and transmit the work, under the following conditions:

**Attribution:** You must attribute the work in the manner specified by the author or licensor (but not in any way that suggests that they endorse you or your use of the work).

**Non-commercial:** You may not use this work for commercial purposes.

**No derivative works:** You may not alter, transform, or build upon this work.

For any reuse or distribution, you must make clear to others the license terms of this work, which can be found at http://creativecommons.org/licenses/by-nc-nd/3.0/legalcode. Any of the above conditions can be waived if you get permission from the copyright holder. Nothing in this license impairs or restricts the author’s moral rights.
TAXONOMIC TREATMENT

Cercosporoid species on dicots s. lat. (Acanthaceae to Amaranthaceae)

Acanthaceae

Cercospora

Key to Cercospora species on Acanthaceae

1 Conidia in chains, 8–40 × 2–3 µm, (0–)1–3(–4)-septate, hyaline; on Justicia adhatoda [Adhatoda vasica] ................................................................. Cercospora justiciae-adhatodae .......................... 2

2 (1) Stromata large, 20–85 µm; conidiophores short, 10–40 × 3–5 µm, 0–4-septate; conidia short, acicular-subcylindrical, 20–50 × 2–4 µm, 0–5-septate .......................................................... C. balaghatensis
   Stromata lacking or smaller, 10–30 µm diam; and/or conidiophores much longer, 10–500 µm, pluriseptate throughout; conidia longer, 15–360 µm, pluriseptate ........................................ 3

3 (2) Conidia obclavate-cylindrical with obconically truncate base .......................................................... 4
   Conidia consistently acicular, base truncate, or at least longer conidia acicular, mixed with shorter obclavate-cylindrical conidia ................................. 6

4 (3) Conidiophores short, 10–40 × 2.5–5 µm, 0–1-septate; conidia 25–80 µm long; on Justicia spicigera, Central America (Guatemala) ..................................................................................... C. jacobiniicola
   Conidiophores longer, about 30–185 µm, with more than two septa; conidia longer, about 20–170 µm .................. 5

5 (4) Stromata 30–50 µm diam; conidiophores long and aseptate, 35–120 × 3–6 µm;
   on Justicia betonica, Asia (India) ................................................................................ C. justiciigena
   Stromata lacking or very small, < 25 µm diam; length of the conidiophores similar, but 1–6-septate;
   on Lepidagathis spp. ................................................................................................ C. lepidagathidis

6 (3) Conidia acicular to obclavate-cylindrical, base truncate to obconically truncate ................. 7
   Conidia consistently acicular, base truncate ................................................................. 10

7 (6) Conidiophores relatively short, 10–30(–70) µm; stromata 10–70 µm diam; on Acanthus spp. .......... C. acanthi
   Conidiophores longer, 25–155 µm; stromata lacking or small, 10–30 µm; on other hosts ...................... 8

8 (7) Conidia narrow, 30–150 × 2–4 µm, average < 3 µm wide; on Andrographis spp. ......................... C. andrographidis
   Conidia wider, 3–5 µm, average > 3 µm; on other hosts .................................................. 9

9 (8) Conidiophores to 195 µm long; on Justicia spp. ................................................................. C. justiciicola
   Conidiophores much shorter, to 62.5 µm; on Crossandra spp. ............................................. C. crossandreae

10 (6) Stromata well-developed, 10–70 µm diam; conidiophores short, 10–40(–7) µm; on Acanthus sp. ........ C. acanthi
   Stromata lacking or small, about 10–45 µm diam; and/or conidiophores much longer, at least partly longer than 50 µm; on other hosts ......................................................... 11

11 (10) Conidiophores 40–310 × 4–8.5 µm; conidia rather broad, 40–360 × 3–8 µm; on Thunbergia spp. ...... C. thunbergiana
   Conidiophores and conidia narrower; conidia about 1.5–5 µm wide; on other hosts or
   if on Thubergia conidia only 2–3 µm wide ................................................................ 12

12 (11) Conidia (2–)2.5–5.5(–6) µm wide, average > 3 ................................................................. 13
   Conidia narrower, 1.5–4 µm, average < 3 µm ................................................................. 17

13 (12) Conidiophores long, 30–500 × 3–8 µm; on Hygrophiella spp. ............................................... C. hygrophilae
   Conidiophores much shorter, to 250 µm; on other hosts .................................................. 14

14 (13) Stromata well-developed, about 15–55 µm diam; on Andrographis spp. ......................... C. andrographidicola
   Stromata lacking or small, about 10–25 µm diam; on other hosts .................................... 15
15 (14) Lesions formed as brown leaf spots with indefinite margin; on Asystasia spp. .............................................. C. asystasiana
Leasion visible as greyish white leaf spots; on other hosts ......................................................................................... 16

16 (15) Conidiogenous loci 2–3 µm wide; on Justicia adhatoda ................................................................................... C. adhatodae
Conidiogenous loci somewhat narrower, 1.5–2.5 µm wide; on Strobilathes ................................................................. C. strobilanthis

17 (12) Stromata well-developed, to 60 µm diam; on Barleria and Blepharis spp. .......................................................... 18
Stromata lacking or small, 10–25 µm diam; on other hosts .......................................................................................... 19

18 (17) Conidiophores long, 10–210 µm; on Barleria spp. ................................................................. C. barleriicola
Conidiophores much shorter, 15–65 µm; on Blepharis spp. ..................................................................................... C. blepharidicola

19 (17) Conidia very narrow, about 1.5–2.5 µm; on Ruellia spp. ............................................................... C. ruellina
Conidia wider, 2–4 µm; on other hosts .............................................................................................................................. 20

20 (19) Conidiophores 10–100 µm long; on Justicia and Rhytiglossa spp. ............................................................... C. diantherae
Conidiophores longer, to 220 µm; on other hosts ........................................................................................................... 21

21 (20) Leaf spots yellowish brown to dark brown, later greyish white with dark border, 0.5–4 mm diam; on Peristrophe spp. .................................................................................................................. C. peristrophes
Leaf spots dark brown to black, vein-limited, 3–10 mm diam; on Thunbergia .......................................................... C. thunbergiigena

Tabular key to Cercospora species on Acanthaceae according to host genera

Acanthus
A single species .................................................................................................................................................................. C. acanthi

Adhatoda, see Justicia

Andrographis
1 Conidia acicular, base truncate, 200–240 × 3.3–5 µm ..................................................................................... C. andrographidicola
Conidia acicular to obclavate, base truncate to obconically truncate, shorter and narrower,
30–150 × 2–4 µm ................................................................................................................................. C. andrographidis

Asystasia
A single species ................................................................................................................................................................. C. asystasiana

Barleria
A single species ................................................................................................................................................................. C. barleriicola

Blepharis
A single species ................................................................................................................................................................. C. blepharidicola

Crossandra
A single species ................................................................................................................................................................. C. crossandrae

Hygrophila
A single species ................................................................................................................................................................. C. hygrophilae

Justicia
1 Conidia in chains, 8–40 × 2–3 µm, (0–)1–3(–4)-septate, hyaline;
on Justicia adhatoda [Adhatoda vasica] ................................................................. Cercospora justiciae-adhatodae
Conidia formed singly, much longer and pluriseptate ........................................................................................... 2

2 (1) Stromata large, 20–85 µm diam; conidiophores short, 10–40 × 3–5 µm, 0–4-septate; conidia short,
acicular-subcylindrical, 20–50 × 2–4 µm, 0–5-septate ................................................................. C. balaghatensis
Stromata lacking or smaller, 10–30 µm diam; conidiophores much longer, 10–195 µm,
pluriseptate throughout; conidia longer, 15–250 µm, pluriseptate ................................................... 3
3 (2) Conidia consistently acicular, base truncate, or at least longer conidia acicular, mixed with shorter obclavate-cylindrical conidia ................................................................. 4
Conidia obclavate-cylindrical with obconically truncate base, acicular conidia lacking .................................................. 6

4 (3) Conidia acicular to obclavate-cylindrical, base truncate to obconically truncate ........................................ C. justiciicola
Conidia consistently acicular, base truncate ................................................................................................................... 5

5 (4) Conidia, 35–250 × 2.5–5 µm; on Justicia adhatodae [Adhatoda vasica], Asia (India) ............................................. C. adhatodae
Conidia narrower, 40–180 × 2–4 µm; on Justicia spp., common in North America
(records from Africa and Asia rare and unproven) .................................. C. diantherae

6 (3) Stromata 30–50 µm diam; conidiophores long and aseptate, 35–120 × 3–6 µm; conidia 50–100 µm long;
on Justicia betonica, Asia (India) .............................................................. C. justiciigena
Stromata smaller, 10–25 µm diam; conidiophores much shorter, 10–40 × 2.5–5 µm, 0–1-septate;
conidia 25–80 µm long; on Justicia spicigera, Central America (Guatemala) ......................................................... C. jacobiniicola

**Lepidagathis**
A single species .................................................................................. C. lepidagathidis

**Pachystachys**
A single species ................................................................................ C. justiciicola

**Peristrophe**
A single species ................................................................................ C. peristrophes

**Rhytiglossa**
A single species ................................................................................ C. diantherae

**Ruellia**
A single species ................................................................................ C. ruellina

**Rungia**
A single species ................................................................................ C. justiciicola

**Strobilanthes**
A single species ................................................................................ C. strobilanthis

**Thunbergia**
1 Conidia acicular to somewhat obclavate, 40–360 × (2–)3–8 µm,
base truncate to somewhat obconically truncate .............................................. C. thunbergiana
Conidia acicular, narrower, 45–155 × 2–3 µm, base truncate .............................................. C. thunbergiigena

**Cercospora species on Acanthaceae**

**Cercospora acanthi** Pass., in Rabenh., *Fungi Eur. Exs.*, Edn Nov., Ser. Sec., Cent. 3 (Resp. Cent. 23),
no. 2273 (1876).

(Fig. 1)


**Illustration:** Chupp (1954: 21, fig. 1).


**Description:** Leaf spots amphigenous, circular, subcircular to angular-irregular, 1–12 mm diam, sometimes confluent and larger, sometimes zonate, at first pale greenish, later yellowish to ochraceous, brown, finally greyish brown to greyish white, usually with a narrow darker border, occasionally somewhat raised. Caespituli amphigenous, punctiform, dark brown, later greyish white by abundant conidiation, scattered. Mycelium internal. Stromata well-
developed, 10–70 µm diam, substomatal to immersed, large stromata often rupturing the stomata, somewhat erumpent, pale, subhyaline to yellowish brown, later dark brown, composed of swollen hyphal cells, 2.5–10 µm diam, circular to somewhat irregular in shape. \textit{Conidiophores} in small to large fascicles, dense, arising from stromata, through stomata or erumpent, erect, straight to curved or somewhat geniculate-sinuous, unbranched, 10–40(–60) × 3–7 µm, 0–2-septate, subhyaline, yellowish to pale olivaceous, paler towards the tip, somewhat darker in mass, thin-walled, smooth; conidiogenous cells integrated, terminal or conidiophores reduced to conidiogenous cells, 10–30 µm long, mostly with a single or two, occasionally several conspicuous conidiogenous loci, 1.5–2.5 µm diam. \textit{Conidia} solitary, acicular, smaller conidia narrowly obclavate-cylindrical, straight to curved, 20–130 × 2–5 µm, rarely longer, shorter conidia 1–5-septate, longer ones indistinctly pluriseptate, hyaline, thin-walled, smooth, apex pointed, base truncate to short obconically truncate, (1.5–)2–2.5(–3) µm wide, hila thickened and darkened.


\textit{Host range and distribution}: On \textit{Acanthus} (\textit{hungaricus} [balcanicus], \textit{mollis} [longifolius, nigre], \textit{spinosus} [spinosisimus], \textit{Acanthus} spp., \textit{Ruttya fruticosa}, \textit{Acanthaceae}, Africa (Algeria, Ethiopia), Europe (Germany, Hungary, Italy, Romania).

\textit{Notes}: The first valid description of \textit{Cercospora acanthi} dates back to 1876 (in Rabenh., Fungi Eur. Exs. 2273), with a brief description on the label. The secondary description in “\textit{Hedwigia} 16. 123 (1877)”, usually cited as the original description, is younger; this is a consequence of Saccardo not accepting publication in exsiccate labels as acceptable. This species is a true \textit{Cercospora} readily distinguishable from the \textit{C. api s. lat} complex by its short, aseptate or sparingly septate, pale conidiophores and acicular to obclavate conidia. Records of this species on \textit{Peristrophe bicalyculata} from Myanmar (Thaung 1984) are very doubtful and excluded.


(Fig. 2)


\textit{Illustration}: Chowdhury (1956: 85, fig. 1).

\textit{Description}: Leaf spots amphigenous, 0.5–8 mm diam, often confluent, forming larger patches, subcircular to irregularly shaped, greyish white. \textit{Caespituli} amphigenous, fine, dark. \textit{Mycelium} internal. \textit{Stromata} lacking or small, substomatal, brown. \textit{Conidiophores} in small, divergent fascicles, arising from internal hyphae or stromatic hyphal aggregations, through stomata or erumpent, erect, straight, subcylindrical to geniculate in the upper half, 40–190 × 4–6 µm, 0–7-septate, brown to dark brown, paler towards the tip, thin-walled, smooth; conidiogenous cells integrated, terminal, conidiogenous loci conspicuous, about 2–3 µm wide, thickened and darkened. \textit{Conidia} solitary, acicular, straight to curved, 35–250 × 2.5–5.5 µm, 2–22-septate, hyaline, thin-walled, smooth, apex pointed, base truncate, about 2–3 µm wide, hila thickened and darkened.


\textit{Host range and distribution}: On \textit{Justicia adhatoda} [\textit{Adhatoda vasica}], \textit{Acanthaceae}, Asia (India, Asom, Karnataka, Uttar Pradesh, West Bengal).
Notes: This is a species of the *Cercospora apii s. lat.* complex. Several Indian collections from Karnataka, West Bengal and Uttar Pradesh have been examined (K(M) IMI 135861, 293593 and 330436).


(Fig. 3)

**Literature:** Chi (1994: 94).

**Illustration:** Chi (1994: 94, fig. 86).

**Description:** Leaf spots amphigenous, circular, elliptical to irregular, centre pale, margin indistinct, greyish green below. *Caespituli* amphigenous. *Mycelium* internal. Stromata well-developed, 16–57 μm diam, brown. Conidiophores in loose fascicles, 4–15, arising from stromata, erect, straight, subcylindrical, non-geniculate, unbranched, 150–233 × 3.3–6.7 μm, 2–10-septate, olivaceous; conidiogenous cells integrated, terminal, usually with a single terminal conidiogenous locus, thickened and darkened. **Conidia**...
solitary, acicular, straight to somewhat curved, 200–240 × 3.3–5 µm, hyaline, thin-walled, smooth, apex pointed, base truncate, hila thickened and darkened.


**Host range and distribution:** On *Andrographis paniculata*, Acanthaceae, Asia (China).

**Notes:** Belonging to the *Cercospora apii s. lat.* complex, but type material was not available for a re-examination. To-anun *et al.* (2011) described and illustrated “*C. andrographidicola*” on *Andrographis paniculata* from Thailand. This material is, however, morphologically distinct by lacking or small stromata, up to 30 µm diam, shorter, geniculate conidiophores, and much shorter, acicular to obclavate conidia, and rather belongs to *C. andrographidis*.


(Fig. 4)

**Literature:** Vasudeva (1963: 36), Braun & Crous (2003: 57), Kamal (2010: 16), To-anun *et al.* (2011: 30), as “*C. andrographidicola*”.

**Illustrations:** Thirumalachar & Govindu (1953: plate VI, figs 5–6), To-anun *et al.* (2011: 30, fig. 12), as “*C. andrographidicola*”.

**Description:** Leaf spots amphigenous, circular to somewhat angular-irregular, 2–6 mm diam, at first dingy greenish, olivaceous or brown, later greyish white, surrounded by a narrow brown margin or marginal line, slightly raised or margin broader, pinkish brown. Caespituli amphigenous, mainly epiphyllous, not very conspicuous. Mycelium internal. Stromata lacking or inconspicuous and small, only a few swollen hyphal cells, 5–20 µm diam, usually intraepidermal, if present to 30 µm diam, brown, cells 3–8 µm diam, wall slightly thickened. Conidiophores solitary or in small divergent fascicles, arising from internal hyphae or aggregations of swollen hyphal cells, erumpent, erect, straight to geniculate, unbranched, 15–165 × 3–6.5 µm, 1–9-septate, light to medium dark brown, wall thin to slightly thickened, smooth; conidiogenous cells integrated, terminal, occasionally intercalary, 15–30 µm long, sympodial, conidiogenous loci conspicuous, thickened and darkened, 2–3 µm diam. *Conidia* solitary, acicular to somewhat obclavate, straight to curved, 30–150 × 2–4 µm, 1–16-septate, hyaline, thin-walled, smooth, apex subacute, base truncate to somewhat obconically truncate, 2–2.5 µm wide, hila thickened and darkened.


**Host range and distribution:** On *Andrographis* (paniculata, *Andrographis* sp.), Acanthaceae, Asia (India, Bihar, Andhra Pradesh, West Bengal; Thailand).

---

**Fig. 4. Cercospora andrographidis** (BPI 432654). A. Conidiophore fascicle. B. Solitary conidiophore. C. Conidia. Bar = 10 µm.

**Note:** The conidia of this species were described to be “acicular” with obconically truncate base, i.e. they are, at least partly, obclavate as depicted in Thirumalachar & Govindu (1953: plate VI, fig. 6). This could be confirmed on the base of an examined collection from India (West Bengal, Midnapur, Daspur, 20 Mar. 1967, M. Mandal, BPI 432654). To-anun *et al.* (2011) described and illustrated “*C. andrographidicola*” on *Andrographis paniculata* from Thailand. This material, characterised by lacking or small stromata and acicular to obclavate conidia, is quite distinct from true *C. andrographidicola* and rather belongs to *C. andrographidis*. An additional sample from India (Daspur, BPI 432654) has been examined.

(Fig. 5)


Illustration: Yen (1967: 181, fig. 2), Yen & Lim (1980: 206, fig. 9).

Description: Leaf spots amphigenous, on faded leaves, scattered, subcircular, 2–5 mm diam, brown, margin indefinite. Caespituli hypophyllous, rather inconspicuous. Mycelium internal. Stromata lacking or almost so. Conidiophores solitary or in small divergent fascicles, 2–5, arising from internal hyphae or small substomatal hyphal aggregations, through stomata, erect, straight to somewhat curved, subcylindrical to distinctly geniculate-sinuous, unbranched, 30–120(–135) × 3–6 µm, 0–5-septate, brown to dark brown, thin-walled, smooth; conidiogenous cells integrated, terminal or intercalary, about 15–30 µm long, conidiogenous loci thickened and darkened, about 2–2.5 µm diam. Conidia solitary, acicular to somewhat obclavate, straight to curved or somewhat sigmoid, 45–185 × 2.5–5 µm, 4–20-septate, hyaline, thin-walled, smooth, apex subacute, base truncate or slightly obconically truncate, 2–3 µm wide, hila thickened and darkened.


Host range and distribution: On Asystasia (chelonoides, nemorum), Acanthaceae, known from the type collection [records of Cercospora justiciicola on Asystasia gangetica and C. cf. malloti on A. salicifolia might belong to this species – see notes].

Notes: This species is part of the Cercospora apii s. lat. complex. Records of Cercospora justiciicola on Asystasia gangetica [coromandeliana] (see Cous & Braun 2003: 234) are unclear but might belong to C. asystasiana.

Nguathom et al. (2015) examined Cercospora species from northern Thailand using molecular methods. Sequences derived from several collections on various unrelated host species, including Asystasia salicifolia, clustered in a clade tentatively denominated as C. cf. malloti in Groenewald et al. (2013: 157). Taxa belonging to this clade represented the most common Cercospora encountered in this study. Cercospora cf. malloti is morphologically part of the C. apii complex. The true C. malloti was based on North American Cercospora material infecting Mallotus japonicus. The tentative allocation of this clade comprising plurivorous C. apii-like races to C. malloti is neither settled nor finally proven since cultures and sequences based on North American collections retrieved from Mallotus are not yet available. Due to the wide but hitherto little known host range of this taxon, it is not yet possible to exclude that several other host species and older species names of Cercospora might be involved. Therefore, it is currently impossible to resolve the clade concerned.


(Fig. 6)

Fig. 5. Cercospora asystasiana (PC, holotype). A. Conidiophore fascicle. B. Conidiophore. C. Conidia. Bar = 10 µm.


Illustration: Singh (1977: 18, fig. 1).

Description: Leaf spots dingy grey with light to dark brown irregular margin. Caespituli amphigenous, scattered, punctiform, dark. Mycelium internal. Stromata 20–85 µm diam, immersed, olivaceous-brown. Conidiophores in large,
loose to usually dense fascicles, arising from stromata, erect, straight, subcylindrical, barely geniculate, unbranched, about 10–40 × 3–5 µm, 0–4-septate, pale olivaceous to brownish, thin-walled, smooth; conidiogenous cells integrated, terminal, conidiogenous loci conspicuous, thickened and darkened, about 2–2.5 µm diam. Conidia solitary, acicular or subcylindrical, straight to slightly curved, 20–50 × 2–4 µm, 0–5-septate, hyaline, thin-walled, smooth, apex subacute or subobtuse, base truncate, 1.5–3 µm wide, hila thickened and darkened.


Host range and distribution: Only known from the type collection.

Notes: A true Cercospora s. str. distinct from C. api s. lat. by having very large stromata, short, densely fasciculate conidiophores and short, 0–4-septate conidia.

Cercosporoid fungi 4

Cercospora barleriicola Payak & Thirum., Indian Phytopathol. 2: 191 (1949); as “barlericola” (Fig. 7)


Illustrations: Govindu & Thirumalachar (1957: plate VIII, fig. 10), Vasudeva (1963: 47, fig. 16), To-anun et al. (2011: 31, fig. 13).

Description: Leaf spots amphigenous, subcircular to angular-irregular, 2–8 mm diam, diffuse discolorations, yellowish to dark reddish brown, later pale brownish to greyish brown or greyish white with darker border. Caespituli amphigenous, subeffuse to punctiform, dark brown. Mycelium internal. Stromata almost lacking or small to moderately large, 10–60 µm diam, olivaceous-brown to dark brown, substomatal to immersed. Conidiophores in small to moderately large fascicles, loose to dense, arising from stromata, through stomata or erumpent, erect, straight, subcylindrical to...
Braun et al.

moderately geniculate-sinuous, unbranched, 10–210 × 2.5–6 µm, 1- to pluriseptate throughout, pale to medium olivaceous-brown or brown throughout or paler towards the tip, wall thin to slightly thickened, smooth; conidiogenous cells integrated, terminal, 10–30 µm long, conidiogenous loci conspicuous, thickened and darkened, (1.5–)2–3 µm diam. Conidia solitary, longer conidia acicular with truncate base, shorter ones may be obclavate-cylindrical with obconically truncate base, straight to curved, 30–220 × 2–4 µm, 3–18-septate, hyaline, thin-walled, smooth, apex pointed, base truncate to somewhat obconically truncate, 1.5–3 µm wide, hila thickened and darkened.


Host range and distribution: On Barleria (cristata, priofitis, Barleria sp.), Acanthaceae, Asia (India, Jammu and Kashmir, Kamataka, Maharashtra, Madhya Pradesh, Uttar Pradesh; Thailand), West Indies (Jamaica).

Notes: Several Indian collections and a sample from Jamaica on Barleria spp. (K(M) IMI 102433, 163693, 226980, 265815) have been examined and proved to belong to a single variable Cercospora s. str. species belonging to the C. api s. lat. complex. Since phylogenetic data are not yet available, it remains unclear if a single polymorphous species or several cryptic species are involved.

Cercospora blepharidicola U. Braun, nom. nov. MycoBank MB814564


Illustration: Dubey et al. (2011: 516, fig. 3).

Description: Leaf spots amphigenous, mainly epiphyllous, small to large, scattered, dark brown. Caespituli amphigenous, effuse, uniformly distributed. Mycelium internal. Stromata well-developed, to 45 µm diam, dark olivaceous. Conidiophores in loose fascicles, erect, straight, subcylindrical, flexuous, unbranched, arising from a swollen base, about 15–65 × 4–6 µm, 1–3-septate, light olivaceous, thin-walled, smooth; conidiogenous cells integrated, terminal, cylindrical, cicatrized, thickened and darkened. Conidia solitary, acicular, straight to curved, 20–112 × 1–4 µm, to 10-septate, hyaline, thin-walled, smooth, apex pointed, base truncate or subtruncate, hila thickened and darkened.


Host range and distribution: Only known from the type collection.

Notes: The name C. blepharidis R.K. Dubey et al. is a homonym of C. blepharidis Chidd. (Chiddarwar 1960).


Illustration: Jaganathan et al. (1972: 671, fig. 1).

Description: Leaf spots circular to irregular, 2–5 mm diam, confluent, foliage finally drying up, brown, border yellow, sometimes with concentric rings. Colonies white, later with dark brown margin. Mycelium internal. Conidiophores fasciculate, geniculate, unbranched, about 37.5–62.5 × 6 µm, 4–7-septate, brown. Conidia solitary, acicular to obclavate (“filliform according to the original description), about 53–106 × 3–6 µm, 5–11-septate, hyaline, apex pointed, base truncate to short obconically truncate, probably thickened and darkened.
Holotype: **India**: Tamil Nadu: Coimbatore, College Orchard of the Tamil Nadu Agricultural University, on *Crossandra infundibuliformis*, *Acanthaceae* (T.N. Agric. Univ., Coimbatore, Pl. Pathol. Herb. No. 231).

**Host range and distribution**: Only known from the type collection.

**Notes**: It is unknown if type material of this species is maintained. It was not available for examination. Based on the original description and illustration, we suppose that this species belongs to *Cercospora s. str.* although details of the conidiogenous loci and hila were not described.

**Cercospora diantherae** Ellis & Kellerm., *J. Mycol*. 1: 2 (1885).

(Fig. 9)

**Synonym**: *Cercospora jacobinae* Mendoza, *Philipp. J. Sci.* 75: 169 (1941) [holotype: **Philippines**: Manila, on *Justicia carnea*, Mendoza, no. 7124 (not traced)].


**Illustration**: Chupp (1954: 23, fig. 4).


**Description**: Leaf spots circular to somewhat angular-irregular, 1–5 mm diam, occasionally confluent and larger, slightly zonate, centre greyish white with narrow to often broad brown border. *Caespituli* amphigenous, dark brown. *Mycelium* internal. *Stromata* lacking to small, 10–25 µm diam, composed of a few swollen hyphal cells, brown, substomatal to intraepidermal. *Conidiophores* in small to moderately large fascicles, 2–15, divergent to moderately dense, arising from internal hyphae or stromatic hyphal aggregations, through stomata or erumpent, erect, straight, subcylindrical to sinuous or somewhat geniculate, unbranched or rarely branched, 10–100 × 3.5–6 µm, aseptate to pluriseptate throughout, pale to medium brown, wall thin to slightly thickened, smooth; conidiogenous cells integrated, terminal, 10–25 µm long, with a single to several conidiogenous loci, 2.5–3.5 µm diam. *Conidia* solitary, acicular or subacicular, straight to curved or occasionally somewhat sigmoid, 40–160(–180) × 2–4 µm, pluriseptate, distance between septa 5–15 µm, hyaline, thin-walled, smooth, apex acute to subobtuse, base usually truncate, occasionally somewhat obconically truncate, 2–3 µm wide, hila thickened and darkened.


**Host range and distribution**: On *Justicia* (*americana*, *carnea*, *ovata*, *Justicia* sp.), *Rhytiglossa humilis* [*Justicia humilis*], *Acanthaceae*, Asia (Philippines), North America (USA, Delaware, Florida, Illinois, Indiana, Kansas, Maryland, Missouri, Oklahoma, Texas, Washington, West Virginia), South America (Venezuela).

**Notes**: This species belongs to the *Cercospora apii* (*s. lat.*) complex. Type material (collected in Sep. 1884) is not preserved at NY. There are numerous topotypes from 1886, e.g. B; BPI 435404, 435692, 435700, 435701; NY 270701. However, the type material collected in Sep. 1884 was traced in CUP and FH. The CUP material is designated as lectotype. Records of *C. diantherae* on *Jacobinia* spp. are doubtful. The hosts concerned probably refer to *Justicia* spp. in the current sense.

(Fig. 10)

**Literature:** Crous & Braun (2003: 222), Kamal (2010: 52).

**Illustration:** Ponnappa (1968: 32, fig. 1).

**Description:** Leaf spots amphigenous, often marginal, oblong to irregular, 5–15 mm diam, dark brown to blackish. *Caespituli* amphigenous, fine, dark. *Mycelium* internal. *Stromata* lacking or small, substomal, about 10–25 μm diam, dark olivaceous to brown. *Conidiophores* in small to moderately large fascicles, divergent to dense, arising from internal hyphae or stromata, through stomata, erect, straight, subcylindrical-conical to slightly geniculate-sinuous, unbranched, 10–40 × 2.5–5 μm, 0–1-septate, subhyaline to pale olivaceous-brown, thin-walled, smooth; conidiophores reduced to conidiogenous cells or conidiogenous cells integrated, terminal, 10–30 μm long, conidiogenous loci conspicuous, formed as minute circles, about 1 μm diam, only margin slightly thickened and darkened (paracercosporoid). *Conidia* solitary, cylindrical to subclavate-subcylindrical, straight to slightly curved, 25–80(–105) × (2.5–)3–4(–5) μm, 2–7(–9)-septate, hyaline to very pale greenish or olivaceous, thin-walled, smooth, apex obtuse to subacute, base short obconically truncate, occasionally truncate, 1–1.5 μm wide, barely thickened and darkened.

**Holotype:** Guatemala: Chimaltenango, on Justicia spicigera [Jacobiina spicigera], Acanthaceae, 2 Oct. 1941, A. S. Muller 42 (CUP 40082).
Cercosporoid fungi 4

Host range and distribution: Only known from the type collection.

Note: Tentatively maintained in Cercospora. The conidia are colourless or almost so, the conidiogenous loci are conspicuous, but minute, ca. 1 µm wide, and somewhat paracercospora-like. Cultures and results based on molecular sequence analyses are necessary to resolve the true generic affinity of this species.

Cercospora justiciae-adhatodae U. Braun, sp. nov.
MycoBank MB814556
(Fig. 12a,b)

Diagnosis: Differs from Cercospora adhatodae and all other Cercospora species on hosts of the Acanthaceae in forming small catenate conidia, (8–)12–35(–40) × 2–3 µm, (0–)1–3(–4)-septate.

Description: Leaf spots amphigenous, circular, subcircular to slightly angular-irregular, 0.5–4 mm diam, at first brown, but soon turning greyish white to white, margin narrow, somewhat raised, dark, brown, dark violet to almost blackish. Caespituli amphigenous, punctiform, scattered to dense, brown to dark brown. Mycelium internal. Stromata lacking or almost so to well-developed, 10–30 µm diam, substomatal to intraepidermal, brown, cells 2–5 µm diam, wall at first thin, later slightly thickened. Conidiophores in small, loose to moderately large and dense fascicles, arising from substomatal or intraepidermal hyphae or stromata, emerging through stomata or erumpent, erect, straight to curved, subcylindrical or somewhat attenuated towards the tip to moderately geniculate-sinuous, unbranched, 10–50 × 2–5 µm, 0–3-septate, pale olivaceous to olivaceous-brown, paler towards the tip, thin-walled, smooth; conidiogenous cells integrated, terminal or conidiophores reduced to conidiogenous cells, 10–25 µm long, proliferation sympodial, conidiogenous loci conspicuous, 1–2 µm wide, somewhat thickened and darkened. Conidia solitary and catenate, in simple or occasionally branched chains, narrowly cylindrical-fusiform, short obclavate, (8–)12–35(–40) × 2–3
µm, (0–)1–3(–4)-septate, hyaline, thin-walled, smooth, apex subacute, subobtuse or short conically truncate, base subtruncate to short obconically truncate, 1–1.5 µm wide, hila slightly thickened and darkened.


Host range and distribution: Only known from the type collection.

Notes: Type material of this species was originally deposited as Cercospora adhatodae, but it is quite distinct from the latter species, which belongs to the C. api complex, by much shorter, narrower, usually 1–3-septate conidia formed in chains. These characteristics distinguish the new species from all other Cercospora species described on hosts belonging to the Acanthaceae. Previously one were inclined to put this species in Passalora s. lat. (including Phaeoramularia), but catenate, colourless conidia are rather in favour of Cercospora s. str., which has recently been shown in the course of phylogenetic studies of cercosporoid hyphomycetes (see Braun et al. 2013). Therefore, the present new species is, at least for the interim, placed in Cercospora although cultures and molecular data are not yet available.

Cercospora justiciigena U. Braun, nom. nov.
MycoBank MB814565
(Fig. 13)
Basionym: Cercospora acanthacearum var. macrospora

Illustration: Karan & Manoharachary (1978: 158, fig. 1–2).

Description: Leaf spots epiphyllous, zonate, 4–6 mm diam, white, surrounded by a pinkish border. Mycelium internal. Stromata about 30–50 µm diam, dark brown, pseudoparenchymatic. Conidiophores fasciculate, arising from stomata, erect, straight to curved, unbranched, geniculate in the upper half, about 35–120 × 3–6 µm, aseptate, brown, colourless towards the tip; conidiophores reduced to conidiogenous cells, apex rounded with dark annular conidiogenous loci. Conidia solitary, obclavate, straight, about 50–100 × 3–4.5 µm, pluriseptate, hyaline, thin-walled, smooth, tips pointed, base short obconically truncate, hila somewhat thickened and darkened.


Host range and distribution: Only known from the type collection.

Notes: This fungus was introduced as variety of C. acanthacearum, although quite different from the latter species by its much longer conidiophores and much wider conidia. Owing to the description and illustration of conspicuous, dark conidiogenous loci and hila as well as colourless conidia formed singly, C. acanthacearum var. macrospora is undoubtedly a true Cercospora (s. str.) species. The generic affinity of C. acanthacearum is unclear, but this species might rather be a member of Pseudocercospora.

Cercospora justiciicola F.L. Tai, Lloydia 11: 47 (1948); as “justiciaecola”.

(Fig. 14)


Illustrations: Tai (1948: 44, fig. 9), Ellis (1976: 243, fig. 183 A), Guo et al. (2005: 17, fig. 1).

Description: Leaf spots amphigenous, subcircular, 3–10 mm diam, yellowish, ochraceous, pale brown to finally greyish white, often somewhat zonate, sometimes with yellowish halo. Caespituli amphigenous, punctiform, brown. Mycelium internal. Stromata almost lacking to developed, 10–30 µm diam, brown, substomatal to immersed. Conidiophores in small to moderately large fascicles, divergent, arising from stomata, through stomata or erumpent, erect, straight, subcyldindrical to usually geniculate-sinuous, unbranched, 25–195 × 3–6 µm, 2–10-septate, olivaceous-brown, thin-walled, smooth; conidiogenous cells integrated, terminal to intercalary, about 15–35 µm long, conidiogenous loci thickened and darkened, 1.5–3 µm diam. Conidia solitary, acicular to obclavate-subcyldindrical, straight to curved, (15–)40–140(–155) × 3–4.5(–5) µm, (2–)3–10(–17)-septate, hyaline, thin-walled, apex subacute, base truncate to short obconically truncate, 2–3 µm wide, hila thickened and darkened.


Host range and distribution: On Justicia (betonica, carnea [Jacobinia obtusior], diffusa, flava, gendarussa, procumbens, simplex, Justicia sp.), Pachystachys lutea [Justicia lutea], Rungia (pectinata [Justicia pectinata, Rungia parviflora], repens [Justicia repens], Rungia sp.), Acanthaceae, Africa (Guinea, Mauritius), Asia (Brunei, China; India, Andhra Pradesh, Madhyar Pradesh, Uttar Pradesh; Nepal), North America (USA, Florida).

Notes: A true Cercospora s. str. close to or conspecific with C. api s. lat. Records of C. justiciicola on Asystasia gangetica [coromandeliana] (Crous & Braun 2003) are unclear, doubtful and might belong to C. asystasiana.


Illustrations: Govindu & Thirumalachar (1955: plate 3, fig. 3), Vasudeva (1963: 134, fig. 92).

Description: Leaf spots amphigenous, circular or subcircular, 4–7 mm diam, centre greyish white, margin pinkish or brown. Caespituli amphigenous. Mycelium internal. Stromata lacking or small, composed of a few swollen hyphal cells, brown. Conidiophores in well-developed, dense fascicles, erect, mostly unbranched, strongly geniculate-sinuous, 33–183 × 2.8–5.7 µm, 1–6-septate, pale olivaceous to brown, tips colourless; conidiogenous cells integrated, terminal or intercalary, with several conspicuous conidiogenous loci. Conidia solitary, obclavate-cylindrical, straight to slightly curved, 23–170 × 2.8–4.2 µm, 1–14-septate, hyaline, thin-walled, smooth, apex pointed, base short obconically truncate, hila thickened.

Holotype: India: Karnataka, Mysore, Bababudans, on Lepidagathis cuspidata, 20 May 1953, H. C. Govindu (not traced).

Host range and distribution: On Lepidagathis (cuspidata, incurva [hyalina]), Acanthaceae, Asia (India, Karnataka, Maharashtra, Uttar Pradesh; Pakistan).

Notes: In the original publication, Govindu & Thirumalachar (1955) mentioned that type material of this species was deposited at BPI, HCIO and IMI. However, type collections could neither be traced at BPI nor IMI. Based on the original description and illustration, this species is undoubtedly a true Cercospora s. str. Records of this species from West Indies (Puerto Rico, and Virgin Islands) on Lepidagathis alopecuroides are results of misidentifications and belong to Pseudocercospora lepidagathidis.

Cercospora peristrophes Thirum. & Govindu, Sydowia 7: 47 (1953).

(Fig 15)


Description: Leaf spots amphigenous, circular to somewhat angular-irregular, scattered, 0.5–8 mm diam, yellowish brown, brown to dark brown, centre finally dingy grey to greyish white, margin indefinite, marginal slightly raised or surrounded by a darker border. Caespituli amphigenous, scattered, finely punctiform to effuse, greyish white by abundant conidal formation or brownish. Mycelium internal; hyphae branched, septate, pale olivaceous or brownish. Stromata almost lacking or small, 10–25 µm diam, substomatal, olivaceous-brown or brown. Conidiophores solitary or in small fascicles, 2–7, divergent, arising from internal hyphae or stromata, through stomata, erect, straight, somewhat curved to geniculately-sinuous, unbranched, 30–220 × (2.5–)3–7 µm, 1–16-septate, pale olivaceous.

Fig. 15. Cercospora peristrophes (BPI 439429, isolecotype). A. Conidiophore fascicle. B. Conidiophore tips. C. Conidia. Bar = 10 µm.
to olivaceous brown, paler towards the tip, thin-walled, smooth; conidiogenous cells integrated, terminal and intercalary, 10–35 µm long, conidiogenous loci thickened and darkened, 2.5–3.5 µm diam. Conidia solitary, acicular or subacicular to somewhat obclavate-subcylindrical when shorter, 30–130–150) × 2.5–4.5 µm, 1–15-septate, hyaline, thin-walled, smooth, apex subacute to obtuse, base truncate or almost so, 2–3 µm wide, hila thickened and darkened.

Lectotype (designated here, MycoBank, MBT202781):

Host range and distribution: On Peristrophe bicalyculata, Acanthaceae, Asia (India, Bihar, Maharashtra, Uttar Pradesh; Myanmar), Africa (Somalia).

Notes: This species belongs to the Cercospora apii s. lat. complex. Records from “Nepal” (Crous & Braun 2003, MycoBank and Index Fungorum) refer to the type of C. peristrophigena (Rao et al. 1999), which is, however, incorrect since this species was described from India (Uttar Pradesh).

(Fig. 16)


Illustration: Rao et al. (1999: 87, fig. 13).

Description: Leaf spots amphigenous, circular or almost so, 1–6 mm diam, brown to blackish on the upper leaf surface, whitish green with olivaceous margin below. Caespituli amphigenous, effuse. Mycelium internal; hyphae branched, septate, hyaline to pale olivaceous. Stromata substomatal, 10–35 µm diam, olivaceous to olivaceous-brown. Conidiophores solitary or in small fascicles, 2–5, arising from stromata, through stomata, erect, straight to geniculate-sinuous, unbranched, about 20–125 × 2.5–5 µm, 2–9-septate, light olivaceous to olivaceous-brown, thin-walled, smooth; conidiogenous cells integrated, terminal and intercalary; conidiogenous loci conspicuous, thickened and darkened. Conidia solitary, narrowly acicular, shorter conidia sometimes subcylindrical to somewhat obclavate, straight to curved, often with short lateral germ tubes which may give rise to secondary conidia, about 30–145 × 1.5–2.5 µm, 3–18-septate, hyaline, thin-walled, smooth, apex obtuse to subacute, base truncate, hila thickened and darkened.


Host range and distribution: Only known from the type collection.

Notes: This species is a typical member of the Cercospora apii s. lat. complex with acicular conidia. A morphologically well agreeing North American sample on Ruellia ciliosa has been examined (USA, Illinois, Chandlerville, 18 Aug. 1886, A. B. Seymour, BPI 435186), although the conidiophores in the latter collection are much longer and somewhat broader, 60–280 × 4–7 µm. However, if Indian and North America collections are conspecific requires phylogenetic examination and confirmation. Indian as well as North American samples seems to pertain to plurivorous species of the C. apii complex.
**Cercospora strobilanthis** Chidd., *Mycopathol. Mycol. Appl.* **17**: 77 (1962); as “strobilanthidis”.

*Description:* Leaf spots amphigenous, circular, subcircular or somewhat irregular, 1–7 mm diam, brown, greyish brown, finally greyish white, with dark brown border, sometimes with diffuse brownish halo, finally sometimes with shot-hole symptoms. *Caespituli* amphigenous, mostly hypophyllous, not very distinct or finely punctiform, dark. *Mycelium* internal. *Stromata* lacking or small, forming small aggregations of swollen hyphal cells, 10–30 µm diam, brown. *Conidiophores* in small fascicles, 2–15, divergent, occasionally solitary, arising from internal hyphae or small stromata, through stomata, erect, straight to geniculate-sinuous, unbranched, 40–220 × (3–)4–8 µm, 2–9-septate, brown, paler towards the tip, thin-walled, smooth; conidiogenous cells integrated, terminal and intercalary, 10–40 µm long, conidiogenous loci conspicuous, thickened and darkened, (1.5–)2–5 µm wide. *Conidia* solitary, acicular to somewhat obclavate, straight to curved, 40–360 × (2–)3–8 µm, 3–34-septate, occasionally somewhat constricted at septa, hyaline, thin-walled, smooth, base truncate or slightly obconically truncate, 2–5 µm wide, hila thickened and darkened.

**Holotype:** India: Maharashtra: Mumbai, Matheran, on *Strobilanthes* sp., 12 Dec. 1956, P. P. Chiddarwar 29 (K(M) IMI 83190).

**Host range and distribution:** On *Strobilanthes* sp., Acanthaceae, Asia (China, Guangdong; India, Maharashtra).

**Note:** A true *Cercospora* s. str. belonging to the *C. apii* s. lat. complex, but well characterised by conidia that are often somewhat attenuated at the very base.

**Cercospora thunbergiana** J.M. Yen, *Rev. Mycol.* **30**: 198 (1965); as “thunbergiaena”.

(Fig. 18)

*Description:* Leaf spots amphigenous, circular to irregular, 2–10 mm diam, at first pale greenish, later brownish, finally greyish white or white, with darker border, narrow to moderately wide, brown to almost black. *Caespituli* hypophyllous, scattered, punctiform, fine, dark. *Mycelium* internal. *Stromata* almost absent or small, mainly substomatal, 10–25 µm diam, brown. *Conidiophores* in divergent fascicles, 2–15, rarely solitary, arising from stromata, through stomata, erect, straight, subcylindrical to distinctly geniculate, 1–6 times, unbranched, 35–250 × 4–5.5 µm, 1–7-septate throughout, pale brown or olivaceous-brown, wall thin or slightly thickened, smooth; conidiogenous cells integrated, terminal and intercalary, conidiogenous loci thickened and darkened, 1.5–2.5 µm diam. *Conidia* solitary, acicular to slightly obclavate, straight to curved, 30–195 × 3–5(–5.5) µm, 3–16-septate, hyaline, thin-walled, smooth, apex pointed, base truncate to usually somewhat attenuated at the very base (very short obconically truncate), 1.5–2.5 µm, hila thickened and darkened.

**Illustrations:** Yen (1965: 199, fig. 13), Yen & Lim (1980: 223, fig. 26), Guo et al. (2005: 20, fig. 3).


---

**Fig. 17. Cercospora strobilanthis** (K(M) IMI 83190, holotype). A. Conidiophore fascicle. B. Conidiophore tips. C. Conidia. Bar = 10 µm.
Lectotype (designated here, MycoBank, MBT202782):

Host range and distribution: On Thunbergia (alata, erecta, grandiflora, Thunbergia sp.), Acanthaceae, Asia (Brunei; India, Andhra Pradesh, Uttar Pradesh; Singapore), South America (Venezuela), West Indies (Cuba).

Cercospora thunbergiigena U. Braun & Crous, nom. nov.
MycoBank MB814567
(Fig. 19)


Description: Leaf spots amphigenous, often vein-limited, mostly marginal, 3–10 mm diam, dark brown to blackish. Caespituli amphigenous, fine. Mycelium internal. Stromata absent or small, substomatal, 10–20 µm diam, brown. Conidiophores in small, loose fascicles, arising from small stromata, through stomata, erect, straight to moderately geniculate, unbranched, about 50–220 × 4–5 µm, 3–10-septate, brown, thin-walled, smooth; conidiogenous cells integrated, terminal and intercalary, conidiogenous loci conspicuous, thickened and darkened, about 2 µm diam. Conidia solitary, narrowly acicular, straight to curved, 45–155 × 2–3 µm, 4–14-septate, hyaline, thin-walled, smooth, apex pointed, base truncate, 1.5–2 µm wide, hila thickened and darkened.


Illustration: Srivastava et al. (1995: 41, fig. 4).

Fig. 18. Cercospora thunbergiana (PC, lectotype). A. Conidiophore fascicle. B. Conidiophore tips. C. Conidia. Bar = 10 µm.

Fig. 19. Cercospora thunbergiigena (K(M) IMI 345300, holotype). A. Conidiophore fascicle. B. Conidia. Bar = 10 µm.
**Holotype:** India: Uttar Pradesh: Gorakhpur, Ramgarh area, on *Thunbergia grandiflora*, Acanthaceae, Dec. 1989, V. P. Pandey (K(M) IMI 345300).

**Host range and distribution:** Only known from the type collection.

**Notes:** This species belongs to the *C. apiī s. lat.* complex. It resembles *C. thunbergiana*, but differs in having much narrower conidia.

**Doubtful, excluded and insufficiently known species**


**Illustration:** Govindu & Thirumalachar (1954: pl. 6, fig. 1).

**Description:** Leaf spots circular to irregular, 2–4 mm diam, centre greyish white, surrounded by a pinkish or light brown border. *Caespituli* mostly epiphyllous. *Mycelium* internal. *Stromata* small, only composed of a few brown swollen hyphal cells, 15–30 µm diam. *Conidiophores* fasciculate, arising from stromatic hyphal aggregations, erect, straight to sinuous, geniculate, unbranched, 14–28.5 × 2.8–4.2 µm, aseptate, pale brown, thin-walled, smooth; conidiogenous cells integrated, terminal and intercalary, conidiogenous loci conspicuous, thickened and darkened, about 1.5–2 µm diam. *Conidia* solitary, narrow obclavate, 14–35.5 × 2–3 µm, 1–6-septate, hyaline, thin-walled, smooth, apex pointed, base short obconically truncate.

**Holotype:** India: Karnataka: Mysore, Nandi Hills, on *Justicia betonica*, Acanthaceae, 31 Jan. 1953, H. C. Govindu (not traced).

**Host range and distribution:** Only known from the type collection.

**Notes:** According to Govindu & Thirumalachar (1954), type material of new species described in their paper had been deposited at BPI, IMI, and HClO. However, type material could not be traced, neither in BPI nor in IMI (now K). Details of the structure of the conidiogenous loci were not provided by Govindu & Thirumalachar (1954), but based on the original description this species does probably not belong to *Cercospora s. str.* It might be a species of *Pseudocercospora*, but a re-examination of type material or new collections agreeing with the original description are necessary to answer this question.


**Literature:** Crous & Braun (2003: 316).

**Note:** *Cercospora peristrophes* E. Castell. is a homonym of *C. peristrophes* Thirum. & Govindu, 1953.

**Passalora**

**Key to Passalora species on Acanthaceae**

1 Conidia solitary, obclavate, short, 25–50 × 3.5–5 µm, 1–3(–4)-septate; on *Barleria lupulina* ................. *P. barleriigena*

Conidia in chains, either much longer, 20–95 µm, or much narrower, 2–3 µm; on other hosts .................................... 2

2 (1) Conidiophores 100–325 µm long, pluriseptate; conidia cylindrical or somewhat cylindrical-obclavate, 20–95 × 4–6 µm, 1–6-septate, pale olivaceous; on *Acanthus arboreus* .............................................. *P. acanthicola*

Conidiophores much shorter, 10–50 µm; conidia 8–40 × 2–3 µm, (0–)1–3(–4)-septate, hyaline; on *Adhatoda vasica* .............................................. see *Cercospora justiciae-adhatodae*

**Passalora species on Acanthaceae**


(Fig. 20)


**Literature:** Chupp (1954: 22).

**Illustration:** Deighton (1987: 387, fig. 17).

**Description:** Leaf spots at first diffuse, yellowish, later angular, 5–8 mm diam, brown. *Caespituli* hypophyllous, effuse, deep reddish brown, more or less vein-limited. *Mycelium* internal; hyphae 2.5–4 µm wide, colourless. *Stromata* lacking or almost so. *Conidiophores* in small loose fascicles, to 10, arising from internal hyphae or small aggregations of swollen hyphal cells, emerging through stomata, erect, geniculate-sinuous, simple or branched, 100–325 × 4–6 µm, pluriseptate throughout, moderately brown or reddish brown, thin-walled, smooth; conidiogenous cells integrated, terminal and intercalary, conidiogenous loci conspicuous, thickened and darkened, about 1.5–2 µm diam. *Conidia* catenate, in simple or branched chains, cylindrical or somewhat obclavate-cylindrical, straight or slightly curved, 20–95 × 4–6
µm, 1–6-septate, pale olivaceous, thin-walled, smooth, apex obtuse or subtruncate, base short obconically truncate, 1.5–2 µm wide, hila thickened and darkened.

Holotype: **Uganda**: Kampala, Kawandra, on *Acanthus arboreus*, *Acanthaceae*, Jan. 1943, C. G. Hansford 3144 (K(M) IMI 4557a).

**Host range and distribution**: Only known from the type collection.


(Fig. 21)

**Description**: Leaf spots amphigenous, subcircular to irregular, 1–14 mm diam, pale to pale brown, margin at first indefinite, later conspicuous, dark. *Caespituli* amphigenous. *Mycelium* internal. *Stromata* substomatal, small, 7.5–20 µm diam, composed of 3–7 swollen hyphal cells, brown. *Conidiophores* in dense fascicles, 4–7, arising from stromata, through stomata, erect, straight, subcylindrical, somewhat attenuated towards the tip, unbranched, 1–2 times geniculate, about 20–65 × 3.5–5 µm, 1–3-septate, darker brown below, paler towards the tip, thin-walled, smooth; conidiogenous cells integrated, terminal, conidiogenous loci conspicuous, thickened and darkened, 1–2 µm diam. *Conidia* solitary, mostly obclavate, occasionally oblong cylindrical, straight to occasionally somewhat curved, 25–50 × 3.5–5 µm, 1–3(–4)-septate, subhyaline to pale brown, thin-walled, smooth, apex obtuse, base short obconically truncate, 1–2 µm wide, hila thickened and darkened.

**Illustration**: Meeboon et al. (2007a: 141, fig. 1).

**Pseudocercospora**

**Key to Pseudocercospora species on Acanthaceae**

1. Mycelium internal and external; superficial hyphae with solitary conidiophore *in vivo* developed ........................................ 2
   Mycelium internal; superficial hyphae with solitary conidiophores *in vivo* not developed .................................................. 12

2 (1) Stromata lacking; conidiophores 50–130 × 4–6.5 µm; conidia 3.5–6.5 µm long; on *Justicia* spp. .................. P. justiciae
   Stromata developed; and/or conidiophores shorter, to about 80 µm, and/or narrower, 2–4 µm;
   and/or conidia much narrower, 1.5–4 µm ................................................. 3

3  (2) Stromata lacking or almost so ....................................................................................................................... 4
   Stromata developed, 10–40 µm diam .............................................................. 7

4 (3) Conidiophores relatively short, 5–50(–60) µm .......................................................................................... 5
   Conidiophores longer and broader, to 120 × 2.5–6 µm, longer conidiophores with more than two septa
   (pluriseptate); on other hosts ................................................................. 6

5 (4) Conidiophores short, 5–30 × 1.5–3 µm, 0–1-septate; on *Thunbergia* spp. ................................. P. thunbergiae
   Conidiophores 5–50(–60) × 2–5 µm, 0–3-septate; on *Rhinacanthus nasutus* .................. P. rhinacanthi

6 (4) Conidia cylindrical or subcylindrical, base truncate to somewhat obconically truncate;
   conidiophores to 120 µm long; on *Strobilanthes cusia* ................................................................. P. baphiacanthi
   Conidia obclavate-cylindrical, base consistently obconically truncate; conidiophores shorter, to 80 µm long;
   on *Lepidagathis alopecuroidea* ........................................................................ P. lepidagathidis

7 (3) Conidiophores 10–80 × 3–5 µm, 0–5-septate; conidia rather long, 60–175 × 3.5–5 µm, 4–11-septate,
   hila 2–2.5 µm wide; on *Thunbergia alata* .................................................. P. thunbergiicola
   Conidiophores shorter, 5–50 × 1.5–5 µm; conidial hila 0.8–2 µm wide; on other hosts .................... 8

8 (7) Conidiophores 20–50 µm long, 2–6-septate; on *Justicia japonica* .................................................. P. justiciicola
   Conidiophores 0–3-septate; on other hosts .................................................. 9

9 (8) Conidia obclavate-cylindrical to subacicular; conidia with pointed apex; on *Acanthus guineensis* ............ P. acanthi
   Conidia obclavate-cylindrical, subacicular conidia lacking; on other hosts ..................... 10

10 (9) Conidial base truncate to obconically truncate; on *Odontonema callistachyum* .................. P. odontonematis
   Conidial base consistently obconically truncate .............................................. 11

11 (10) Conidiophores 5–30 × 1.5–4 µm, 0–1(–2)-septate; on *Justicia galapagana* ................ P. consociata var. dimorpha
   Conidiophores to 60 µm long, 0–3-septate; on *Rhinacanthus nasutus* .................... P. rhinacanthi

12 (1) Stromata lacking; conidia 15–65 × 2–4 µm, hila 1–1.5 µm wide; on *Blechum pyramidatum* .................. P. blechi
   Stromata developed; and/or conidia longer, to 150 µm, hila broader, 1.5–2.5 µm; on other hosts .................. 13

13 (12) Stromata lacking or almost so; conidiophores 20–130 × 4–7 µm; on *Barleria cristata* .................. P. barleriæ
   Stromata developed; and/or conidiophores shorter, to 75 µm, and above all narrower, 2–5 µm wide;
   on other hosts ......................................................................................... 14

14 (13) Conidiophores short, 5–40 µm, 0–1(–2)-septate; on *Cynarospermum, Dyschoriste, Justicia, Rhinacanthus, Ruellia* ................................................................. 15
   Conidiophores longer, to 125 µm, and/or (if shorter) 0–5-septate ......................... 17

15 (14) Conidia acicular to obclavate-cylindrical, base truncate to long obconically truncate, 2–2.5 µm wide;
   on *Cynarospermum* ............................................................................... P. blepharidis
Conidia obclavate-cylindrical, acicular conidia not formed, base 1.5–2 µ wide; on other hosts .......................... 16

16 (15) Conidiophores 5–30 × 1.5–4 µm, 0–1(–2)-septate (external mycelium lacking);
Conidiophores to 60 µm long, 0–3-septate (external mycelium usually developed);
on Dyschoriste, Justicia, Ruellia ................................................................. P. consociata var. consociata
on Rhinacanthus nasutus ................................................................. P. rhinacanthi

17 (14) Conidiophores long, 20–125 µm; on Asystasia gangetica ................................................................. P. asystasiae
Conidiophores shorter, to 75 µm, average < 50 µm; on other hosts
(see “Tabular key to Pseudocercospora species on Acanthaceae according to host genera”
– further identification just based on morphology barely possible)

Tabular key to Pseudocercospora species on Acanthaceae according to host genera

**Acanthus**
A single species ......................................................................................................................... P. acanthi

**Asystasia**
A single species ......................................................................................................................... C. asystasiae

**Baphicacanthus**, see **Strobilanthes**

**Barleria**
A single species ......................................................................................................................... P. barleriae

**Blechum**
A single species ......................................................................................................................... P. blechi

**Blephris**, see **Cynarospermum**

**Cynarospermum**
A single species ......................................................................................................................... P. blepharidis

**Dicliptera**
A single species ......................................................................................................................... P. diclipterae

**Dyschoriste**
A single species ......................................................................................................................... P. consociata var. consociata

**Ecbolium**
A single species ......................................................................................................................... P. ecbolii

**Justicia**
1 Mycelium internal, superficial hyphae with solitary conidiophores lacking ...................... P. consociata var. consociata
Mycelium internal and external, superficial hyphae with solitary conidiophores developed ......................................................................................................................... 2

2 (1) Stroma lacking; conidiophores 50–130 × 4–6.5 µm; conidia cylindrical to obclavate-subcylindrical,
40–110 × 3.5–6.5 µm, hila 2–2.5 µm wide ................................................................................ P. justiciae
Stromata developed, 10–25 µm diam; conidiophores shorter and narrower, 20–50 × 1–5 µm;
conidia narrower, obclavate, 35–120 × 1.5–5 µm, hila 1–2 µm wide ........................................ P. justiciicola

**Lepidagathis**
A single species ......................................................................................................................... P. lepidagathidis

**Odontonema**
A single species ......................................................................................................................... P. odontonematis

**Rhinacanthus**
A single species ......................................................................................................................... P. rhinacanthi
**Ruellia**
A single species ........................................................................................... P. consociata var. consociata (and ? P. justiciicola)

**Rungia**
A single species ........................................................................................... P. rungiae

**Strobilanthes**
A single species ........................................................................................... C. baphicacanthi

**Thunbergia**

1 Stromata lacking; conidiophores short and narrow, 5–30 × 1.5–3 µm; conidia 20–95 × 1.5–4 µm, 0–8-septate, subhyaline, pale yellowish green to very pale olivaceous ................................................................. P. thunbergiae

   Stromata to 30 µm diam; conidiophores longer and broader, 10–80 × 3–5 µm; conidia also longer and broader, 60–175 × 3.5–5 µm, 4–11-septate, pale olivaceous-brown ................................................................. P. thunbergiicola

**Pseudocercospora species on Acanthaceae**


(Fig. 22)


*Description:* Leaf spots amphigenous, subcircular, to 5 mm diam, grey-brown, with narrow dark brown border, somewhat raised. *Caespituli* amphigenous rather pale olivaceous, punctiform, scattered. *Mycelium* internal and external; internal hyphae 2.5–6.5 µm wide, subhyaline, superficial hyphae 1.5–3 µm wide, pale olivaceous, septate, thin-walled, smooth. *Stromata* well-developed, 20–40 µm diam, compact, brown. *Conidiophores* in small to sometimes large and dense fascicles, 8–50, arising from stromata, or occasionally solitary, arising from superficial hyphae, erect, straight, subcylindrical-conical to somewhat geniculate-sinuous, unbranched, 20–40 × 3–4.5 µm, 0–1(-2)-septate, pale brown, thin-walled, smooth; conidiogenous cells integrated, terminal or conidiophores reduced to conidiogenous cells, 20–30 µm long, conidiogenous loci inconspicuous to subdenticulate, but always unthickened and not darkened. *Conidia* solitary, obclavate-subcylindrical to subacicular, straight to curved or somewhat sigmoid, 30–90 × 2.5–4 µm, 3–8-septate, pale olivaceous, apex pointed, base obconically truncate to almost rounded, 1.5–2 µm wide, hila unthickened, not darkened.

*Holotype:* Sierra Leone: Gorahun (Tunkia), on *Acanthus guineensis*, Acanthaceae, 3 Apr. 1939, F. C. Deighton, M 1937 (K(M) IMI 7695).

*Host range and distribution:* Only known from the type collection.


(Fig. 23)


*Illustrations:* Yen (1967: 179, fig. 1), Yen & Lim (1980: 228, fig. 31).

*Description:* Leaf spots amphigenous, subcircular to somewhat irregular, 2–10 mm diam, at first pale greenish, greyish green,
later yellowish brown to brownish, greyish brown, finally greyish white, occasionally zonate, margin indistinct to distinct, dark brown to blackish. *Caespituli* amphigenous, indistinct to punctiform, brown, scattered to confluent and denser. *Mycelium* internal. *Stromata* lacking or small, substomatal, 10–30 µm diam, subglobose, brown. *Conidiophores* in small to moderately large fascicles, loose to moderately dense, arising from stromata, emerging through stomata, erect, straight, subcylindrical to flexuous, sinus or somewhat geniculate-sinuous, unbranched, 20–125 × 3–5 µm, 0–5-septate, olivaceous-brown, thin-walled, smooth; conidiogenous cells integrated, terminal, about 10–40 µm long, conidiogenous loci inconspicuous to subdenticulate, neither thickened nor darkened. *Conidia* solitary, obclavate-cylindrical, straight to curved, 25–90(–110) × (2.5–)3–4.5(–5) µm, 2–8(–9)-septate, subhyaline to pale olivaceous-brown, thin-walled, smooth, apex obtuse to subacute, base short obconically truncate, 1–2 µm wide, hila unthickened, not darkened.

**Holotype:** Singapore: Botanical Garden, on *Asystasia gangetica*, 5 Jan. 1966, J. M. Yen 731 (PC).

**Host range and distribution:** On *Asystasia* (*gangetica* [*coromandeliana*], *nemorum*), Acanthaceae, Africa (Ivory Coast), Asia (Indonesia, Japan, Singapore).

**Note:** A second collection on *Asystasia gangetica* from Ivory Coast (29 Dec. 1974, G. Gilles 47), deposited at PC, has been examined.


**Fig. 23.** *Pseudocercospora asystasiae* (PC, holotype). **A.** Conidiophore fascicle. **B.** Conidiophores. **C.** Conidia. Bar = 10 µm.

**Fig. 24.** *Pseudocercospora baphicacanthi* (based on Hsieh & Goh 1990: 13, fig. 2). **A.** Solitary conidiophore, arising from superficial hyphae or emerging through stomata. **B.** Conidiophores. **C.** Conidia. Bar = 10 µm.
**Illustration:** Hsieh & Goh (1990: 13, fig. 2), Guo & Hsieh (1995: 2, fig. 1), Guo et al. (1998: 10, fig. 1).

**Description:** Leaf spots irregularly shaped, 2–12 mm diam, sometimes confluent, forming yellowish patches on the upper leaf surface, yellowish brown below, margin indefinite. *Caespituli* hypophyllous. *Mycelium* internal and external; superficial hyphae emerging through stomata. *Stromata* lacking. *Conidiophores* solitary or in small, loose fascicles, emerging through stomata or solitary, arising from superficial hyphae, lateral, erect to decumbent, straight to curved, geniculate-sinuous, simple or branched, 20–120 × 3–6 µm, septate, pale brown throughout or paler towards the tip, thin-walled, smooth; conidiogenous cells integrated, terminal, conidiogenous loci inconspicuous or visible as truncate tips, about 2 µm wide, unthickened, not darkened. *Conidia* solitary, cylindrical or subcylindrical, straight to somewhat curved, 30–100 × 3–4 µm, 2–7-septate, greenish to pale olivaceous, thin-walled, smooth, apex obtuse or subobtuse, base truncate to short obconically truncate, hila unthickened, not darkened.

**Holotype:** Taiwan: Hsitou, Nantou Hsien, on *Strobilanthes cusia* [Baphicacanthus cusia], Acanthaceae, 28 Mar. 1985, W. H. Hsieh (NCHUPP-162). Isotype: K(M) IMI 312069.

**Host range and distribution:** Only known from the type collection.


(Fig. 25)


**Literature:** Yen & Lim (1980: 156), Crous & Braun (2003: 76).

**Illustrations:** Yen & Lim (1973: 110, fig. 3; 1980: 207, fig. 10), Braun (2001: 58, fig. 15).

**Description:** Leaf spots amphigenous, angular, vein-limited, 1–5 mm diam or confluent and larger, blackish on the upper leaf surface, dark brown below, sometimes forming large blackish brown patches, covering large leaf segments. *Caespituli* hypophyllous, effuse, dark brown, sometimes velvety. *Mycelium* internal. *Stromata* lacking or almost so. *Conidiophores* in small to moderately large fascicles, loose, arising from internal hyphal or small hyphal aggregations, through stomata, erect to decumbent, flexuous, simple or occasionally branched, somewhat geniculate-sinuous, 20–130 × 4–7 µm, (0–)1–7-septate, olivaceous to olivaceous-brown, tips paler, thin-walled, smooth; conidiogenous cells integrated, terminal, 10–40 µm long, conidiogenous loci inconspicuous or subconspicuous by being subdenticulate or somewhat refractive, but neither thickened nor darkened. *Conidia* solitary, obclavate to obclavate-cylindrical, straight to somewhat curved, (10–)15–80 × 4–5 µm, 1–8-septate, subhyaline to pale olivaceous or olivaceous-brown, thin-walled, smooth, apex subacute to obtuse, base obconically truncate, 2–2.5 µm wide, hila unthickened, not darkened.


**Host range and distribution:** On *Barleria cristata*, Acanthaceae, Asia (Singapore).

**Notes:** Yen & Lim (1980) retained this species in *Cercospora* and described “brown scars”. However, the examination of type material showed that this species has to be reallocated to *Pseudocercospora*.


(Fig. 26)


**Literature:** Chupp (1954: 22), Crous & Braun (2003: 82).

**Illustrations:** Chupp (1954: 21, fig. 2), Braun et al. (2002: 121, fig. 8).
Description: Leaf spots mainly epiphyllous, indistinct to irregular and somewhat reddish, 0.5–3 mm diam, sometimes confluent, margin indefinite. *Caespituli* hypophyllous, sub-effuse, dark olivaceous to blackish. *Mycelium* internal. *Stromata* lacking or small, composed of a few swollen hyphal cells, brown. *Conidiophores* in small, loose fascicles, arising from internal hyphae or hyphal aggregations, emerging through stomata, erect, flexuous, geniculate-sinuous, simple or rarely branched, 10–90 × 2–4 μm, 0–3-septate, pale olivaceous or olivaceous-brown, thin-walled, smooth; conidiogenous cells integrated, terminal or conidiophores reduced to conidiogenous cells, 10–30 μm long, conidiogenous loci inconspicuous, unthickened, not darkened. *Conidia* solitary, acicular to obclavate-cylindrical, straight to slightly curved, 15–65 × 2–4 μm, 1–6-septate, subhyaline to pale olivaceous, apex obtuse to subacute, base obconically truncate, 1–1.5 μm wide, hila unthickened, not darkened.


Host range and distribution: On *Blechum pyramidatum* [brownei], *Acanthaceae*, Central and South America (Panama, Venezuela), West Indies (Cuba, Puerto Rico, Virgin Islands).

**Pseudocercospora blepharidis** (Chidd.) U. Braun & Crous, *Mycosphaerella and Anam.*: 82 (2003). (Fig. 27)


Illustration: Chiddarwar (1960: plate V, figs 4–5).
Holotype: India: Maharashtra: Khandala, on Cynarospermum asperrimum [Blepharis asperrima], Acanthaceae, 9 Nov. 1956, P. P. Chiddarwar 2 (K(M) IMI 83163).

Host range and distribution: Only known from the type collection.

var. *consociata* (Fig. 28a)


Illustration: Chupp (1954: 21, fig. 3), Guo & Hsieh (1995: 3, fig. 2), Guo et al. (1998: 12, fig. 2), Phengsintham et al. 2013: 111, fig. 67).

Exsiccate: Ellis & Everh., N. Amer. Fungi 2477.

Description: Leaf spots lacking or almost so, inconspicuous to diffuse yellowish to brownish discolorations, or forming

---

Fig. 28a, b. a. *Pseudocercospora consociata* var. *consociata* (BPI 435187, neotype). A. Conidiophore fascicles. B. Conidiophores. C. Conidia.  

subcircular to angular-irregular lesions, 1–10(–15) mm diam, brownish, finally greyish brown to greyish white, margin indefinite or darker brown, occasionally reddish brown, sometimes slightly raised, narrow, often only formed as marginal line. Caespituli amphiogenous, punctiform to effuse, dark brown to blackish, later greyish by abundant conidiation. Mycelium internal; hyphae branched, septate, occasionally constricted at septa, 1.5–5 µm wide, subhyaline to brownish, thin-walled, smooth. Stromata variable, almost lacking, small to medium in size, subglobose to somewhat irregular, 10–30 µm diam, occasionally larger, to 80 µm diam, substomatal to immersed, olivaceous-brown to dark brown, cells subglobose to somewhat angular-irregular in outline, 2–6(–11) µm diam, wall to 0.8 µm wide. Conidiophores in small to moderately large fascicles, occasionally in large fascicles, divergent to dense, arising from substomatal swollen hyphal cells or stromata, emerging through stomata or erumpent, erect, straight, subcylindrical or attenuated towards the tip, slightly to distinctly geniculate-sinuous, unbranched or rarely branched, 5–50(–80) × 2–6 µm, 0–4-septate, longer ones plainly pluriseptate, subhyaline to pale or medium olivaceous, olivaceous-brown or brown, thin-walled, smooth; conidiogenous cells integrated, terminal or conidiophores reduced to conidiogenous cells, 5–20 µm long, conidiogenous loci inconspicuous or visible as truncate tips or shoulders, always unthickened and not darkened. Conidia solitary, obclavate-cylindrical, straight to curved, 20–110(–150) × 2–4.5(–5) µm, 0–10(–12)-septate, subhyaline to pale olivaceous, olivaceous-brown or brown, thin-walled, smooth, apex acute to obtuse, base short to sometimes long obconically truncate, 0.8–2 µm wide, hila unthickened, not darkened.


Host range and distribution: On Dyschoriste oblongifolia, Justicia (gendarussa, procumbens), Ruellia (ciliosa, prostrata, strepens, tuberosa), Acanthaceae, Asia (China; India, Uttar Pradesh, West Bengal; Japan, Thailand), North America (USA, Alabama, Florida, Iowa, Illinois, Mississippi, Missouri, Oklahoma), South America (Brazil, Venezuela).


Description: Differs in vivo from var. consociata by the formation of superficial hyphae with solitary conidiophores (superficial hyphae emerging through stomata, branched, septate, 1.5–2.5(–3) µm wide, subhyaline to pale olivaceous, thin-walled, smooth; solitary conidiophores arising from superficial hyphae, lateral, erect, straight, subcylindrical-conical to somewhat geniculate-sinuous, unbranched, 5–30 × 1.5–4 µm, 0–1(–2)-septate, subhyaline to pale olivaceous-brown, thin-walled, smooth; conidiogenous cells integrated, terminal or conidiophores often reduced to conidiogenous cells, 5–20 µm long, conidiogenous loci inconspicuous); otherwise as in var. consociata.


Host range and distribution: Only known from the type collection.

Notes: Pseudocercospora consociata, known from Asia, North and South America on hosts of various genera of the Acanthaceae, is probably taxonomically heterogeneous. This species is morphologically rather variable in all basic traits. However, a further splitting needs cultures and molecular sequence analyses based on material from all continents and host genera involved. Type material of this species, collected by Seymour before 1884, could not be traced. Records of Pseudocercospora consociata on Dicliptera chinensis from China (Guo & Hsieh 1995, Guo et al. 1998) are undoubtedly incorrect and belong to P. dicipetrae. The two species are morphologically barely distinguishable.


(Fig. 29)


Illustration: Kar & Mandal (1969: 338, fig. 1).

Description: Leaf spots amphiogenous, subcircular, 1–6 mm diam, scattered, sometimes confluent, uniformly yellowish to dull yellowish brown, later olivaceous by abundant fructification. Caespituli amphiogenous, mostly hypophyllous, punctiform to effuse, brown or deep olivaceous. Mycelium internal. Stromata lacking or small, 10–20 µm diam, subglobose or somewhat oblong, substomatal, brown. Conidiophores in small to moderately large fascicles, usually 2–15, divergent, arising from substomatal hyphae or stromata, through stomata, erect, straight to curved or geniculate-sinuous, unbranched, 10–55 × 3–5 µm, 0–5-septate, yellowish brown, thin-walled, smooth; conidiogenous cells integrated, terminal or conidiophores sometimes reduced to conidiogenous cells, 10–20 µm long, conidiogenous loci inconspicuous to subdenticulate, unthickened, not darkened. Conidia solitary, obclavate-subcylindrical, straight to curved, 30–150 × 3–4 µm, 2–16-septate, pale yellowish brown, olivaceous, thin-walled, smooth, apex subacute or subobtuse, base short obconically truncate, 1.5–2 µm wide, hila unthickened, not darkened.

Holotype: India: West Bengal, Darjeeling, Sevak Road, on Dicliptera chinensis [roxburghiana], 11 Nov. 1967, M. Mandal (K(M) IMI 135116). Isotype: BPI 435712.

Host range and distribution: On Dicliptera chinensis, Acanthaceae, Asia (?China; India, West Bengal).
**Note:** Records of *Pseudocercosora consociata* on *Dicliptera chinensis*, from China belong possibly to *P. diclipterae* (see notes under *P. consiciata*).


**Literature:** Crous & Braun (2003: 168), Kamal (2010: 172).

**Illustration:** Kar & Mandal (1969: 339, fig. 2).

**Description:** Leaf spots amphigenous, formed as vein-limited, yellowish discolorations, 4–10 mm diam, scattered, finally deep olivaceous below by abundant fructification. *Caespituli* hypophyllous, effuse, olivaceous. *Mycelium* internal. *Stromata* lacking or small, substomatal, 5–25 µm diam, subglobose to oblong, dark olivaceous to olivaceous-brown. *Conidiophores* in small to moderately large fascicles, mostly 2–15, arising from stromata, through...
stomata, divergent to moderately dense, erect, straight, subcylindrical-conical to geniculate-sinuous, unbranched or occasionally 1–2 times branched, 15–65 × 3–5 µm, 0–6-septate, pale olivaceous to olivaceous-brown, thin-walled, smooth; conidiogenous cells integrated, terminal or conidiophores sometimes reduced to conidiogenous cells, 10–25 µm long, conidiogenous loci inconspicuous to subdenticulate, unthickened, not darkened. Conidia solitary, narrowly obclavate-cylindrical, straight to curved, 20–100 × 2.5–4 µm, 1–10-septate, pale olivaceous to very pale olivaceous-brown, thin-walled, smooth, apex subacute or subobtuse, base obconically truncate, 1–2 µm wide, hila neither thickened nor darkened.


Host range and distribution: Only known from the type collection.


(Fig. 31)


Illustrations: Tai (1948: 44, fig. 11), Guo & Hsieh (1995: 4, fig. 3), Guo et al. (1998: 13, fig. 3).

Description: Leaf spots lacking or only formed as yellowish to yellowish brown discolorations on the upper leaf surface, circular, 2–5 mm diam, grey to greish brown below, margin indefinite. Caespituli hypophyllous, effuse, sooty. Mycelium internal and external; superficial hyphae emerging through stomata, branched, septate, 2–3.5 µm wide, pale olivaceous to olivaceous-brown, thin-walled, smooth. Stromata not developed. Conidiophores in loose fascicles, 2–11, arising from internal hyphae, through stomata or solitary, arising from superficial hyphae, erect, subcylindrical, flexuous, curved-sinuous to somewhat geniculate-sinuous, unbranched or branched, 50–130 × 4–6.5 µm, 3–8-septate, olivaceous-brown to pale brown, thin-walled, smooth; conidiogenous cells integrated, terminal, conidiogenous loci inconspicuous, occasionally subdenticulate. Conidia solitary, cylindrical to obclavate-subcylindrical, straight to curved, 40–110 × 3.5–6.5 µm, 3–11-septate, pale olivaceous to olivaceous, thin-walled, smooth, apex obtuse to subacute, base obconically truncate, about 2–2.5 µm wide, hila neither thickened nor darkened.

Holotype: China: Sichuan: Chengtu, on Justicia procumbens, 1943, Lee Ling 125 (HMAS 12126).

Host range and distribution: On Justicia (procumbens, Justicia sp.), Acanthaceae, Asia (China), ?North America (USA, Florida).

Notes: A record of P. justiciae on Justicia sp. from Florida, USA (Alfieri et al. 1984) is unclear and doubtful. Material could not be traced.


(Fig. 32)

Illustration: Singh et al. (1996: 1129, figs 1–5).

Description: Leaf spots lacking or almost so, lesions indistinct, occasionally formed as greish disolorations. Caespituli hypophyllous, greish, discrete to effuse. Mycelium internal and external; superficial hyphae branched, septate,
subhyaline, 1–3 µm diam, thin-walled, smooth. Stromata developed, small, about 10–25 µm diam, subglobose to somewhat oblong, brown. Conidiophores in loose fascicles, arising from stromata or solitary arising from superficial hyphae, lateral, occasionally terminal, erect, straight to flexuous, somewhat geniculate-sinuous, unbranched, 20–50 × 1–5 µm, 2–6-septate, olivaceous-brown, thin-walled, smooth; conidiogenous cells integrated, terminal, conidiogenous loci unthickened, not darkened. Conidia solitary, obclavate, straight to curved, occasionally with short lateral branchlets, 35–120 × 1.5–5 µm, 4–14-septate, olivaceous, thin-walled, smooth, apex acute to subobtuse, base short obconically truncate, about 1–2 µm wide, hila unthickened, not darkened.

**Host range and distribution:** Only known from the type collection.

**Notes:** There is a second collection from Nepal that probably pertains to *P. justiciicola*. Verma & Kamal (1991) cited it as paratype material of *P. rungiae* [on *Ruellia prostrata*] (on the original label as *Justicia* sp.), Nepal, Kathmandu Valley, Jan. 1986, R. K. Verma (GPU, KK 221, K(M) IMI 303480)]. See discussion under *P. rungiae*.


(Fig. 33)

**Illustration:** Braun & Crous (2005: 402, fig. 5).

**Description:** Leaf spots amphigenous, 1–5 mm diam, subcircular to somewhat irregular or even diffuse, brownish, dingy olivaceous-brown to blackish brown or with blackish purple tinge, margin indistinct. Caespituli hypophyllous, subeffuse, rather inconspicuous. Mycelium internal and external; superficial hyphae emerging through stomata, sparingly branched, 1–3 µm wide, septate, subhyaline to pale olivaceous or olivaceous-brown, thin-walled, smooth. Stromata lacking or only with very small substomatal hyphal aggregations, brown. Conidiophores in small, usually loose fascicles, arising from internal hyphae or stromatic hyphal aggregations, through stomata, or conidiophores solitary, arising from superficial hyphae, lateral or terminal, erect, straight to flexuous, geniculate-sinuous, unbranched or only rarely branched, 5–80 × 2.5–5.5 µm, 0–4-septate, pale olivaceous-brown, thin-walled, smooth; conidiogenous cells integrated, terminal or conidiophores reduced to conidiogenous cells, 5–25 µm long, conidiogenous loci neither thickened nor darkened, occasionally subdenticulate. Conidia solitary, straight to curved, narrowly obclavate-cylindrical, 20–120 × 2–4 µm, 1–9-septate, pale subhyaline to pale olivaceous, thin-walled, smooth, apex obtuse to subacute, base obconically truncate, 1–2 µm wide, hila unthickened, not darkened.

**Host range and distribution:** Only known from the type collection.

**Note:** The material on *Lepidagathis alopecuroidea* was previously falsely referred to as *Cercospora lepidagathidis* (Stevenson 1975, Minter et al. 2001, Crous & Braun 2003), which is, however, a genuine species of *Cercospora* s. str.


(Fig. 34)

**Basionym:** *Cercospora odontonematis* Chupp, Monograph of *Cercospora*: 25 (1954); as “odontonemae”.

**Literature:** Chupp (1954: 25).
Description: Leaf spots amphigenous, circular to somewhat angular-irregular, 2–5 mm diam, brown, later with greyish brown to dingy grey centre and darker border. *Caespituli* hypophyllous, punctiform to effuse. *Mycelium* internal and external; superficial hyphae branched, septate, 1–3 µm wide, subhyaline or pale olivaceous, thin-walled, smooth; *stromata* substomatal, 10–40 µm diam, brown. *Conidiophores* in small to moderately large fascicles, loose to dense, arising from *stromata*, through *stromata* or erumpent, also solitary, arising from superficial hyphae, lateral, rarely terminal, erect, straight, subcylindrical-conical to strongly geniculate-sinuous, unbranched, 10–40 × 2–4.5 µm, 0–2-septate, very pale or pale olivaceous to olivaceous-brown, thin-walled, smooth; *conidiophores* reduced to conidiogenous cells or integrated, terminal, 10–25 µm long, conidiogenous loci inconspicuous to subdenticulate, but always unthickened and not darkened. *Conidia* solitary, narrowly cylindrical to obclavate-cylindrical, 20–90 × 2–4 µm, (2–)3–6-septate, very pale olivaceous, thin-walled, apex obtuse to subacute, base more or less truncate to distinctly obconically truncate, 1.5–2 µm wide, hila unthickened, not darkened.

*Lectotype* (designated here, MycoBank, MBT202784): *Mexico*: Veracruz: Cordoba, on *Odontonema callistachyum*. 

Fig. 33. *Pseudocercospora lepidagathidis* (BPI 437637, holotype). A. Solitary conidiophores arising from superficial hyphae. B. Conidiophore fascicles. C. Conidiophores. D. Conidia. Bar = 10 µm.

Fig. 34. *Pseudocercospora odontonematis* (CUP 40422, lectotype). A. Superficial hypha, B. Solitary conidiophores arising from superficial hyphae. C. Conidiophore fascicle. D. Conidiophores. E. Conidia. Bar = 10 µm.
Isolate type: CUP 60863.

Host range and distribution: Only known from the type collection.


(Fig. 35)


**Description:** Leaf spots amphigenous, formed as diffuse yellowish to brown discolorations or diffuse brown spots with yellowish halo to circular or angular-irregular, 2–10 mm diam, brown or later with dingy grey centre, rather indistinct on dry leaves. *Caespituli* amphigenous, mainly hypophyllous. *Mycelium* internal and external; superficial hyphae branched, septate, 1–4 µm wide, subhyaline to olivaceous-brown, thin-walled, smooth. *Stromata* lacking or small, substomatal, 10–25 µm diam, olivaceous-brown to brown. *Conidiophores* in loose to moderately dense, small fascicles, usually 2–6, arising from stromata, through stomata, erect to decumbent, or solitary, arising from superficial hyphae, lateral, straight, subcylindrical to moderately geniculate-sinuous, unbranched or occasionally branched, 5–60(–80) × 2–5 µm, 0–3-septate, pale olivaceous to olivaceous-brown, thin-walled, smooth; conidiophores reduced to conidiogenous cells or integrated, terminal, about 10–25 µm long, conidiogenous loci inconspicuous or visible as truncate, subdenticulate tips or shoulders. *Conidia* solitary, narrowly obclavate-cylindrical, straight to somewhat curved, (25–)40–120(–130) × 2–5 µm, indistinctly (2–)3–10(–12)-septate, subhyaline to pale olivaceous, thin-walled, smooth, apex more or less pointed, base usually long obconically truncate, 1–1.5 µm wide, hila unthickened, not darkened.


Host range and distribution: On *Rhinacanthus* (nasutus [Justicia nasuta], *Rhinacanthus* sp.), Acanthaceae, Asia (Indonesia, Philippines, Thailand).

Notes: Chinese records of *P. rhinacanthi* on *Justicia procumbens* (Tai 1979, Guo & Hsieh 1995, Guo *et al.* 1998) are unclear, unproven and might belong to *P. consociata*. The two species are morphologically barely distinguishable.


(Fig. 36)


**Description:** Leaf spots mainly hypophyllous, circular to angular-irregular or diffuse, 3–5 mm diam, greenish white, yellowish. *Caespituli* hypophyllous, scattered, rather...
inconspicuous. Mycelium internal; hyphae branched, septate, colourless, 1–2 µm wide, somewhat wider near stromata. Stromata lacking or poorly developed, substomatal, small, to about 25 µm diam. Conidiophores fasciculate, 2–10, divergent, arising from small stromata, through stomata, erect, straight, subcylindrical-conical to distinctly geniculate-sinuous, unbranched to branched, 10–75 × 2–5 µm, 0–3(–6)-septate, pale olivaceous to olivaceous-brown, thin-walled, smooth; conidiophores occasionally reduced to conidiogenous cells, but mostly integrated, terminal, 10–30 µm long, conidiogenous loci inconspicuous to subdenticulate, but always unbranched and not darkened. Conidia solitary, obclavate-subcylindrical, straight to curved, 30–145 × 2–4 µm, 1–10-septate, subhyaline to pale olivaceous, thin-walled, smooth, apex obtuse or subacute, base short obconically truncate, 1–2 µm wide, hila unthickened, not darkened.


**Host range and distribution**: On *Rungia* (pectinata [parviflora], repens), Acanthaceae, Asia (India, Maharashtra, Uttarakhand; Nepal).

**Note**: The identity of *Cercospora rungiae* and *Pseudocercospora rungiae* has been proven by Kamal (2010). A record of *C. rungiae* from Andhra Pradesh (Braun et al. 1992: 363) refers to a true *Cercospora* (see *C. justiciicola*) and not to this species. Verma & Kamal (1991) cited a collection on “Ruellia prostrata” (on the original label as *Justicia* sp.) as paratype material (Nepal: Kathmandu Valley, Jan. 1986, R. K. Verma (GPU, KK 221, K(M) IMI 303480). This collection, characterised by forming superficial hyphae with solitary conidiophores is excluded from *P. rungiae*. The identification of the host is uncertain, and the identity of this fungus is not quite clear, but it probably belongs to *Pseudocercospora justiciicola*.


**(Fig. 37)**


**Illustrations**: Boedijn (1961: tab. 108, fig. 1), Braun & Sivapalan (1999: 20, fig. 13).

**Description**: Leaf spots amphigenous, angular-irregular, often vein-limited, 1–10 mm diam, sometimes confluent and larger, dark brown, later greyish brown to dingy grey, margin indefinite. *Caesalpinia* hypophyllous, inconspicuous. Mycelium internal and external; superficial hyphae sparingly branched, 1–3 µm wide, septate, subhyaline to pale olivaceous, thin-walled, smooth. Stromata lacking or very small, only composed of a few swollen hyphal cells, 1.5–4 µm diam, olivaceous to brownish. Conidiophores solitary, arising from superficial hyphae, lateral, rarely terminal, occasionally emerging through stomata, solitary or in small, loose fascicle, erect, straight, subcylindrical-conical to somewhat geniculate-sinuous, unbranched, 5–30 × 1.5–3 µm, 0–1-septate, subhyaline to pale olivaceous-brown, thin-walled, smooth; conidiogenous cells integrated, terminal or conidiophores aseptate, reduced to conidiogenous cells, 5–20 µm long, conidiogenous loci inconspicuous, neither thickened nor darkened. Conidia solitary, narrowly obclavate-cylindrical, 20–95 × 1.5–4 µm, 0–1–8-septate, subhyaline, pale yellowish green to very pale olivaceous, thin-walled, smooth, apex subacute, base obconically truncate, 1–2.5 µm wide, hila unthickened, not darkened.

Host range and distribution: On Thunbergia (alata, erecta, grandiflora), Acanthaceae, Asia (Brunei; India, Uttar Pradesh; Indonesia).


(Fig. 38)

*Basionym:* *Cercospora thunbergiicola* J.M. Yen, *Rev. Mycol.* **30**: 195 (1965); as “thunbergiicola”.


*Fig. 38. Pseudocercospora thunbergiicola* (PC, holotype). A. Conidiophore fascicles. B. Solitary conidiophores arising from superficial hyphae. C. Conidiophores. D. Conidia. Bar = 10 μm.

*Illustrations:* Yen (1965: 197, fig. 12), Yen & Lim (1980: 258, fig. 68).

*Description:* Leaf spots amphigenous, circular to angular-irregular, 0.5–3 mm diam, centre pale, whitish, margin dark brown. *Caespituli* amphigenous, not very conspicuous. *Mycelium* internal and external; superficial hyphae emerging through stomata, sparingly branched, 2–3 μm wide, pale olivaceous-brown, thin-walled, smooth. *Stromata* almost lacking or small, 10–30 μm diam, substomatal, subglobose, brown. *Conidiophores* in small to moderately large fascicles, 2–22, arising from substomatal hyphae or stromata, emerging through stomata, divergent to moderately dense, or solitary, arising from superficial hyphae, lateral, erect, straight, subcylindrical or somewhat narrower towards the tip, to geniculate-sinuous, unbranched or occasionally once branched, 10–80 × 3–5 μm, 0–5-septate, pale olivaceous or olivaceous-brown, somewhat paler towards the tip, thin-walled, smooth; conidiogenous cells integrated, terminal, 10–30 μm long, conidiogenous loci inconspicuous or visible as truncate tips or shoulders. *Conidia* solitary, cylindrical or obclavate-cylindrical, straight to curved, about 60–175 × 3.5–5 μm,
4–11-septate, pale olivaceous-brown, thin-walled, smooth, apex subacute, base short to long obconically truncate, 2–2.5 µm wide, hila unthickened, not darkened.


Host range and distribution: On Thunbergia alata, Acanthaceae, Asia (Singapore).

Semipseudocercospora

A single species.


(Fig. 39)


Description: Leaf spots lacking or rather indistinct, forming diffuse dark brown discolorations. Caespituli hypophyllous, rarely amphigenous, effuse, brown. Mycelium internal. Stromata lacking. Conidiophores solitary or in small, divergent fascicles, arising from internal hyphae, through stomata, erect, straight to curved or somewhat flexuous, long, filiform, not or barely geniculate, unbranched, 140–260(–300) × 5–7 µm, 3–15-septate throughout, medium brown, paler towards the tip, wall somewhat thickened, smooth; conidiogenous cells integrated, terminal, about 10–30 µm long, conidiogenous loci subconspicuous, 1.5–2 µm diam, in front view visible as small circle with darker rim and minute central pore, unthickened, but somewhat refractive or slightly darkened-refractive, loci somewhat bulging, denticle-like. Conidia solitary, short obclavate or clavate, ellipsoid, subcylindrical or obvoid, usually straight, 30–45 × 5.5–8.5 µm, pale olivaceous to olivaceous-brown, thin-walled, smooth, apex broadly rounded, base short obconically truncate, 1.5–2 µm wide, hila unthickened, but occasionally slightly refractive or darkened-refractive.


Host range and distribution: Only known from the type collection.

Notes: The generic affinity of Semipseudocercospora is quite unclear, and it is unknown if it is a true cercosporoid genus (Braun et al. 2013). Molecular sequence analyses are necessary to elucidate the phylogenetic position of this genus.

Fig. 39. Semipseudocercospora peristrophes-acuminatae (PC, holotype). A. Conidiophore fascicle. B. Conidiophore tips. C. Conidia. Bar = 10 µm.
**Actinidiaceae**

**Cercospora species on Actinidiaceae**

A single species


(Fig. 40)

*Literature:* Guo et al. (2005: 23).

*Illustrations:* Liu & Guo (1987: 354, fig. 1), Guo et al. (2005: 23, fig. 6).

**Description:** Leaf spots amphigenous, subcircular to irregular, 2–8 mm diam, centre greyish brown or yellowish brown, with narrow dark brown border. *Caespituli* amphigenous. *Mycelium* internal. *Stromata* lacking or small, composed of a few swollen hyphal cells, brown. *Conidiophores* solitary or in small fascicles, 2–8, divergent, arising from internal hyphae or swollen hyphal cells, erect, straight, curved to geniculate, usually unbranched, about 60–280 × 4–4.5(–5.5) µm, pluriseptate, medium brown to brown, thin-walled, smooth; conidiogenous cells integrated, terminal and intercalary, conidiogenous loci thickened and darkened, 2.2–2.6 µm diam. *Conidia* solitary, acicular, straight to curved, sometimes sigmoid, 40–160(–220) × 2–3(–4) µm, pluriseptate, hyaline, thin-walled, smooth, apex pointed, base truncate or slightly attenuated, 1–2.5 µm wide, hila thickened and darkened.


*Host range and distribution:* Only known from the type collection.

*Note:* Belonging to the C. api s. lat. complex.

**Pseudocercospora species on Actinidiaceae**

**Key to Pseudocercospora species on Actinidiaceae**

1 Conidia broadly obclavate-subcylindrical, 25–85(–100) × 5–8.5 µm, base 2–2.5 µm wide;
   on *Actinidia* spp. .................................................................................................................. P. actinidiae
   Conidia narrowly obclavate-filiform, 15–110 × 1–4 µm, base 1–2 µm wide .................................. P. hangzhouensis


(Fig. 41)


*Exsiccatea:* Reliquiae Farlowianae 829.

**Description:** Leaf spots at first lacking, later subcircular to angular-irregular, 1–10 mm diam, sometimes confluent and larger, brown to dark brown, later with paler centre, pale brown to greyish brown, margin indefinite. *Caespituli* amphigenous, mainly hypophylloous, finely punctiform to effuse, deep olivaceous, floccose. *Mycelium* internal and external; superficial hyphae emerging through stomata, branched, 1–3.5 µm wide, sometimes anastomosing,
pale olivaceous to pale brown, thin-walled, smooth. Stromata lacking below to well-developed on the upper leaf surface, 10–50 µm diam, substomatal, subglobose, pale brown. Conidiophores in loose to dense fascicles, to 100 conidiophores or even more, arising from stromata, through stomata, above all when epiphyllous, and solitary, arising from superficial hyphae, lateral, occasionally terminal, differentiation between hypophyllously formed erect to decumbent branched conidiophores and superficial hyphae difficult, fertile threads to 700 µm long, simple or branched, individual solitary conidiophores formed as lateral branchets 2–45 µm long, 3–6.5 µm wide, aseptate to pluriseptate, pale to medium olivaceous, thin-walled, smooth; conidiogenous cells integrated, terminal, conidiogenous loci inconspicuous to subdenticulate, 1.5–2 µm diam, unthickened, not darkened. Conidia solitary, obclavate-subcylindrical, straight to curved or somewhat sigmoid, 25–85(–100) × 5–8.5 µm, 3–9-septate, pale to medium olivaceous, thin-walled, smooth, apex obtuse, base short or rarely long obconically truncate, about 2–2.5 µm wide, hila unthickened, not darkened.


Host range and distribution: On Actinidia (callosa, chinensis, Actinidia sp.), Actinidiaceae, Asia (China, Anhui, Fujian, Guizhou; Korea, Japan, Taiwan).


A single species ........................................................................................................ Viburnum
Conidia cells, 2–20 µm long, conidiogenous loci inconspicuous. Integrated, terminal or conidiophores reduced to conidiogenous olivaceous-brown, thin-walled, smooth; conidiogenous cells 2–40 × 1–3.5(–4.5) µm, 0–3-septate, subhyaline to pale curved, subcylindrical to geniculate-sinuous, unbranched, or solitary, arising from superficial hyphae, erect, straight to oblong, mainly epiphyllous, brown. Conidiophores in dense fascicles, arising from stromata, through stomata or erumpent, or solitary, arising from superficial hyphae, erect, straight to curved, subcylindrical to geniculate-sinuous, unbranched, 2–40 × 1–3.5(–4.5) µm, 0–3-septate, subhyaline to pale olivaceous-brown, thin-walled, smooth; conidiogenous cells integrated, terminal or conidiophores reduced to conidiogenous cells, 2–20 µm long, conidiogenous loci inconspicuous. Conidia solitary, narrowly obclavate-filiform, 15–110 × 1–4 µm, 2–11-septate, hyaline to pale olivaceous, thin-walled, smooth, apex subacute, base subtruncate to short obconically truncate, 1–2 µm wide, hila unthickened, not darkened.


Host range and distribution: On Actinidia (arguta, chinensis, Actinidia sp.), Actinidiaceae, Asia (China, Anhui, Zhejiang; Japan, Korea, Taiwan).

Doubtful, excluded and insufficiently known species


Adoxaceae

Cercospora

Tabular key to Cercospora species on Adoxaceae according to host genera

Sambucus
A single species ........................................................................................................ C. sambucicola

Viburnum
A single species ........................................................................................................ C. viburnicola

Cercospora species on Adoxaceae

Cercospora sambucicola Y.L. Guo, nom. nov. MycoBank MB814566 (Fig. 43)


Illustrations: Guo & Jiang (2000: 263, fig. 3), Guo et al. (2005: 56, fig. 32).

Description: Leaf spots amphigenous, subcircular to angular-irregular and vein-limited, 1–18 mm diam, brown to dark brown, yellowish brown below, later centre greyish white with dark margin, sometimes with yellowish brown halo. Caespituli brown, yellowish brown below, later centre greyish white with irregular and vein-limited, 1–18 mm diam, brown to dark grayish white centre on the upper leaf surface, surrounded by a greyish black marginal line, pale yellowish brown internal. Stromata lacking or small, only formed as aggregations of a few swollen hyphal cells, globose, 10–25 µm diam, substomatal or intraepidermal, brown, cells 2–8 µm diam, wall slightly thickened. Conidiophores solitary or in small, loose fascicles, 2–13, arising from internal hyphae or hyphal aggregations, through stomata or erumpent, erect, straight, subcylindrical to geniculate-sinuous, unbranched, width irregular, 42.5–140(–400) × 3.5–7 µm, to 8 µm wide at the base, 1–6(–13)-septate, medium brown, paler and narrower towards the tip, thin-walled, smooth; conidiogenous cells integrated, terminal and intercalary, conidiogenous loci thickened and darkened, 2–3.5(–4) µm diam. Conidia solitary, acicular, straight to somewhat curved or slightly sigmoid, 37.5–175 × 3–5 µm, 5- to pluriseptate, hyaline, thin-walled, smooth, apex pointed or subtubose, base truncate, 2–3.5 µm wide, hila thickened and darkened.

Holotype: China: Jilin Province: Yongji, on Sambucus williamsii, Adoxaceae, Sep. 1962, J. K. Bai (HMAS 77346).

Host range and distribution: On Sambucus (?canadensis, javanica, williamsii [buergeriana]). Adoxaceae, Asia (China, Jilin, Zhejiang), ?North America (USA, Alabama).

Notes: This species belongs to the Cercospora apii s. lat. complex. Type material of Passalora catenospora contains a second cercosporoid hypomycete which is morphologically barely distinguishable from C. sambucicola (conidiophores in small fascicles, to 140 µm long and 4–7 µm wide; conidia...
acicular, hyaline, 3–4.5 µm wide). The conspecificity of Chinese collections and material from the USA remains to be proven via phylogenetic analyses.


(Fig. 44)


*Illustration*: Guo et al. (2005: 57, fig. 33).

*Description*: Leaf spots amphigenous, subcircular to angular-irregular, 2–12 mm diam, different shades of brown, pale to medium brown, reddish brown to greyish brown. *Caespituli* amphigenous, fine, dark. Mycelium internal. Stromata lacking or small, about 10–25 µm diam, brown. Conidiophores in small to moderately large fascicles, mostly dense, arising from internal hyphae or stromata, through stomata or erumpent, erect, subcylindrical to geniculate-sinuous, unbranched, 25–175 × 4–6 µm, septate throughout, uniformly pale olivaceous-brown or somewhat paler towards the tip, thin-walled, smooth; conidiogenous cells integrated, terminal and intercalary, about 15–35 µm long, conidiogenous loci conspicuous, thickened and darkened, 2–3 µm diam. Conidia solitary, acicular, shorter conidia narrowly obclavate, straight to curved, 20–90 × 2–4 µm, pluriseptate, hyaline, thin-walled, smooth, apex acute or subacute, base truncate or somewhat obconically truncate in obclavate conidia, 2–2.5 µm wide, hila thickened and darkened.

Host range and distribution: On *Viburnum* (carlesii, odoratissimum, opulus, plicatum var. tomentosum [tomentosum], suspensum, *Viburnum* spp.), Adoxaceae, ?Asia (China, Jiangsu, Shaanxi, Sichuan), USA (Florida, Oklahoma, Wisconsin).

Notes: *Cercospora viburnicola* W.W. Ray is morphologically part of the *Cercospora api s. lat.* complex. Records of this species on *Viburnum cylindricum* from China are incorrect and based on a confusion with *C. viburnicola*. The collection concerned has been re-examined and turned out to belong to *Pseudocercospora opuli* which is common on *Viburnum opulus* and also known from Poland. Chinese collections on *Viburnum buddlefolium* and *Viburnum* sp. (Guo 1996: 92; Guo & Jiang 2000: 264; Guo et al. 2005: 56–57, fig. 33), referred to as *C. viburnicola*, represent typical *C. api s. lat.*, but these specimens are probably not conspecific with the latter species. The conidiophores are formed in small, divergent fascicles, to about 450 µm long, and the conidia are acicular, about 35–385 µm long. Without any molecular data based on Asian and North American samples, the Chinese collections are currently better classified as *Cercospora api s. lat.*

### Doubtful, excluded and insufficiently known species


**Literature:** Chupp (1954: 98), Crous & Braun (2003: 46).

**Type:** France: on *Adoxa moschatellina*, Roum., *Fungi Sel. Gall. Exs.* 1871 (e.g., B, HBG, PC).

**Host range and distribution:** On *Adoxa moschatellina*, Adoxaceae, Europe (France).

**Notes:** Although listed in literature, this species was never described (Chupp 1954). Numerous duplicates of the original material have been examined, but all were without any trace of fructification. The name *C. adoxae* might refer to *Ramularia adoxae* (Rabenh.) P. Karst.


**Synonym:** *Cercosporella prolificans* (Ellis & Holw.) Sacc., *Syll. Fung.* 15: 84 (1901).


**Illustration:** Braun (1995: 79, fig. 75).

**Exsiccatea:** Calif. Fungi 403.

**Lectotype** (designated by Braun 1995: 80): **USA:** California: San Bernardino Valley, San Bernadino County, on *Sambucus cerulea*, Aug. 1893, Parish 2735 (NY 234139). **Isolectotypes:** BPI 439986, NY 234138.

**Host range and distribution:** On *Sambucus* (cerulea, glauca, melanocarpa), Adoxaceae, North America (USA, Alaska, California, Minnesota, Missouri, New Mexico, Oregon).

**Notes:** *Cercospora prolificans* has colourless conidiophores and conidia as well as cercosporelloid conidiogenous loci and belongs to *Cercosporella* (Braun 1995). This species was recorded and described from Bulgaria on *Sambucus ebulus*.

---

*Fig. 44. Cercospora viburnicola* (CUP 29236, holotype). **A.** Conidiophore fascicle. **B.** Conidia. Bar = 10 µm.

(Fig. 45)


Cercospora affinis G. Winter, in herb. [USA: Pennsylvania, on Sambucus canadensis, ex herb. G. Winter (B 700016202)].


Illustration: Ellis (1976: 318, fig. 240 A).

Exsiccate: Barthol., Fungi Columb. 2309.

Description: Leaf spots indistinct. Caespituli forming effuse, irregular patches, hypophyllous, ochraceous, dark olivaceous to brownish. Mycelium internal. Stromata lacking, only formed as small aggregations of swollen hyphal cells or small, 10–25 μm diam, substomatal, yellowish to ochraceous-brown. Conidiophores in small to moderately large fascicles, usually 3–12, arising from swollen hyphal cells or small stromata, emerging through stomata, erect, straight, subcylindrical or attenuated towards the tip, not or only slightly geniculate, somewhat curved-sinuous, unbranched, 15–80 × 4–6 μm, rarely longer, usually 0–4-septate, subhyaline, pale ochraceous to golden brown or olivaceous-brown, thin-walled, smooth; conidiogenous cells integrated, terminal or conidiophores reduced to conidiogenous cells, 10–30 μm long, conidiogenous loci conspicuous, thickened and darkened, (1–)1.5–2 μm wide. Conidia solitary to catenate, in simple or branched chains, cylindrical, subcylindrical, occasionally almost obclavate or broadly fusiform, straight to curved or slightly sinuous, 20–100(–125) × 3–5.5 μm, 1–8-septate, subhyaline, pale olivaceous to brownish, thin-walled, smooth, apex obtuse, rounded (in primary conidia) or short conically truncate in catenate conidia (with a single or two hila), base short obconically truncate, (1–)1.5–2 μm wide, hila somewhat thickened and darkened.


Passalora Key to Passalora species on Adoxaceae

1 Colonies in pale reddish brown to fuliginous patches; mycelium internal and external, superficial; conidiophores fasciculate as well as solitary, arising from superficial hyphae;

conidia subhyaline to reddish brown; on Sambucus spp. .......................................................... P. lateritia

Colonies (caespituli) without reddish tinge; mycelium internal, superficial hyphae lacking in vivo;

conidia not reddish brown .......................................................... 2

2 (1) Conidiophores short, 5–25 × 2–4 μm, 0–1-septate; conidia 8–45 × 1.5–4 μm, 0–4-septate;

on Viburnum spp. ....................................................................................................................... P. viburni

Conidiophores much larger, 15–80 × 4–6 μm, 0–4-septate; conidia much longer and somewhat broader,

20–100(–125) × 3–5.5 μm, 1–8-septate; on Sambucus spp. .......................................................... P. catenospora

Fig. 45. Passalora catenospora (CUP-A-002255a, lectotype). A. Conidiophore fascicles. B. Conidiophore. C. Conidia. Bar = 10 μm.
Host range and distribution: on Sambucus (cerulea, canadensis, intermedia, Sambucus spp.), Adoxaceae, Central America (Dominican Republic), North America (Canada; USA, Alabama, Florida, Kansas, Mississippi, North Carolina, Oklahoma, Pennsylvania, Texas), West Indies (Haiti).

Note: The lectotype is a collection from October 1891. Other syntypes are from August 1891 (CUP-A-002245#1–3(AL)). Records of this species from Taiwan on Sambucus javanica [formosana] (Tai 1979) are incorrect and refer to Pseudocercospora ebulicola (Cercospora ebulicola) which was erroneously reduced to synonymy with C. catenospora in Chupp (1954). Type material of C. catenospora contains a second cercosporoid hyphomycete which might cause confusion. The second fungus belongs to the Cercospora apii s. lat. complex and is morphologically barely distinguishable from the Chinese Cercospora sambucicola. This species is readily distinguishable from P. catenospora by its much darker conidiophores with larger conidiogenous loci, 2–3 µm diam, as well as hyaline, acicular conidia formed singly.

Passalora lateritia (Ellis & Halst.) U. Braun & Crous, Mycosphaerella and Anam.: 244 (2003).

(Fig. 46)
Basionym: Cercospora lateritia Ellis & Halst., J. Mycol. 4: 7 (1888).


Description: Leaf spots lacking or indefinite. Colonies hypophyllous, effuse, forming reddish, pale reddish brown to ferruginous patches. Mycelium internal and external; superficial hyphae lacking or present, emerging through stomata, occasionally climbing leaf hairs, rarely anastomosing, unbranched or sparingly branched, 1–7 µm wide, septate, concolorous with conidiophores or pale, thin-walled, smooth. Stromata lacking or formed as stomatic aggregations of swollen hyphal cells, substomatal, 10–50 µm diam, reddish brown. Conidiophores in small to moderately large fascicles, divergent to moderately dense, arising from substomatal hyphae or stromata, through stomata or conidiophores solitary, arising from superficial hyphae, lateral or terminal, 5–75 × 3–8 µm, 0–4-septate, subhyaline to pale reddish brown, thin-walled, smooth; conidiogenous cells integrated, terminal or conidiophores reduced to conidiogenous cells, 5–30 µm long, conidiogenous loci conspicuous, thickened and darkened, about 1–2 µm diam. Conidia solitary to catenate, usually in simple chains, cylindrical or subcylindrical or somewhat

Fig. 46. Passalora lateritia (NY 838232, lectotype). A. Superficial hypha. B. Conidiophore fascicles. C. Conidiophore. D. Solitary conidiophores arising from superficial hyphae. E. Conidia. Bar = 10 µm.

Fig. 47. Passalora viburni (NY 830558, lectotype). A. Conidiophore fascicle. B. Conidiophores. C. Conidia. Bar = 10 µm.
obclavate-cylindrical, straight to slightly curved, 15–70(–80) × 3–7 µm, (0–)1–6-septate, occasionally constricted at the septa, subhyaline to pale reddish brown, thin-walled, smooth, apex obtuse, rounded in solitary conidia, short conically truncate in catenate conidia, base obconically truncate, (1–)1.5–2(–2.5) µm wide, hila somewhat thickened and darkened.


Host range and distribution: On Sambucus (canadensis, nigra, pubens, racemosa), Adoxaceae, North America (USA, Iowa, Kansas).

Notes: This is a typical mycovelloioid member of Passalora, with fasciculate and solitary conidiophores arising from superficial hyphae, thickened, darkened conidiogenous loci and obclavate-cylindrical, pigmented conidia. This species is very characteristic by its effuse, reddish to reddish brown colonies and reddish tinge of conidiophores and conidia.

Passalora viburni (Ellis & Everh.) U. Braun & Crous, Mycosphaerella and Anam.: 474 (2003). (Fig. 47)

Basionym: Ramularia viburni Ellis & Everh., J. Mycol. 5: 69 (1889).


Pseudocercospora

Key to Pseudocercospora species on Adoxaceae

1 Stromata lacking; superficial hyphae developed, but without solitary conidiophores; conidiophores fasciculate, 50–190 µm long, pluriseptate; on Sambucus javanica, Asia .................................................. P. ebulicola
Stromata developed, 10–100 µm diam; superficial hyphae lacking or, if present, with conidiophores which are much shorter, 5–35 µm, and only 0–1-septate .......................................................... 2

2 (1) Superficial hyphae with solitary conidiophores developed, 5–35 µm long, 0–1-septate, geniculate, i.e. proliferation symiodial; on Viburnum spp. ................................................................................. P. tinea
Superficial hyphae with solitary conidiophores lacking; conidiophores longer, 10–200 µm, aseptate to pluriseptate (if short and mostly aseptate, then non-geniculate, proliferation percurrent, with annelations .................................................................................................. 3

3 (2) Conidiophores subcylindrical, non-geniculate, proliferation percurrent, with fine annelations, rather short, 5–30(–50) × 2–5(–6) µm, 0(–2)-septate; conidia cylindrical-filiform or occasionally somewhat obclavate; on Viburnum spp. .............................................................. P. viburnigena
Conidiophores at least partly geniculate-sinuous, proliferation symiodial, without annelations or occasionally with a single enteroblastic rejuvenation, much longer, 10–200(–300) µm, 0–3-septate or pluriseptate throughout; conidia obclavate-cylindrical to somewhat fusiform ....................................... 4

Description: Leaf spots subcircular to angular-irregular, sometimes vein-limited, 2–10 mm diam, greyish green to brown, later centre greyish white, with darker margin. Caespituli amphiogenous, punctiform, greyish white to brownish. Mycelium internal. Stromata intraepidermal, occasionally substomatal, 10–50 µm diam, occasionally confluent and larger, pale yellowish brown to medium brown, composed of swollen hyphal cells, 2–6 µm diam. Conidiophores in small to rather large fascicles, arising from stromata, erumpent, occasionally emerging through stomata, erect, straight, subcylindrical to geniculate-sinuous, unbranched, 5–25 × 2–4 µm, 0–1-septate, hyaline, yellowish, greenish to pale olivaceous or yellowish brown, thin-walled, smooth; conidiogenous cells integrated, terminal or conidiophores mostly reduced to conidiogenous cells, 5–20 µm long, conidiogenous loci conspicuous, minute, 1–1.5 µm diam, barely or very slightly thickened, somewhat darkened-refractive. Conidia solitary or catenate, in simple or rarely branched chains, shape and size variable, narrowly ellipsoid-ovoid, fusiform, subcylindrical, straight to slightly curved, 8–45 × 1.5–4 µm, 0–4-septate, hyaline to pale yellowish or greenish, smooth to faintly verruculose, thin-walled, ends attenuated, short obconically truncate when in chains, about 1 µm wide, hila minute, very slightly thickened and darkened-refractive. Notes: This is a typical mycovelloioid member of Passalora, with fasciculate and solitary conidiophores arising from superficial hyphae, thickened, darkened conidiogenous loci and obclavate-cylindrical, pigmented conidia. This species is very characteristic by its effuse, reddish to reddish brown colonies and reddish tinge of conidiophores and conidia. Lectotype (designated by Braun 1993): USA: Wisconsin: Racine, on Viburnum lentago, 17 Jun. 1888, J. J. Davis (NY 830558). Isolectotype: NY 830559, WIS.

Host range and distribution: On Viburnum (lentago, prunifolium), Adoxaceae, North America (USA, Tennessee, Wisconsin).

Notes: The generic affinity of this species, which is intermediate between Passalora and Ramularia, is not quite clear. The general habit of this species is rather ramularioid, but the stromata and conidiophores are pigmented. Results of molecular sequence analyses would be helpful to elucidate the true generic affinity.
4 (3) Conidia short, 15–40 × 3–7 µm, 1–5-septate, pigmented; conidiophores in divergent fascicles, walls thickened, to 1 µm, medium to dark brown, occasionally with a single enteroblastic rejuvenation leaving a coarse annellation; on Viburnum nudum, North America ................................. P. viburni-nudi
Conidia longer, 25–150 µm, 1–12(–15)-septate; on other hosts .................................................. 5

5 (4) Conidia broadly obclavate or obclavate-cylindrical, 25–125 × 4.5–8 µm, 2–10-septate, usually pale to medium olivaceous or olivaceous-brown, wall thin to somewhat thickened, to 0.8 µm; on Sambucus spp., North America .................................................... P. viburnicola
Conidia narrower, 2–5 µm, if wider [2–6.5(–7) µm] conidia consistently pale, usually hyaline or subhyaline and/or thin-walled, or conidia at least partly catenate or disarticulating .......................... 6

6 (5) Conidiophores relatively short, usually 10–80 µm, average < 60 µm .................................................. 7
Conidiophores longer, about 50–155 µm, average > 60 µm .................................................. 9

7 (6) Conidia hyaline or subhyaline, finally sometime pale olivaceous, solitary or in short chains or disarticulating, conidiogenous loci inconspicuous to subconspicuous, i.e. unthickened, but somewhat refractive or even slightly darkened-refractive; on Viburnum spp.
(Viburnum sect. Opulus, mainly V. opulus s. lat. (including subsp. calvescens and trilobum) .................. P. opuli
Conidia formed singly ................................................................. 8

8 (7) Conidiogenous loci inconspicuous to subconspicuous, i.e. unthickened but somewhat refractive or even darkened-refractive; on Sambucus spp. ................................................................. P. depazeoides
Conidiogenous loci inconspicuous (conidiophores usually in small fascicles, 2–15, divergent, North American collections = var. varia; conidiophores in larger, sense fascicles, to 50 per fascicle, Asian collections on Viburnum sargentii = var. viburni-sargentii); on Viburnum spp. ................................................................. P. varia

9 (6) Conidiogenous loci consistently inconspicuous; conidiophores fasciculate to coremioid; on Viburnum spp. ................................................................. 10

10 (9) Conidia cylindrical-filiform, 50–130 × 2.5–4 µm, 3–12-septate; on Viburnum erosum ......................... P. viburni-erosi
Conidia obclavate-cylindrical, 25–85 µm long, 2–7-septate ................................................................. 11

11 (10) Conidia 2.5–4 µm wide, 2–5-septate; on Viburnum cylindricum, China ................................. P. viburni-cylindrici
Conidia wider, 3–6.5 µm, 3–7-septate; on Viburnum sp., India .......................................................... P. caprifoliacearum

**Tabular key to Pseudocercospora species on Adoxaceae according to host genera**

**Sambucus**
1 Stromata lacking; superficial hyphae developed; conidiophores loosely fasciculate, simple to branched; on Sambucus javanica, Asia ................................................................. P. ebulicola
Stromata developed, 10–100 µm diam; superficial hyphae lacking; conidiophores mostly in denser fascicles, unbranched; on Sambucus spp., northern hemisphere ........................................ P. depazeoides

**Viburnum**
1 Superficial hyphae with solitary conidiophores developed, 5–35 µm long, 0–1-septate, geniculate, i.e. proliferation sympodial ................................................................. P. tinea
Superficial hyphae with solitary conidiophores lacking; conidiophores longer, 10–200 µm, aseptate to pluriseptate (if short and mostly aseptate, then non-geniculate, proliferation percurrent, with annellations ................................................................. 2

2 (1) Conidiophores subcylindrical, non-geniculate, proliferation percurrent, with fine annellations, rather short, 5–30(–50) × 2–5(–6) µm, 0(–2)-septate; conidia cylindrical-filiform or occasionally somewhat obclavate ................................................................. P. viburnigena
Conidiophores at least partly geniculate-sinuous, proliferation sympodial, without annellations or only with a single enteroblastic rejuvenation, much longer, 10–200(–300) µm, 0–3-septate or pluriseptate throughout; conidia obclavate-cylindrical to somewhat fusiform .......................... 3
3 (2) Conidia broadly obclavate or obclavate-cylindrical, 25–125 × 4.5–8 µm, 2–10-septate, usually pale to medium olivaceous or olivaceous-brown, wall thin to somewhat thickened, to 0.8 µm; on Viburnum spp., North America ................................................................. P. viburnicola

Conidia narrower, 2–5 µm, if wider [2–6(–7) µm] conidia consistently pale, usually hyaline or subhyaline and thin-walled ....................................................... 4

4 (3) Conidiophores relatively short, usually 10–80 µm, average < 60 µm ......................................................... 5
Conidiophores longer, about 50–155 µm, average > 60 µm ................................................................. 7

5 (4) Conidia short, 15–40 × 3–7 µm, 1–4(–5)-septate, pigmented; conidiophores in divergent fascicles, walls thickened, to 1 µm, medium to dark brown, occasionally with a single enteroblastic rejuvenation leaving a coarse annellation; on Viburnum nudum, North America ................................................. P. viburni-nudi

Conidia longer, 30–150 µm, 1–12(–15)-septate; on other hosts .................................................................. 6

6 (5) Conidia solitary, in short chains or disarticulating, conidiogenous loci inconspicuous to subconspicuous, i.e. unthickened, but somewhat refractive or even slightly darkened-refractive; on Viburnum spp. (Viburnum sect. Opulus), mainly V. opulus s. lat. (including subsp. calvescens and trilobum) ........................................ P. opuli

Conidia solitary; conidiogenous loci inconspicuous, not darkened-refractive (conidiophores usually in small fascicles, 2–15, divergent, North American collections = var. varia; conidiophores in larger, sense fascicles, to 50 per fascicle, Asian collections on Viburnum sargentii = var. viburni-sargentii); on Viburnum spp. ......................................................... P. varia

7 (4) Conidia cylindrical-filiform, 50–130 × 2.5–4 µm, 3–12-septate; on Viburnum erosum .......................... P. viburni-erosi

Conidia obclavate-cylindrical, 25–85 µm long, 2–7-septate .................................................................. 8

8 (7) Conidia 2.5–4 µm wide, 2–5-septate; on Viburnum cylindricum, China ............................................ P. viburni-cylindrici
Conidia wider, 3–6.5 µm, 3–7-septate; on Viburnum sp., India ............................................................... P. caprifoliacearum

Pseudocercospora caprifoliacearum (C. Gupta et al.) Kamal, Cercosporoid Fungi of India: 159 (2010). (Fig. 48)


Synonym: Pseudocercospora khasiana B.K. Gupta & Kamal, Perspectives in Mycological Research, Prof. G.P. Agarwal Festschrift Volume 1: 25 (1987) [holotype: India: Meghalaya, Shillong, on Viburnum sp., B. K. Gupta KB 58 (K(M) IMI 274850)]


Illustrations: Gupta et al. (1987: 16, fig. 2), Gupta & Kamal (1987: 33, fig. 4).

Description: Leaf spots amphigenous, subcircular to angular-irregular, 2–20 mm diam, brown, olivaceous-brown, later greyish brown to dingy grey, margin indefinite or with narrow to broad darker border or halo. Caespituli amphigenous, scattered, finely punctiform, brown to blackish. Mycelium immersed, composed of subhyaline to pale brown, septate, thin-walled, smooth hyphae. Stromata about 10–50 µm diam, dark brown, substomal, cells 2–6 µm diam. Conidiophores in loose to dense, almost corediom fascicles, 7–18, arising from stromata, though stromata, erect, when corediom then dense below and looser towards the apex, straight to flexuous, unbranched or only rarely branched, subcylindrical-filiform, width uniform throughout or tips somewhat swollen, genulate-sinuous in the upper half, about 60–155 µm long and 2.5–4.5 µm wide, apex sometime to 6 µm wide, pluriseptate throughout, olivaceous-brown, wall thin-walled to somewhat thickened, to 1 µm, at the very base occasionally to 1.3 µm, smooth; conidiogenous cells integrated, terminal, occasionally intercalary, sympodially proliferating, with several conidiogenous loci, inconspicuous or visible as truncate tips or shoulders, subdenticulate, but not thickened. Conidia solitary, obclavate-cylindrical, broadly fusiform, straight to slightly curved, about 30–85 × 3–6.5 µm, 1–7-septate, subhyaline to pale olivaceous or olivaceous-brown, thin-walled, smooth, apex obtuse, base obconically truncate, 1.5–2.5 µm wide, hila unthickened, not darkened.


Host range and distribution: On Viburnum spp., Adoxaceae, Asia (India, Meghalaya, Uttarakhnad, Uttar Pradesh).

Notes: Pseudocercospora caprifoliacearum and P. khasiana, both described from India on Viburnum sp., are morphologically barely distinguishable and undoubtedly conspecific. The two species have been simultaneously published in a single book. It is proposed to give precedence to Phaeoisariopsis caprifoliacearum, and to reduced P. khasiana to synonymy. Braun (1992) examined type material of Pseudocercospora khasiana and compared it with P. viburni-cylindrici, described from China. The two species are morphologically very similar. Therefore, Braun (1992) proposed to reduce P. khasiana to synonymy with P. viburni-cylindrici, a treatment followed by Crous & Braun (2003) and Kamal (2010). Differences...
between Chinese and Indian collections were considered to be variation within a single species. The Chinese type material of *P. viburni-cylindrici* is characterised by having loose to often dense or even corymbose conidiophores, similar to *P. caprifoliacearum*, but narrower conidia, 2–4.5 µm wide. We prefer to maintain *P. caprifoliacearum* (including *P. khasiana*) as separate species, at least for the interim until cultures and molecular data will be available.

**Pseudocercospora depazeoides** (Desm.) U. Braun & Crous, *combin. nov.*

MycoBank MB814570 (Fig. 49)


**Synonyms:** *Passalora penicillata* Ces., in Rabenh., *Herb. Viv. Mycol.*, no. 587 (1857), with description on label *[lectotype (designated here), MycoBank, MBT202789]:


**Cercospora penicillata** (Ces.) Fresen., *Beitr. Mykol.* 93 (1863).

**Cercospora depazeoides** (Desm.) Sacc., *Myoth. Ven.*, *Cent. Ill.*, no. 280 (1875).

**Cercospora sambucina** Ellis & Kellerm., *Amer. Naturalist* 17: 1166 (1883) *[lectotype (designated here), MycoBank, MBT202790]: USA; Ohio, on *Sambucus canadensis*, Jul. 1883, W. A. Kellerman 401 (NY 2343036); isolecotypes: CUP 41160, NY 2343037*.


**Cercospora tícinosensis** Cavara, in Briosi & Cavara, *Fungi Paras.* Piante Colt. *Util. Ess.*, no. 336 (1900) *[lectotype (designated here), MycoBank, MBT202792]:


**Cercospora sambuci** F. Stevens & King, *Illinois Biol. Monogr.* 11: 59 (1927) *[holotype: Costa Rica]: Cartago, on *Sambucus canadensis* [mexicana], 7 Jul. 1923, F. L. Stevens (ILL 15175); isotypes: CUP 14660, NY 2343299.


Description: Leaf spots amphigenous, subcircular to angular-irregular, 1–10 mm diam, sometimes confluent and larger, at first pale greenish or greyish green, later brownish, finally grey to greyish white, often zonate, margin narrow, often formed as marginal line, somewhat raised, dark olivaceous-brown to blackish or dark purplish violet. Caespituli amphigenous, punctiform, dark brown to blackish, scattered. Mycelium internal; hyphae branched, septate, 2–4 µm wide, subhyaline to pale olivaceous, thin-walled. Stromata well-developed, substomatal to intraepidermal, subglobose to somewhat irregular, 10–100 µm diam, immersed to somewhat erumpent, medium to dark brown, composed of swollen hyphal cells, 3–7 µm diam. Conidiophores in small to large fascicles, loose to very dense, occasionally even almost coremioid, arising from stromata, emerging through stomata or erumpent, erect, straight, subcylindrical to flexuous, somewhat geniculate-sinuous, unbranched, 20–200(–300) × 3–7 µm, short conidiophores 0–1-septate, longer ones pluriseptate throughout, pale olivaceous to dark olivaceous-brown or brown, tips often paler, thin-walled, smooth or almost so; conidiogenous cells integrated, terminal, occasionally conidiophores reduced to conidiogenous cells, 10–30 µm long, sympodially or occasionally percurrently proliferating, conidiogenous loci inconspicuous, unthickened or almost so and not darkened to subconspicuous by being substidicate, tips truncate or subtruncate, 1.5–2.5 µm wide, or slightly

Conidia paracercosporoid, visible as minute circle, 1.5–2 µm diam. 

Culture characteristics: Colonies on MEA reaching 16 mm diam after 30 d in the dark at 24°C, circular to subcircular, with smooth to slightly irregular margin, prominently convex, with moderate aerial mycelium, pale greenish grey to pale discolorations, yellowish to brownish, or circular to irregular spots, 1–8 mm diam, pale brown, yellowish brown to brown, with moderate aerial mycelium, pale greenish grey to pale yellowish white, margin indefinite or brown on the upper surface. Caespituli hypophyllous, effuse, olivaceous to brownish, forming irregular patches. Stromata wide, septate, pale olivaceous.


Notes: The generic affinity of Cercospora depazeoides has been confused and misinterpreted. Owing to the structure of the conidiogenous loci of this species, it cannot be excluded that the cercosporoid as well as the alternarioid fungus were included in the very meagre original description. However, we follow Saccardo’s treatment on the occasion of his introduction of the combination Cercospora depazeoides and confine this name to the cercosporoid element. Saccardo’s combination is usually cited as “Nuovo Giorn. Bot. Ital. 8: 187 (1876)” but it was first validly published in Mycotheca Veneta 280 (1875). The designated epitype, the ex-epitype culture and sequences obtained from this material help to fix the phylogenetic position and affinity of this species. The reallocation of C. depazeoides to Pseudocercospora has serious consequences on genetic level since Cercospora penicillata (i.e. C. depazeoides) being the type species of the genus Cercospora (Braun 1995, Braun et al. 2013). Thus, the allocation of C. depazeoides to Pseudocercospora, based on morphological reassessments and results of molecular sequence analyses, renders Cercospora an older heterotypic synonym of Pseudocercospora, which is undesirable and requires a proposal to conserve Cercospora with C. api as conserved type under Art. 14.9, which is being made.

Eriksson, Fungi Paras. Scand. Exs. 42, deposited at HAL, has been examined and turned out to be a mixture of Ramularia sambucina and Cladosporium herbarum.


Illustrations: Hsieh & Goh (1990: 50, fig. 34), Guo & Hsieh (1995: 49, fig. 46), Guo et al. (1998: 60, fig. 46).

Description: Leaf spots amphigenous, indefinite discolorations, yellowish to brownish, or circular to irregular spots, 1–8 mm diam, pale brown, yellowish brown to brown, later greyish white, margin indefinite or brown on the upper surface. Caespituli hypophyllous, effuse, olivaceous to brownish, forming irregular patches. Mycelium internal or partly external; superficial hyphae branched, 2–3 µm wide, septate, pale olivaceous. Stromata lacking or very small, only a few substomatal swollen hyphal cells, brown. Conidiophores in loose fascicles, 3–15, arising from internal hyphae or small hyphal aggregations, through stomata, erect, subcyllindrical, curved, geniculate-sinuous to somewhat tortuous, simple or branched, 50–190 × 4–5.5
µm, 2–8-septate, olivaceous to olivaceous-brown, thin-walled, smooth; conidiogenous cells integrated, terminal and intercalary, conidiogenous loci inconspicuous or subdenticulate, but always unthickened, not darkened. Conidia solitary, obclavate-cylindrical, straight to curved, 20–130 × 3–5 µm, 2–11-septate, subhyaline, yellowish to pale olivaceous, thin-walled, smooth, apex obtuse to subacute, base subtruncate to short obconically truncate, 1.5–2.5 µm wide, hila unthickened, not darkened.


Fig. 50. Pseudocercospora ebulicola (CUP-039732, lectotype). A. Conidiophore fascicle. B. Solitary conidiophore arising from a superficial hypha. C. Conidiophore tips. D. Conidia. Bar = 10 µm.

Host range and distribution: On Sambucus (javanica [formosana]), Adoxaceae, Asia (China, Sichuan, Zhejiang; Japan, Taiwan).

Notes: Chupp (1954) reduced Cercospora ebulicola to synonym with C. catenospora which is, however, incorrect. Cercospora ebulicola is a species of the genus Pseudocercospora whereas C. catenospora is a Phaeoramularia-like species of Passalora.

Pseudocercospora opuli (Fuckel) U. Braun & Crous, Mycophaearella and Anam.: 299 (2003).

(Fig. 51)
Basionym: Cercospora penicillata f. opuli Fuckel, Fungi Rhen. Exs. 118 (1863).

Host range and distribution: On Viburnum (edule [pauciflorum], opulus subsp. opulus, opulus subsp. calvescens [opulenti var. calvescens], opulus subsp. trilobum [opuleni var. americanum, trilobum]), Adoxaceae, Asia (China, Kazakhstan, Russia, Turkey), Caucasus (Armenia, Azerbaijan, Georgia), Europe (Austria, Belarus, Bulgaria, Czech Republic, Denmark, Estonia, Finland, Germany, Great Britain, Italy, Latvia, Poland, Portugal, Romania, Russia, Slovakia, Sweden, Ukraine), Central America (Costa Rica), North America (Canada; USA, Idaho, Iowa, Kansas, Mississippi, Oklahoma, Wisconsin).

Notes: A record of this species on V. burejaeticum from the Far East of Russia (Egorova 2007) is uncertain and unproven and might rather pertain to P. varia. Viburnum burejaeticum does not belong to Viburnum sect. Opulus, but P. opuli seems to be confined to species of this section, which has been confirmed as well supported clade in phylogenetic studies (Winkworth & Donoghue 2005, Clement et al. 2014). Viburnum lantana and V. orientale (Crous & Braun 2003) are additional unproven and doubtful hosts of P. opuli not belonging to sect. Opuli. The taxonomic history of this species is complicated and characterised by confusions and misinterpretations. Passalora penicillata (syn. Cercospora penicillata, now Pseudocercospora depaezoides) was introduced for a cercosporoid fungus on Sambucus nigra. Fuckel (1863) added C. penicillata f. opuli, and Fuckel (1870) treated the fungus on Viburnum opulus as sole species of C. penicillata, i.e. “f. penicillata” on Sambucus nigra was not mentioned, which seems to be the reason for Saccardo’s (1886) misinterpretation of the name C. penicillata. Chupp (1954) followed Saccardo’s misinterpretation, although the confusion of the names involved had already been discussed and corrected by Lindau (1910). The proposed combination Phaeoramularia penicillata (Liu & Guo 1982) was also based on Chupp’s wrong interpretation of the name Cercospora penicillata. Höhnel’s name C. opuli can be interpreted as new species (see Braun & Mel’nik 1997, Crous & Braun 2003) or as new combination at new rank according to Art. 41.4. We prefer to follow the second version. The morphology of this species is unusual and caused additional confusion. The conidia are formed singly as well as in chains or disarticulate in smaller units. The conidigenous loci range from being truncate and somewhat refractive or darkened-refractive, but unthickened, in front view sometimes visible as minute circles, about 1.5–2.5 µm diam. Conidia solitary to catenate, in simple, short acropetal chains or disarticulating, obclavate-subcylinidrinal to cylindrical, straight to somewhat curved, (20–)30–90–(145) × (2.5–)3–6–(7) µm, 1–10-septate, occasionally somewhat constricted at the septa, hyaline, subhyaline, later pale olivaceous, thin-walled, smooth, apex of primary and solitary conidia obtuse, rounded, truncate to short conically truncate in catenate conidia, base truncate to short obconically truncate, 2–3 µm wide, hila unthickened, not to slightly refractive or darkened-refractive.


Illustrations: Chupp (1954: 103), Ellis (1976: 248, fig. 186A), Braun (1992: 216, fig. 9), Braun & Mel’nik (1997: appendix, fig. 82), Guo (2012: 161, fig. 2).


Description: Leaf spots amphigenous, subcircular to somewhat angular-irregular, 1–8 mm diam, sometimes confluent and larger, occasionally somewhat zonate, dull greenish, ochraceous to brown, centre later greyish to greyish white, with narrow dark margin. Caespituli amphiogenous, punctiform, scattered, dark brown to blackish. Mycelium internal. Stromata substomatal or immersed, 10–80 µm diam, brown, composed of swollen hyphal cells, 2–7 µm diam, walls somewhat thickened. Conidiophores in small to fairly large fascicles, loose to dense, arising from stroma, emerging through stomata or erumpent, erect, straight, subcylindrical to moderately geniculate-sinuous, unbranched or occasionally with a single branchlet, 15–70–(80) × (2.5–)3–7–(8) µm, aseptate or sparingly septate, usually with 0–3 septa, at first subhyaline and thin-walled, later pale olivaceous to olivaceous-brown and walls somewhat thickened, smooth; conidiogenous cells integrated, terminal or conidiophores reduced to conidiogenous cells, about 10–40 µm long; conidiogenous loci inconspicuous to subconspicuous by being truncate and somewhat refractive or darkened-refractive, but unthickened, in front view sometimes visible as minute circles, about 1.5–2.5 µm diam. Conidia solitary to catenate, in simple, short acropetal chains or disarticulating, obclavate-subcylindrical to cylindrical, straight to somewhat curved, (20–)30–90–(145) × (2.5–)3–6–(7) µm, 1–10-septate, occasionally somewhat constricted at the septa, hyaline, subhyaline, later pale olivaceous, thin-walled, smooth, apex of primary and solitary conidia obtuse, rounded, truncate to short conically truncate in catenate conidia, base truncate to short obconically truncate, 2–3 µm wide, hila unthickened, not to slightly refractive or darkened-refractive.
has been examined (Kütahya, on Viburnum opulus, 9 Jul. 1953, ex herb. Petrak, GZU).

*Pseudocercospora varia* is morphologically very close to and confusable with *P. opuli*, but the conidia are formed singly. The latter species seems to be confined to species on *Viburnum* sect. *Opuli*. The taxonomic meaning of conidial catenation, which occurs in varying degrees in *P. opuli*, is not quite clear and has to be elucidated by means of molecular methods. Other morphological characters of *P. opuli* and *P. varia* are very similar. The two species are treated as separate species, at least tentatively.

(Fig. 52)  

**Literature:** Hsieh & Goh (1990: 51–52, as *Stigmina tinea*), Guo et al. (1998: 63–64).  

**Illustrations:** Hsieh & Goh (1990: 53, fig. 36, as *Stigmina tinea*), Guo (1995: 125, fig. 4), Guo et al. (1998: 64, fig. 49).  

**Description:** Leaf spots amphigenous, subcircular to angular-irregular and vein-limited, 0.5–7 mm diam, often confluent and along the leaf margin, brown to dark brown, margin indefinite or centre later greyish brown, dingy grey to greyish white, margin dark brown above and paler brown below, sometimes with diffuse yellowish to greyish brown halo. *Caespituli* amphigenous, punctiform, dark brown. *Mycelium* internal and external; superficial hyphae hypophyllous, emerging through stomata or arising from the base of conidiophore fascicles, branched, septate, pale olivaceous, thin-walled, smooth, 1.5–2.5 µm wide. *Stromata* well-developed, substomatal, 15–75 µm diam, brown. *Conidiophores* in small to moderately large fascicles, divergent to usually dense, arising from stromata, through stomata or solitary, arising from superficial hyphae, erect, straight to curved, subcylindrical, somewhat attenuated towards the tip or irregular in width, geniculate-sinuous, unbranched, relatively short, 5–35 × 2–5 µm, 0–1-septate, pale olivaceous, olivaceous-brown to brownish, thin-walled, smooth; conidigenous cells integrated, terminal or conidiophores often reduced to conidigenous cells, 5–20 µm long, conidigenous loci inconspicuous or subdenticulate, but always unthickened and not darkened. *Conidia* solitary, obclavate-cylindrical, 10–120 × 2–3.5(–4) µm, 1–11-septate, subhyaline to pale olivaceous or olivaceous-brown, thin-walled, smooth, apex obtuse to subacute, base subtruncate to usually obconically truncate, 1–2.5 µm wide, hila unthickened, not darkened.

**Holotype:** *China:* Guangdong Province: Guangzhou, on *Viburnum macrocephalum*, 6 Nov. 1961, Q. M. Ma & X. J. Liu 1079 (HMAS 67254).

**Host range and distribution:** On *Viburnum* (*luzonicum, macrocephalum, plicatum* var. *tomentosum* [tomentosum], *suspensum, Viburnum sp.*), Adoxaceae, Asia (China, Anhui, Guangdong, Sichuan, Zhejiang; Japan, Taiwan).

Notes: Guo and Hsieh (in Guo 1995) compared this species with *Cercospora tinea*, supposed that the two species could be synonymous, but hesitated to reduce them to synonymy. Therefore, they did not propose a new combination based on *Cercospora tinea*, but preferred to describe a new species with a Chinese type collection. Hence, the name *Pseudocercospora tinea* is valid. However, as already stated in Braun & Hill (2002), *Cercospora tinea* (now *Pseudocercospora viburnigena*) is clearly distinct from the Chinese species by having non-geniculate, only percurrently proliferating conidigenous cells with fine annellations. Superficial hyphae are only rarely formed. *Pseudocercospora tinea* is possibly rather common in China and Taiwan. Two additional Taiwanese collections on *Viburnum* sp. have been examined and identified by C. Nakashima (TUA 40, 56), and Japanese collections on *Viburnum suspensum* and *V. plicatum* var. *tomentosum* [V. tomentosum] have been revised and confirmed as *P. tinea* by C. Nakashima (CNS567, CNS976).

*var. varia* 

(Fig. 53a)


*Description*: Leaf spots amphigenous, subcircular to angular-irregular, 1–8 mm diam, sometimes confluent and larger, pale to medium dark brown or reddish brown, later grey to greyish white, margin indistinct, formed as marginal line or with narrow to moderately wide, dark brown margin, sometimes somewhat raised, and sometimes with ochraceous, yellowish brown to brownish halo. *Caespituli* amphigenous, punctiform, scattered, brown, dark brown to almost blackish. *Mycelium* internal (in some collections with superficial hyphae probably formed by conidial germination); hyphae branched, septate, pale, 1.5–3 µm wide, thin-walled, smooth. *Stromata* substomatal or intraepidermal, small to well-developed, 10–60 µm diam, subglobose, pale to medium brown or yellowish brown, cells globose or subglobose to somewhat irregular, 3–10 µm diam, wall thickened, to 1 µm. *Conidiophores* solitary or in usually small fascicles, mostly 2–15, usually divergent, rarely dense, arising from stromata, through stomata or erumpent, erect, straight to somewhat curved, subcylindrical or attenuated towards the tip, mostly not geniculate-sinuous, occasionally somewhat geniculate-sinuous in the upper half, unbranched, (5–)10–80(–100) × 2–9 µm, usually 0–3-septate, at first very pale, hyaline to subhyaline, later pale olivaceous, olivaceous-brown or yellowish brown, above all below, mostly paler towards the tip, wall thin to usually somewhat thickened, to 0.5 µm, above all below, sometimes to 1 µm thick, smooth; conidiogenous cells integrated, terminal or conidiophores reduced to conidiogenous cells, 15–40 µm long, conidiogenous loci inconspicuous or visible by being truncate or subdenticulate, but always unthickened and not darkened, occasionally somewhat refractive or loci visible as minute circle. *Conidia* solitary, obclavate-cylindrical, cylindrical, rarely subacicular, straight to somewhat curved, (15–)20–85(–90) × 2–6.5 µm, 1–7-septate, hyaline to subhyaline, thin-walled, smooth, apex obtuse, occasionally subacute, base truncate to short or long obconically truncate, 1–2.5 µm wide, hila unthickened, not darkened.


*Host range and distribution*: On *Viburnum* (*acerifolium*, *alnifolium*, *cassinoides*, *dilatatum*, *lentago*, *odoratissimum*, *prunifolium*, *pubescens*, *rafinesquianum* var. *affine* [affine], *suspressum*, Adoxaceae, North America (Canada; USA, Alabama, Alaska, Florida, Idaho, Illinois, Iowa, Kansas, Massachusetts, Mississippi, New York, Oklahoma, Oregon, South Carolina, Texas, Vermont, Virginia, West Virginia, Wisconsin, Wyoming)).

*Notes*: Collections on *Viburnum edule* [var. *pauciflorum*], *V. opulus* and *V. trilobum* have a tendency to form conidia in short chains and short lateral branchlets. They are referred to as *P. opuli*. Records of this species from New Zealand (Gagdil 2005) on *Viburnum lantana* and *V. carlesii* are quite unclear. The collections concerned have been examined (PDD 19826, 19843, 1985), but sufficient fructification for a final identification has not been found. Superficial hyphae with solitary conidiophores found on "V. lantana" are not in accordance with *P. varia*. The host identity is also uncertain (short petioles do not coincide with *V. burejaeticum*). Other records of *C. varia* on *Lonicera* spp. are also based on misidentifications. Collections on *L. japonica* belong to *Pseudocercospora lonicericola* (material examined: BPI 442155), other specimens, e.g. on *L. ciliata* (BPI 442168–442170), refer to *Passalora antipus* (Ellis & Holw.) U. Braun & Crous.
Cercosporoid fungi 4

ARTICLE

427
volume 6 · no. 2
var. viburni-sargentii
U. Braun & H.D. Shin, var. nov.
MycoBank MB814558
(Fig. 53b)

Diagnosis: Morphologically close to North American collections of var. varia, but characterised by having conidiophores in larger, dense fascicles, about 10–30; conidia, 35–145 × 3.5–6.5 µm, 2–15-septate.


Host range and distribution: On Viburnum sargentii, Adoxaceae, Asia (China, Heilongjian, Liaoning; Korea).

Notes: The Asian collections on Viburnum sargentii from China and Korea differ from North American specimens in having much larger, dense fascicles of conidiophores. The taxonomic status of the Asian collections is not quite clear and in urgent need of molecular confirmation. It cannot be excluded that this is a separate species, but for the interim we prefer a conservative treatment as variety.

Pseudocercospora viburnicola U. Braun, sp. nov.
MycoBank MB814559
(Fig. 54)

Diagnosis: Differs from Pseudocercospora viburni-nudi in having much longer, usually obclavate conidia, 25–125 × 4.5–8 µm, with 2–10 septa and somewhat thickened walls and subconspicuous conidiogenous loci. Pseudocercospora caprifoliacearum, known from India on Viburnum sp., has longer conidiophores, 60–155 µm, and the conidia are 3–6.5 µm wide and thin-walled.

Description: Leaf spots amphigenous, subcircular to angular-irregular, 1–8 mm diam, medium to dark brown, finally with pale centre, greyish brown to dingy greyish white, margin indefinite or narrow, dark, or surrounded by darker veins, occasionally somewhat raised. Caespituli amphigenous, punctiform, scattered, blackish. Mycelium internal. Stromata lacking or substomatal to intraepidermal, 10–50 µm diam, brown, swollen hyphal cells 2–7 µm diam, walls slightly thickened. Conidiophores in small, divergent fascicles, mostly 3–12, arising from substomatal hyphae or stromata, through stomata or erumpent, erect, straight, subcylindrical to distinctly geniculate or geniculate-sinuous in the upper fertile portion, unbranched, 20–100 × 3.5–8(–10) µm, (0–)1–4(–5)-septate, medium to dark brown throughout or somewhat paler towards the tip, wall somewhat thickened, to 0.8 µm, smooth; conidiogenous cells integrated, terminal, 10–30 µm long, proliferation sympodial, occasionally with enteroblastic, monopodial proliferation leaving coarse annellations, conidiogenous loci inconspicuous to conspicuous by being denticle-like, 1.5–3 µm diam, often somewhat refractive or even appearing darkened, in front view sometimes visible as minute circle with dark rim (caused by the relatively thick and dark walls of the conidiophores and conidiogenous cells), but loci not thickened. Conidia solitary, obclavate, often with almost rostrate apex, shorter conidia subcylindrical, 25–125 × 4.5–8 µm, 2–10-septate, occasionally subhyaline to pale olivaceous, but usually medium olivaceous or olivaceous-brown, wall thin to somewhat thickened (to 0.8 µm), smooth, apex obtuse to subacute, base obconically truncate, 2–3 µm wide, hila unthickened, not darkened, but often somewhat refractive or ultimate rim slightly darker.


Host range and distribution: On Viburnum (cassinoides, Viburnum sp.), Adoxaceae, North America (USA, Wisconsin, West Virginia).

Notes: An additional collection of this species has been examined [USA: West Virginia: Pocahontas County, on

Fig. 53b. Pseudocercospora varia var. viburni-sargentii (KUS 14011, holotype). A. Conidiophore fascicles. B. Conidia. Bar = 10 µm.
Viburnum cassinoides, 6 Aug. 1909, B. Brooks, hb. J. L. Sheldon 3815 (BPI 439059). This species is easily distinguishable from all Pseudocercospora species on hosts of the Adoxaceae by its consistently solitary, pigmented, much wider conidia with somewhat thickened walls. Other comparable cercosporoid fungi on Viburnum spp., e.g. Pseudocercospora varia, are quite distinct by narrower, mostly subhyaline conidia and unthickened conidiogenous loci and conidial hila. The shape of the conidia with somewhat thickened walls and the structure of the conidiogenous loci in this species are reminiscent of former Prathigada species which have turned out to belong to Pseudocercospora (Braun et al. 2013).


(Fig. 55)  

**Description:** Leaf spots amphigenous, subcircular to angular-irregular, size variable, 1–8 mm diam, sometimes confluent, brown to dark brown or even blackish, margin indefinite or finally centre brown, greyish brown or greyish white with darker margin, brown, sometimes with a diffuse olivaceous halo. **Caespituli** hypophyllous, punctiform, scattered, effuse, dark, brown to dark brown. **Mycelium** internal. **Stromata** well-developed, subglobose to somewhat irregular, substomatal, 20–35 μm diam, brown. **Conidiophores** in small to moderately large, somewhat divergent to dense or even coremioid fascicles, arising from stromata, through stomata, erect, straight, subcylindrical to geniculate-sinuous, unbranched, 50–150 × 3–4.5 μm, 3–7-septate, medium olivaceous-brown to brown, wall somewhat thickened, smooth; conidiogenous cells integrated, terminal, about 10–30 μm long, conidiogenous loci inconspicuous unthickened and not darkened. **Conidia** solitary, obclavate-cylindrical, straight or only slightly curved, 25–75 × 2.5–4 μm, 2–5-septate, pale olivaceous, thin-walled, smooth.


**Illustrations:** Tai (1948: 37, fig. 1), Braun (1992: 216, plate 2, fig. 10), Guo & Hsieh (1995: 53, fig. 49), Guo et al. (1998: 67, fig. 51).

**Fig. 54.** Pseudocercospora viburnicola (BPI 442174, holotype). A. Conidiophore fascicles. B. Conidiophore tips. C. Conidia. Bar = 10 μm.
smooth, apex obtuse to subacute, base short obconically truncate, 1–2 µm wide, hila unthickened, not darkened.

**Holotype:** China: Yunnan Province: Kunming, on *Viburnum cylindricum*, Adoxaceae, Jun. 1938, Xu Ren (HMAS 01929).

**Host range and distribution:** Only known from the type collection.

**Notes:** Braun (1992) reduced *Pseudocercospora khasiana* to synonymy with *P. viburni-cylindrici*, although the conidia in the Indian type material are distinctly wider and the conidiophore fascicles are smaller and not coremoid. Therefore, we prefer to consider *P. khasiana* (= *P. caprifoliacearum*) a distinct species of its own, at least for the interim (see notes under the latter species). Korean material on *Viburnum erosum*, referred to as *P. viburni-cylindrici* by Shin & Kim (2001: 228), differs in having much longer, plurisepate, cylindrical conidia with truncate base. The material concerned is described as a new species, *Pseudocercospora viburni-erosi*.

---

**Pseudocercospora viburni-erosi** U. Braun & H.D. Shin, sp. nov.

MycoBank MB814560

(Fig. 56)

**Literature:** Shin & Kim (2001: 228).

**Illustration:** Shin & Kim (2001: 229, fig 105).

**Diagnosis:** Differs from *P. viburni-cylindrici* in having large, dense, often coremoid conidiophore fascicles and above all much longer, plurisepate, cylindrical-filiform conidia, length 50–130 µm, with truncate base.

---

**Fig. 55.** *Pseudocercospora viburni-cylindrici* (HMAS 01929, holotype). A. Conidiophore fascicle. B. Conidiophore tips. C. Conidia. Bar = 10 µm.

**Fig. 56.** *Pseudocercospora viburni-erosi* (KUS-F14126, holotype). A. Conidiophore fascicle. B. Conidia. Bar = 10 µm.
Description: Leaf spots amphigenous, scattered to confluent, circular or almost so to angular-irregular, 3–10 mm diam, sometimes confluent and larger, to 15 mm diam, at first brown to dark brown, later with greyish brown to grey centre surrounded by a brown to dark brown border, finally centre turning greyish white, with raised brown border line. 

Caespituli hypophyllous, brown, punctiform. Mycelium internal; hyphae branched, septate, hyaline, 2–3 µm wide. Stromata substomatal, small to well-developed, 15–30 µm diam, globose or subglobose, brown. Conidiophores in well-developed fascicles, to 30, dense to very dense, coremioid, arising from stromata, through stomata, erect, straight, subcylindrical or only slightly geniculate-sinuous, above all in the upper portion, unbranched, 60–120 × 3–4 µm, 3–8-septate, olivaceous-brown to brown, paler towards the tip, thin-walled, smooth; conidiogenous cells integrated, terminal, conidiogenous loci inconspicuous, unthickened, not darkened. Conidia solitary, cylindrical-filiform, straight or almost so to moderately curved, 50–130 × 2.5–4 µm, 3–12-septate, subhyaline to very pale olivaceous, thin-walled, smooth, apex obtuse, base truncate or almost so, 2–2.5 µm wide, hila unthickened, not darkened.

Holotype: Korea: Kwachon, on Viburnum erosum var. taquetii, 4 Sep. 1997, H. D. Shin (KUS-F14126).

Host range and distribution: Only known from the type collection.

Notes: Type material of the new species was previously assigned to Pseudocercospora viburni-cylindrici (Shin & Kim 2001). The two species are morphologically very close, but Korean material on Viburnum erosum differs in having large, dense, often coremioid conidiophore fascicles and above all much longer, pluriseptate, cylindrical-filiform conidia, length 50–130 µm, with truncate base. Based on these obvious differences, the Korean material on Viburnum erosum is better excluded from P. viburni-cylindrici and treated as separate species.


(Fig. 57)


Illustrations: Ellis (1976: 117, fig. 83B), Kirk (1999: fig., unnumbered), Crous et al. (2013: 109, fig. 65).

Description: Leaf spots amphiogenous, subcircular to angular-irregular, sometimes vein-limited, 2–15 mm diam, pale to darker brown or centre later greyish brown to dingy grey, margin indefinite or darker, sometimes with reddish tinge, occasionally slightly raised. Caespituli amphiogenous, punctiform, olivaceous to dark or blackish brown, scattered. Mycelium internal, external mycelium lacking or occasionally with some external hyphae; hyphae branched, septate, 1.5–4 µm wide, subhyaline, thin-walled, smooth. Stromata substomatal or immersed, 15–80 µm diam, occasionally larger, to 120 µm diam (hypophyllous stromata smaller and substomatal, epiphyllous ones larger and immersed to somewhat erumpent), subglobose, brown to dark brown, composed of brown swollen hyphal cells, 3–6 µm diam, subglobose to somewhat irregular, walls thickened, 0.5–2 µm. Conidiophores in somewhat divergent to usually dense, sometimes very dense fascicles, larger fascicles sporodochial, arising from stromata, emerging through stomata or erumpent, occasionally with solitary conidiophores arising from superficial hyphae, straight to somewhat curved-sinuous, not geniculate, unbranched, cylindrical, subcylindrical or slightly attenuated towards the tip, sometimes ampulliform, apex at first rounded, later truncate or subtruncate, 5–30(–50) × 2–5(–6), 0(–2)-septate, pale brown or olivaceous-brown, thin-walled, smooth, occasionally slightly rough-walled; conidiophores mostly reduced to conidiogenous cells sympodially proliferating. Katsuki (1965) recorded Cercospora tinea from Japan on Viburnum dilatatum and V. suspensum. Kobayashi (2007) added V. davidi, V. erosum, V. plicatum and V. sieboldii as host species for Japan. Japanese collections on Viburnum suspensum and V. plicatum var. tomentosum [V. tomentosum] have been examined by C. Nakashima (CNS567, CNS976) and turned out to belong to P. tinea. Therefore, all other Japanese records of P. viburnigena [C. tinea] remain unproven and doubtful, i.e, they seem rather to refer to P. tinea.

A single North America collection has been examined and confirmed (USA: Louisiana; Lafayette, on Viburnum plicatum, 21 Sep. 1886, A. B. Langlois, BPI 441939, 441940).

Pseudocercospora viburni-nudii U. Braun, sp. nov. MycoBank MB814562 (Fig. 58)

Diagnosis: Distinguished from all other species of Pseudocercosora on Viburnum spp. and other hosts of the Adoxaceae by having much shorter, 1–4(–5)-septate conidia, 15–40 × 3–7 µm.

Description: Leaf spots amphiogenous, subcircular to angular-irregular, sometimes vein-limited, 2–12 mm diam or confluent and larger, medium to dark brown, margin indefinite, later with paler centre, greyish brown, grey to greyish white, surrounded by a narrow to moderately wide darker border, dark brown to almost blackish, sometimes with diffuse reddish halo. Caespituli amphiogenous, punctiform, scattered, dark brown to blackish. Mycelium internal. Stromata substomatal to intraepidermal, 15–45 µm diam, dark brown. Conidiophores in small to moderately large fascicles, 3–18, arising from stromata, through stomata or erumpent, divergent, erect, straight, subcylindrical, or obliquely bent, terminal part somewhat geniculate-sinuous or subdenticulate, unbranched, 40–105 × 3–5 µm, base occasionally somewhat wider, to 7 µm, apex sometimes slightly swollen, to 8 µm wide,
1–5-septate, medium to dark brown throughout or paler towards the tip, wall somewhat thickened, to 1 µm, conidiophores occasionally with a single enteroblastic rejuvenation leaving a single conspicuous annellation; conidiogenous cells integrated, terminal, 15–40 µm long, sympodially proliferating, conidiogenous loci inconspicuous to conspicuous by being subdenticulate, denticle-like loci about 1.5–2 µm diam, but loci always unthickened and not darkened. 

Conidia solitary, short obclavate-cylindrical, fusiform, ellipsoid, straight to curved, 15–40 × 3–7 µm, 1–4(–5)-septate, often slightly constricted at the septa, subhyaline to mostly pale to medium olivaceous or olivaceous-brown, thin-walled, smooth, apex obtuse, base short obconically truncate, sometimes rounded, 1.5–2.5(–3) µm wide, hila unthickened, not darkened.


Host range and distribution: Only known from the type collection.

Aizoaceae

Cercospora

Key to Cercospora species on Aizoaceae

1 Conidia obclavate-cylindrical to acicular, to 125 µm long, base truncate to obconically truncate; on Trianthema portulacastrum
   Conidia acicular, to 200 µm long, base truncate; on Tetragonia tetragonoides

Cercospora species on Aizoaceae

(Fig. 59)

Cercospora tetragoniae (Speg.) Chupp, in Viégas, Bol. Soc. Bras. Agron. 8: 54 (1945).


Illustration: Sutton & Pons (1980: 207, fig. 1F).

Description: Leaf spots amphigenous, circular or subcircular, 2–10 mm diam, scattered to confluent and larger, up to 20

mm, pale brown, dingy olivaceous, greyish brown to grey or dingy greyish white, margin brown to reddish brown, occasionally somewhat zonate. Caespituli amphigenous, mostly epiphyllous, punctiform, dark brown to blackish. Mycelium internal. Stromata lacking or almost so to small, substomatal, 10–25(–40) µm diam, brown. Conidiophores solitary or fasciculate, 2–10, divergent, arising from internal hyphae or stromata, through stomata or occasionally erumpent, erect, straight, subcylindrical to geniculate-sinuous, unbranched, 20–125 × 3–7 µm, 0–6-septate, pale yellowish, olivaceous-brown to brown, pale towards the tip, thin-walled, smooth; conidiogenous cells integrated, terminal, sometimes conidiophores reduced to conidiogenous cells, 10–30 µm long, conidiogenous loci conspicuous, thickened and darkened, 2.5–4 µm diam. Conidia solitary, acicular, straight to curved, 30–200 × 2.5–5 µm, 3- to pluriseptate, hyaline, thin-walled, smooth, apex pointed, base truncate, 2.5–4 µm wide, hila thickened and darkened.
Holotype: Argentina: La Plata, on Tetragonia tetragonoides, 18 Nov. 1909, C. Spegazzini (LPS 16153). Isotype: K(M) IMI 241730 (slide).

Host range and distribution: On Tetragonia (tetragonoides [expansa]), Aizoaceae, Africa (Cameroon, Kenya, Malawi, Sierra Leone, Tanzania, Uganda, Zimbabwe), Asia (Brunei, Israel, Japan), Caucasus (Georgia), Central and South America (Argentina, El Salvador, Brazil), North America (USA, Maryland, Indiana, Texas).

Notes: This species belongs to the C. api s. lat. complex. Records from Brazil on Spinacia oleracea (Mendes et al. 1998) are based on misidentifications.

Cercospora trianthematis Chidd., Mycopathol. Mycol. Appl. 17: 80 (1962); as “trianthemiae”.


Illustrations: Chiddarwar (1962: 78, plate II, figs 12–13), Bhartiya et al. (1998: 45, fig. 2).

Description: Leaf spots amphigenous, circular to oval or irregular, scattered, 0.5–6 mm diam, dark brown to blackish. Casepsituli amphigenous, mainly hypophyllous, punctiform, brown. Mycelium internal. Stromata substomatal, 15–45 µm diam, olivaceous to brown. Conidiophores in small to moderately large fascicles, 2–20, occasionally solitary, relatively dense to divergent, arising from stromata, through stomata, erect, straight, subcylindrical-conical to geniculate-sinuous, usually unbranched, 15–100 × 3–5 µm, 0–7-septate, dark olivaceous to brown, wall somewhat thickened, smooth; conidiogenous cells integrated, terminal, intercalary or conidiophores reduced to conidiogenous cells, 10–30 µm long, conidiogenous loci thickened and darkened, 2.5–3 µm diam. Conidia solitary, shorter conidia obclavate to subcylindrical, longer ones aciculate, straight to curved, (10–)30–125 × 2.5–4 µm, occasionally broader, (1–)3–11-septate, hyaline, thin-walled, smooth, apex pointed to subobtuse, base truncate to short obconically truncate, about 2–2.5 µm wide, hila thickened and darkened.

Lectotype (designated here, MycoBank, MBT202798): India: Maharashtra: Pune, Wanwari, Military Hospital, on Trianthema portulacastrum, 12 Oct. 1956, P. P. Chiddarwar 32 (K(M) IMI 83193). Isol ectotypes: BPI 441988, HCIO.

Host range and distribution: On Trianthema portulacastrum, Aizoaceae, Asia (India, Maharashtra).

Note: A true Cercospora s. str. distinct from the C. api s. lat. complex by having obclavate-cylindrical to acicular conidia. C. aizoacearum, described from India on the same host, is undoubtedly conspecific. Type material was not available. The conidia were described to be cylindrical, but the illustration shows cylindrical to somewhat obclavate shorter and acicular longer conidia, a range similar to C. trianthematis.

Altingiaceae

Key to cercosporoid species on Altingiaceae

1 Leaf spots absent or almost so, sometimes with slight discolorations on the upper leaf surface; mycelium internal; superficial hyphae lacking; conidiophores fasciculate, 0–1-septate, 3–7 µm wide, conidiogenous loci unthickened, nor darkened; conidia 20–80 × 4–7.5 µm; on Liquidambar styraciflua, North America .............................................................. Pseudocercospora tuberculans

With distinct leaf spots; mycelium internal and often also external; conidiophores fasciculate and often also solitary, arising from superficial hyphae if present, aseptate, narrower, 2–5 µm wide; conidia much narrower, 2–4 µm .............................................................. 2

2 (1) Leaf spots small, 0.5–4 mm diam, dingy greenish grey, brown, grey, finally usually greyish white, with dark, often somewhat raised margin; casepsituli mainly epiphyllous, conspicuous, punctiform to almost postulate, dark brown to blackish; conidiophores pale to medium dark brown, paler towards the tip, wall somewhat thickened (to 1 µm), (10–)25–90 µm long; conidiogenous loci conspicuous, slightly thickened, at least around the outer rim, somewhat darkened-refractive, 1–1.5 µm diam, in front view visible as minute circle; conidia to 150 µm long, subhyaline or very pale olivaceous, hila unthickened to slightly thickened, somewhat darkened-refractive; on Liquidambar styraciflua, North America ................. Cercospora liquidambaris

Leaf spots larger, usually 1–10 mm diam; casepsituli less conspicuous, never postulate; conidiophores paler, subhyaline to pale olivaceous or olivaceous-brown, thin-walled, shorter, 5–35 µm long; conidiogenous loci inconspicuous, neither thickened not darkened; conidia shorter, to 100 µm, subhyaline or pale to medium olivaceous or olivaceous-brown, hila unthickened, not darkened on Liquidambar formosana and L. sty raciflua, Asia and North America ........................................ P. liquidambaricola
Cercospora (s. lat.)

Cercospora liquidambaris Cooke & Ellis ex G.F. Atk., J. Elisha Mitchell Sci. Soc. 8: 48 (1892) (Fig. 61)


Description: Leaf spots amphigenous, subcircular to angular-irregular, small, 0.5–4 mm diam, dingy greyish green, brown, to finally usually greyish white or white, sometimes somewhat raised, margin indefinite or usually darker, narrow. Caespituli amphigenous, usually epiphyllous, very conspicuous, punctiform to almost pustulate, scattered, dark brown to blackish. Mycelium internal and external; superficial hyphae lacking or almost so to well-developed, branched, septate, subhyaline to pale olivaceous or brownish, 1.5–4 µm wide. Stromata almost lacking or small to usually well-developed, substomatal or intraepidermal, 10–60 µm diam, brown. Conidiophores in small to moderately large fascicles, arising from substomatal hyphae or stromata, though stomata or erumpent, divergent to moderately dense, or solitary, arising from superficial hyphae, lateral, erect, straight, subcylindrical or attenuated towards the apex to usually geniculate-sinuous, unbranched, (10–)25–90(–100) × 3–5 µm, (0–)1–8-septate, pale to medium brown, paler towards the tip, wall somewhat thickened, at least below, to 1 µm, smooth or almost so, proliferation sympodial and occasionally percurrent (conidiophores with enteroblastic rejuvenation leaving delicate annellations); conidiogenous cells integrated, terminal, 10–25 µm long, conidiogenous loci conspicuous, in front view visible as minute circle, 1–1.5 µm diam, at least rim slightly thickened, somewhat darkened or refractive. Conidia solitary, narrowly obclavate-cylindrical, filiform-subacicular, straight to curved, occasionally germinating with short to moderately long lateral outgrowths, 40–150 × (2–)2.5–4(–4.5) µm, 3–12-septate,
subhyaline to pale olivaceous, thin-walled, smooth, apex acute to subobtuse, base usually short obconically truncate, sometimes truncate, 1–2 µm wide, hila unthickened or almost so to slightly thickened, somewhat darkened or refractive.


**Host range and distribution**: On Liquidambar styraciflua, Altingiaceae, North America (Mexico; USA, Alabama, Delaware, Florida, Indiana, Louisiana, Maryland, Mississippi, Montana, Massachusetts, North Carolina, Texas).

**Notes**: The taxonomy and nomenclature of Cercospora liquidambaris have been totally confused. Chupp (1954) reduced C. liquidambaris Sawada, based on Taiwanese material causing a leaf spot disease of Liquibambar formosana, to synonymy with C. liquidambaris Cooke & Ellis ex G.F. Atk., which is, however, incorrect since the cercosporoid fungus on these hosts in Asia belongs in Pseudocercospora and is now correctly assigned to P. liquidambaricola (see below). The later species is also known from North America on Liquibambar styraciflua. Several collections from Mexico and the USA have been examined. The identity of the true C. liquidambaris is clarified by lectotypification. Atkinson (1892) cited a specimen collected in Alabama in 1891. A corresponding sample from Atkinson’s herbarium at CUP is designated as lectotype. The generic affinity of C. liquidambaris remains unclear. The conidiogenous loci and conidial hila are thickened and darkened. The conidia are cercospora-like, but pigmented as in the Passalora complex, which recently proved to be phylogenetically quite heterogeneous (S.I.R. Videira, unpubl.). Therefore, this species is tentatively retained in Cercospora s.l. until phylogenetic data will be available.

**Pseudocercospora**

**Pseudocercospora species on Altingiaceae**


(Fig. 62)


**Illustrations**: Yen (1978a: 53, fig. 3), Hsieh & Goh (1990: 151, fig. 113), Guo & Hsieh (1995: 125, fig. 110), Guo et al. (1998: 139, fig. 114), Kobayashi et al. (2002: 224, fig. 6).

**Description**: Leaf spots subcircular to angular-irregular, 1–10 mm diam or confluent and larger, angular spots often vein-limited, pale olivaceous, brown to dark brown or later greyish brown to greyish white, with dark border, brown to blackish, narrow, sometimes raised, with diffuse yellowish halo, occasionally somewhat zonate. Caespituli amphiogenous, delicately punctiform to subelliptic, brown. Mycelium internal and external; superficial hyphae lacking or almost so to developed (mainly hypophyllous when developed), branched, 1.5–4

![Fig. 62. Pseudocercospora liquidambaricola (Hsieh & Goh 1990: 151, fig. 113). A. Conidiophores fascicles. B. Conidiophores arising from superficial hyphae. C. Conidiophores. D. Conidia. Bar = 10 µm.](image-url)
µm wide, subhyaline to pale olivaceous-brown, thin-walled, smooth. Stromata small to moderately large, 15–40 µm diam, rarely larger, subtomatal to immersed, subglobose, brown. Conidiophores in small to moderately large fascicles, loose to dense, arising from stromata, through stromata or erumpent, also solitary, arising from superficial hyphae if present, lateral, erect, straight, subcylindrical-conical to geniculate-sinuous, unbranched, 5–35 × 2–5 µm, 0–2-septate, subhyaline, pale olivaceous to olivaceous-brown, thin-walled, smooth to somewhat rough; conidiophores reduced to conidiogenous cells or integrated, terminal, 5–25 µm long, conidiogenous loci inconspicuous or visible as a truncate tip, but always cells or integrated, terminal, 5–25 µm long, conidiogenous loci inconspicuous or visible as a truncate tip, but always unthickened and not darkened. Conidiophores reduced to conidiogenous cells or integrated, terminal, 5–25 µm long, conidiogenous loci inconspicuous or visible as a truncate tip, but always unthickened and not darkened. Conidia solitary, cylindrical to geniculate-sinuous, also solitary, arising from superficial hyphae if present, lateral, dense, arising from stromata, through stomata or erumpent, Conidiophores rarely larger, substomatal to immersed, subglobose, brown.

Neotype (designated here, MycoBank, MBT202800):

Host range and distribution: On Liquidambar (formosana, styraciflua), Altingiaceae, Asia (China, Japan, Taiwan), North America (Mexico; USA, Alabama, Florida, Louisiana, Texas).

Notes: The holotype material of Cercospora liquidambaricola (Taiwan: Taichung, on Liquidambar formosana, 29 Oct. 1971, J.-M. Yen 71277 bis) could not be traced in PC or UC. Therefore, we designate a neotype here. Collections of Pseudocercospora on Liquidambar formosana in Asia and L. styraciflua in North America are morphologically very similar. If they are truly conspecific or if two morphologically very close, but geographically and genetically distinct species are involved can only be clarified on the base of molecular sequence analyses. Loropetalum chinense (Hamamelidaceae) was reported as host of this species from China (Guo & Hsieh 1995; Guo et al. 1998). The identity of Pseudocercospora on this host is unclear and needs to be confirmed. Chupp (1954), who proposed the original synonymy, found material from the USA and Taiwan to represent the same fungus. However, Hsieh & Goh (1990) did not examine American material. Samples from Taiwan (Taichung, 8 Aug. 1945, K. Sawda, BPI 437752, 437753) are probably toptotypes. Guo & Hsieh (1995) confirmed the synonymy of C. liquidambaricola. They mentioned that they had examined Yen’s type material, but this material was not cited under “material examined”. The material concerned is not preserved at PC. The complicated nomenclature and taxonomy of this species has been discussed by Braun (2000a: 44) who emphasized that identity and application of the name C. liquidambaris can only be clarified by lectotypification. Chupp (1954) interpreted this name in the sense of P. liquidambaricola, which is clearly distinct from P. tuberculans by much narrower conidia, but his interpretation was based on a specimen collected by Geo. V. Nash in 1895 (USA, Florida, Lake City, Plants of Florida No. 2231, 11–19 Jul. 1895, BPI 437755, 437761, 437812), which is non-type material. Atkinson (1892) cited a specimen collected in Alabama in 1891, which can be used as lectotype. There is a single specimen in Atkinson’s herbarium (now CUP) which undoubtedly refers to this collection.

(Fig. 63)
Basionym: Cercospora tuberculans Ellis & Everh., J. Mycol. 4: 115 (1888).


Description: Leaf spots absent or almost so, sometimes with slight discolorations on the upper leaf surface. Caespituli hypophyllous, punctiform, on small, brown, tubercle-like swellings, 0.5–1 mm diam, sometimes effuse between such swellings. Mycelium internal. Stromata almost absent to well-developed, 10–80 µm diam, dark brown to blackish, substomatal to immersed, composed of swollen hyphal cells, 3–8 µm diam. Conidiophores in small to large fascicles, loose to usually dense, subcylindrical to conical, Fig. 63. Pseudocercospora tuberculans (NY 2425377, lectotype). A. Conidiophore fascicles. B. Conidiophores. C. Conidia. Bar = 10 µm.
straight to slightly geniculate-sinuous, unbranched, 10–35 × 3–7 µm, rarely longer, 0–1-septate, pale olivaceous to olivaceous-brown, thin-walled, smooth; conidiogenous cells integrated, terminal or conidiophores reduced to conidiogenous cells, 10–25 µm long, conidiogenous loci inconspicuous or visible as truncate tips, always unthickened and not darkened. Conidia solitary, cylindrical to somewhat obclavate-cylindrical, straight to slightly curved, 20–80 × 4–6.5 µm, 1–6-septate, pale olivaceous to brownish, thin-walled, smooth, apex obtuse, base subtruncate to short obconically truncate, rarely long obconically truncate, 2–3 µm wide, hila unthickened, not darkened.


**Cercospora**

### Key to Cercospora species on Amaranthaceae

1. Conidia cylindrical or obclavate-cylindrical ...................................................................................................................... 2
   - Conidia consistently acicular or at least longer conidia acicular and only shorter ones somewhat obclavate-cylindrical ................................................................. 5

2 (1)  Stromata lacking or almost so; conidiophores short, 5–30 × 3–5 µm, 0(–1)-septate; conidia short, 25–35 × 2.5–3.5 µm, 0–5-septate; on Celosia sp. ................................................... C. celosiigena
   - Stromata developed, 10–60 µm diam; conidiophores longer, 10–150 µm, 0–8-septate; and/or conidia longer, 15–150 µm, pluriseptate ........................................................................ 3

3 (2)  Conidiogenous loci cercospora-like, i.e. distinctly thickened and darkened, in front view visible as small dark circle with minute central pore; conidiophores distinctly brown; on Celosia sp. ......................................................... C. gorakhanathii
   - Conidiogenous loci not cercospora-like, i.e. unthickened to somewhat thickened, but not darkened, at most somewhat refractive, formed on characteristic, bulging, convex tips and shoulders caused by sympodial proliferation; conidiophores hyaline, only pale olivaceous at the base or pale olivaceous-brown, tips often hyaline or subhyaline ........................................................................ 4

4 (3)  Conidiophores hyaline or only pale olivaceous at the base; conidia obclavate-cylindrical, 20–80 × 3.5–7 µm;
   - on Achyranthes spp. ........................................................................................................................................ Cercosporella pseudachyranthis
   - Conidiophores pale olivaceous-brown throughout or tips paler, hyaline or subhyaline; conidia narrowly cylindrical to subacicular, 15–90 × 2–4.5 µm; on Gomphrena spp. ................................................................. C. pretoriensis
   - (see “Doubtful, excluded and insufficiently known species of Cercospora”)

5 (1)  Leaf spots lacking or almost so; conidia acicular to obclavate-cylindrical, broad, 35–195 × (3–)4–6.5(–8) µm;
   - on Achyranthes spp. ........................................................................................................................................ C. achyranthis
   - Leaf spots developed, distinct; conidia consistently acicular and/or narrower, 1.5–5 µm .............................................. 6

6 (5)  Conidia narrowly cylindrical to subacicular, apex obtuse, not distinctly pointed; on Gomphrena spp., South Africa
   - At least longer conidia distinctly acicular with truncate base and acute or subacute apex; on other hosts .......... 7

7 (6)  Conidia consistently acicular, base truncate, various morphologically barely distinguishable species
   - (see **Tabular key to Cercospora species on Acanthaceae according to host genera**)

**Host range and distribution:** On Liquidambar styraciflua, Altingiaceae, North America (USA, Florida, Louisiana, Missouri, Mississippi).

**Notes:** Records of this species from China on Liquidambar formosana (Keissler & Lohwag 1937, Tai 1979) are doubtful, unproven and not included in Guo & Hsieh (1995) and Guo et al. (1998). They refer to Chinese material on dead wood of Liquidambar formosana distributed as Cercospora tuberculans in Petr., Crypt. Exs. 3391 [Hunan, Changcha, Nov., C. Keissler & H. Handel-Mazzetti (W)]. The identity of this wood-inhabiting fungus is not quite clear, but it undoubtedly does not pertain to C. tuberculans.

**Amaranthaceae s. str.**

(The family Chenopodiaceae is phylogenetically close to Amaranthaceae and currently proposed to be included in the latter family (s. lat.); but we prefer to maintain the Chenopodiaceae as separate family, at least tentatively.)
Conidia acicular to obclavate-cylindrical, at least shorter conidia obclavate-cylindrical, with obconically truncate base

8 (7) Stromata well-developed, large, 20–80 µm diam; conidiophores 10–80 µm long; conidial base 1–2 µm wide;
on *Alternanthera* spp. .................................................................................................................................  C. *alternantherae*

Stromata lacking or 10–50 µm diam; conidiophores to 250 µm long; conidial base 1.5–4 µm wide;
on *Amaranthus* spp. ........................................................................................................................................ C. *brachiata*

**Tabular key to Cercospora species on Acanthaceae according to host genera**

**Achyranthes**
1 Conidiophores colourless or only olivaceous at the base; conidiogenous loci conspicuous, but not cercospora-like
(not darkened, only refractive, characteristically bulging, cercosporella-like) ................................................................. Cercosporella *pseudachyranthis*

(see "Doubtful, excluded and insufficiently known species of Cercospora")

Conidiophores brown, distinctly pigmented throughout; conidiogenous loci distinctly thickened and darkened,
*Cercospora* type .......................................................................................................................................................  2

2 (1) Leaf spots lacking or almost so; conidiophores to 450 µm long; conidia acicular to obclavate-cylindrical,
35–195 × (3–)4–6.5(–8) µm ............................................................................................................... C. *achyranthis*

Leaf spots distinct, well-developed; conidiophores to 250 µm long; conidia usually consistently acicular,
much narrower, 25–240 × 2–5 µm .................................................................................................................. C. *achyranthina*

**Aerva**
A single species ..................................................................................................................................................  C. *aervae-lanatae*

**Alternanthera**
A single species ..................................................................................................................................................  C. *alternantherae*

**Amaranthus**
A single species ..................................................................................................................................................  C. *brachiata*

**Celosia**
1 Conidiophores short, 5–30 × 3–5 µm, 0(–1)-septate; conidia short, 25–35 × 2.5–3.5 µm, cylindrical to
obclavate-cylindrical, 0–5-septate, hila 1–2 µm wide ............................................................................. C. *celosiigena*

Conidiophores much longer, 20–220 µm, 0–8-septate; conidia much longer, 15–150 µm, (0–)1–14-septate,
hila 1.5–3 µm wide ...................................................................................................................................................  2

2 (1) Stromata lacking or almost so; conidia acicular ................................................................. C. *celosiae*

Stromata developed, 10–40 µm diam; conidia cylindrical or obclavate-cylindrical ............... C. *gorakhanathii*

**Cyathula**
A single species ................................................................................................................................................ C. *apii s. lat.* (C. *cf. maloti sensu* Groenewald *et al*. 2013)

**Digera**
A single species ................................................................................................................................................ C. *achyranthina*

**Gomphrena**
1 Stromata lacking or small, 10–35 µm diam; conidia acicular, 30–450 µm long, apex pointed; mainly on
*Gomphrena globosa*, Northeast Africa, Asia, North America, West Indies ................................. C. *gomphrenae*

Stromata 15–60 µm diam; conidiogenous loci not cercospora-like (neither distinctly thickened, nor darkened),
conidia narrowly cylindrical to subacicular, apex obtuse; on *Gomphrena* spp.,
South Africa ................................................................................................................................................. C. *pretoriensis*

(see "Doubtful, excluded and insufficiently known species of Cercospora")

**Pupalia**
A single species ................................................................................................................................................ C. *achyranthina*
**Cercospora species on Amaranthaceae**


*Fig. 64*


**Illustrations:** Ellis (1976: 243, fig. 183 B), Guo *et al.* (2005: 27, fig. 9).

**Description:** Leaf spots amphigenous, circular to angular-irregular, 0.5–6.5 mm diam, occasionally confluent and larger, to 10 mm diam, brown to greyish brown, finally sometimes dull grey or greyish white, margin reddish brown to purple-violet, finally very dark, sometimes with diffuse darker halo, often purplish. **Caespituli** amphigenous, scattered, finely punctiform, dark. **Mycelium** internal. **Stromata** almost lacking or small, 10–25 µm diam, substomatal to immersed, brown. **Conidiophores** in fascicles, 2–10, divergent, arising from swollen hyphal cells or stromata, through stomata or erumpent, erect, straight to curved, geniculate-sinuous, above all in the upper half, unbranched, 15–250 × 3–8 µm, (0–1)–10–septate, pale olivaceous-brown or brownish, wall somewhat thickened, smooth; conidiogenous cells integrated, terminal and intercalary, 10–40 µm long, conidiogenous loci thickened and darkened, 2–3.5 µm diam. **Conidia** solitary, acicular, shorter conidia occasionally slightly obclavate-cylindrical, (25–)35–150(–240) × 2–5 µm, usually 3–10-septate, hyaline, thin-walled, smooth, apex acute or subobtuse, base truncate, occasionally obconically truncate, 2–3.5 µm wide, hila thickened and darkened.

**Holotype:** India: Karnataka: Bangalore, on *Achyranthes aspera*, 20 Aug. 1944, M. J. Thirumalachar (BPI 4323844). **Topotype:** 2 Sep. 1945 (CUP 37201).

**Host range and distribution:** On *Achyranthes* (aspera, *Achyranthes* sp.), *Digera muricata*, *Pupalia lappacea* [atropurpurea]. **Amaranthaceae**, Africa (Sudan, Tanzania, Zimbabwe), Asia (China; India, Bihar, Karnataka, Andra Pradesh, Madhya Pradesh, Maharashta, New Delhi, West Bengal; Myanmar, Pakistan, Philippines), South America (Venezuela).

**Notes:** This species is part of the *Cercospora apii* s. lat. complex. Thirumalachar & Chupp (1948) cited a single collection from 1944 as type. Chupp (1954) erroneously mentioned a collection from 2 Sep. 1945 as type material. This specimen, which can be considered topotype material, is maintained and deposited as CUP 37201.


*Fig. 65*


**Illustrations:** Shin & Kim (2001: 25, fig. 1), Guo *et al.* (2005: 28, fig. 10).


**Description:** Leaf spots lacking or almost so, indistinct or only diffuse discolorations, greenish to brownish, finally sometimes vein-limited, greyish, margin indefinite. **Caespituli** hypophyllous, effuse, floccose-velutinuous, dark, greyish to dark grey or brownish. **Mycelium** internal; hyphae branched, septate, somewhat pigmented. **Stromata** lacking or almost so. **Conidiophores** in small, loose fascicles, 2–12, arising from
Cercosporoid fungi 4

Lectotype (designated here, MycoBank, MBT202802):
Japan: Saitama Prefecture: Ome, Musashi, on Achyranthes bidentata, 22 Sep. 1905, I. Miyake (S-F23053). Isolectotypes: LEP.

Host range and distribution.: On Achyranthes (aspera [indica], bidentata [japonica]), Amaranthaceae, Asia (China; India, Andhra Pradesh, Maharashtra, Rajasthan; Japan, Korea, Pakistan, Taiwan), West Indies (Dominican Republ., Puerto Rico).

Note: A true Cercospora s. str. distinct from C. apii s. lat., including C. achyranthina, by having acicular to obclavate-cylindrical conidia with obconically truncate base. Obconically truncate conidial bases are not confined to shorter, young conidia, they are also evident in longer, fully developed conidia. In addition, different from C. achyranthina by lacking or indistinct leaf spots, effuse caespituli and somewhat wider conidia.

Cercospora aervae-lanatae Raghu Ram & Mallaiah, Mycol. Res. 100: 296 (1996); as “aerva-lanatae”. (Fig. 66)

Illustrations: Raghu Ram & Mallaiah (1996: 296, fig. 2), Srivastava et al. (2001: 102, fig. 1).

Description: Leaf spots amphigenous, necrotic, scattered, subcircular to angular-irregular, 2–9(–10) mm diam, greyish, sometimes with reddish or purplish margin. Caespituli epiphyllous. Mycelium immersed; hyphae branched, septate, subhyaline. Stromata well-developed, gobose, 20–40 µm diam, brown to black. Conidiophores in loose fascicles, to 15, arising from stromata, through stomata, erect, subcylindrical to 1–4 times geniculate, unbranched, about 45–230 × 3.5–5.5 µm, 2–11-septate, pale olivaceous to brown, pale towards the tip, thin-walled, smooth; conidiogenous cells integrated, terminal and intercalary, conidiogenous loci thickened and darkened, 2–3.5 µm diam. Conidia solitary, acicular, straight to curved, 55–160 × 1.5–4 µm, 5–15-septate, hyaline, thin-walled, smooth, apex pointed, base truncate, 1.5–3 µm wide, hila thickened and darkened.

Holotype: India: Andhra Pradesh: Nagarjuna Nagar, University Campus, on Aerva lanata, Nov. 1991, M. Raghu Ram (K(M) IMI 351224).

Host range and distribution: On Aerva (lanata, sanguinolenta [scandens]), Amaranthaceae, Asia (India, Andhra Pradesh, Uttar Pradesh).

Note: This species is morphologically assignable to the Cercospora apii s. lat. complex.
Cercospora alternantherae Ellis & Langl., *J. Mycol.* 6: 36 (1890).

(Fig. 67)


**Illustration:** Chupp (1954: 31, fig. 7).

**Description:** Leaf spots amphigenous, circular or subcircular, 0.5–3 mm diam, pale greenish to dingy grey, margin brownish. Caespituli amphigenous, punctiform, scattered, dark brown to blackish. Mycelium internal. Stromata substomatal to immersed, large, 20–80 µm diam, brown. Conidiophores in well-developed, mostly rather large fascicles, divergent to dense, arising from stromata, through stomata or erumpent, erect, straight, subcylindrical-conical to oblong, geniculate-sinuous, unbranched, 10–80 × 3–6 µm, 1–4-septate, subhyaline, pale olivaceous to brown, paler towards the tip, thin-walled, smooth; conidiogenous cells integrated, terminal, occasionally intercalary, 10–30 µm long, conidiogenous loci conspicuous, thickened and darkened, (1–)1.5–2(–2.5) µm diam. Conidia solitary, obclavate-cylindrical, acicular, straight to curved, 20–125 × 2–4 µm, 0–10-septate, hyaline, thin-walled, smooth, apex pointed or subobtuse, base truncate to obconically truncate, 1–2 µm wide, hilum thickened and darkened.

**Lectotype** (designated here, MycoBank, MBT202806): USA: Louisiana: St. Martinsville, on Alternanthera acryana, 18 Jul. 1888, A. B. Langlois 1430 (NY 830163). **Isolectotype:** BPI 432464.

**Host range and distribution:** On Alternanthera (ficoidea,
halimifolia, crucis [portoricensis], pungens [achyrantha, repens]), Amaranthaceae, North America (USA, Louisiana, Texas), South America (Brazil, Venezuela), West Indies (Cuba, Jamaica, Puerto Rico, Virgin Islands).

Notes. This is a true Cercospora s. str. close to C. api s. lat., but distinct by having large stromata (20–80 µm diam) with numerous, densely arranged conidiophores, smaller conidiogenous loci (1–2 µm wide), and obclavate-cylindrical to acicular conidia. Indian records of this species from Uttar Pradesh and West Bengal on A. sessilis (Kamal 2010) are doubtful and need in need of revision and confirmation. They might rather pertain to the illegitimate species Cercospora sessilis or to the invalid C. alternantherina, both described from India on A. sessilis.

Cercospora api s. lat. (C. cf. malloti sensu Groenewald et al. 2013: 157).

Notes. Nguanhom et al. (2015) examined Cercospora species from northern Thailand by means of molecular methods. A plurivorous Cercospora species referred to as Cercospora cf. malloti in Groenewald et al. (2013) turned out to be the most common taxon found during the course of this study. The collections in the clade concerned belong to C. api s. lat., i.e. they are characterised by having consistently acicular conidia. One of the specimens involved was collected on Cyathula prostrata. For further comments, see notes under Cercospora asystasiana.

Cercospora brachiata Ellis & Everh., J. Mycol. 4: 5 (1888).

(Fig. 68)


Illustrations: Tai (1948: 37, fig. 2), Guo et al. (2005: 29, fig. 11), Pirnia et al. (2010: 185, fig. 1).

Exsiccatea: Ellis & Everh., N. Amer. Fung. 2582.

Description. Leaf spots amphigenous, circular, subcircular to somewhat angular-irregular, 0.5–12 mm diam, at first yellowish brown, later brown, reddish or dark brown, or finally with a tan, grey to greyish white centre surrounded by a brown, red or purplish brown or almost blackish margin. Caespituli amphigenous, mostly hypophyllous, punctiform scattered, dark. Mycelium internal. Stromata almost lacking or small to moderately large, substomatal to immersed, subglobose to somewhat irregular, 10–50 µm diam, olivaceous-brown to brown, cells 2–6 µm diam. Conidiophores fasciculate, 2–12(–20), rarely solitary, arising from swollen hyphal cells or stromata, emerging through stomata or erumpent, erect, straight, subcylindrical to usually distinctly geniculate or geniculate-sinuous, above all in the upper half, unbranched or tips occasionally furcate, 20–250 × 3–6.5 µm, usually 1–8-septate, very long conidiophores sometimes with additional septa, pale to medium dark brown or olivaceous-brown throughout or paler towards the tip, wall thin to slightly thickened, smooth; conidiogenous cells integrated, terminal and intercalary, 10–40(–70) µm long, conidiogenous loci conspicuous, thickened and darkened, 1.5–3.5 µm diam. Conidia solitary, acicular, shorter conidia may also be narrowly obclavate-cylindrical, straight to curved, 25–250 × 1.5–5(–
µm, 1–20-septate, hyaline, thin-walled, smooth, apex acute or subacute, sometimes subobtuse, base truncate or obconically truncate in shorter conidia, 1.5–4 µm wide, hila thickened and darkened.


Host range and distribution: On *Amaranthus* (albus, blitoides, cannabinus, caudatus, crassipes, cruentus [paniculatus, hybridus subsp. cruentus], dubius, hybridus, polygamus, retroflexus, spinosus, tamariscinus, tricolor [gangeticus], tuberculatus, viridis [blitum subsp. emarginatus], *Amarathus* sp.), *Amaranthus* sp., *Amaranthaceae*, Africa (Kenya, South Africa, Uganda), Asia (Brunei, China; India, Andhra Pradesh, Delhi, Orissa, Maharashtra, Uttar Pradesh, West Bengal; Indonesia, Iran, Pakistan), Europe (Germany, Russia, Ukraine), Central and South America (Brazil, Ecuador, Panama, Venezuela), North America (USA, Delaware, Florida, Illinois, Nebraska, Texas, Wisconsin), West Indies (Barbados, Cuba, Dominican Republic, Haiti, Puerto Rico, Trinidad and Tobago, Virgin Islands).

Notes: This species belongs to the *Cercospora api s. lat.* complex. *Cercospora brachiata* is morphologically rather variable, above all with regard to the length of conidiophores as well as length and width of conidia. Many host species and a wide distribution range covering different continents are involved. Thus, it remains unclear if all collections pertain to a single species or if we have to do with a complex of cryptic species. *Cercospora acnidae* is morphologically barely distinct from *C. brachiata*. Its introduction and recognition in Chupp (1954) were undoubtedly influenced by assumed host range differences. The hosts of *C. acnidae* were previously assigned to the genus *Acnida*, now a synonym of *Amaranthus* usually treated as subgenus, which reflects the close affinity of former *Acnida* and *Amaranthus* species. A careful search for type material of *Cercospora amaranthi* in LE failed. Records of *C. brachiata* on *Achyranthes bidentata* [japonica] (Crous & Braun 2003) are doubtful and belong probably to *C. achyranthina*. Some newer records not yet included in Crous & Braun (2003) refer to Germany (Jage & Braun 2004), Indonesia (Shivas et al. 1996) and Iran (Pirnia et al. 2010, Hesami et al. 2011).

Ellis & Everh., N. Amer. Fung. 2582 (BPI 432395, FH, GZU, NY, PH and numerous other herbaria) is authentic material (former syntypes) from the type locality, but collected in 1890.


(Fig. 69)


Illustrations: Ellis (1976: 243, fig. 183 C), Hsieh & Goh (1990: 17, fig. 6), Guo et al. (2005: 31, fig. 12).

Description: Leaf spots amphigenous, circular or subcircular, 1–12 mm diam, occasionally larger, tan to pale brown, margin darker, occasionally somewhat raised, sometimes causing shot-hole symptoms. *Caespituli* usually hypophyllous, delicate, dark. *Mycelium* internal. *Stromata* lacking or small, brown, substomatal. *Conidiophores* in fascicles, 2–15, divergent, arising from internal hyphae or small hyphal aggregations, through stomata, erect, straight, usually unbranched, geniculate-sinuous, 20–220 × 3–6 µm, 0–6-septate, pale to medium brown, paler and narrower towards the tip, thin-walled, smooth; conidiogenous cells integrated, terminal and intercalary, conidiogenous loci
conspicuous, thickened and darkened, 1.5–3 µm diam. Conidia solitary, acicular, straight to curved, 25–150 × 2–4(–4.5) µm, hyaline, 2–12-septate, apex acute, base truncate to somewhat obconically truncate, 1.5–3 µm wide, hila thickened and darkened.

**Holotype:** China: Hubei: Wang-Chia-Shau, on *Celosia argentea*, 4 Aug. 1928, T. F. Yu (BPI 434404).

**Host range and distribution:** On *Celosia* (argentea [argentea var. cristata, cristata, plumosa], trigyna [laxa], Celosia sp.), *Amaranthaceae*, Africa (Nigeria, Sudan, Uganda,), Asia (Bangladesh, Brunei, Cambodia, China, India, Indonesia, Malaysia, Myanmar, Pakistan, Papua New Guinea, South Korea, Sri Lanka, Taiwan, Thailand), Central and South America (Brazil, Panama, Venezuela), North America (Mexico; USA, Alabama, Florida, Oklahoma), West Indies (Cuba).

**Notes:** This species is characterised by having colourless acicular conidia and thickened, darkened conidiogenous loci and hila, i.e. it belongs to the *Cercospora apii* s. lat. complex. Japanese records of this species (Katsuki 1965) are wrong and refer to *Pseudocercospora celosiarum* (confirmed by Ch. Nakashima).

*Cercospora celosiigena* U. Braun & Bagyan., sp. nov.

MycoBank MB814563 (Fig. 70)

**Literature:** Bagyanarayana et al. (1991: 324).

**Illustration:** Bagyanarayana et al. (1991: 321, fig. 6).

**Diagnosis:** Differs from *C. celosiae* in having much shorter conidiophores, 5–30 × 3–5 µm, 0(–1)-septate, and short, narrowly obclavate-cylindrical conidia, 25–35 × 2.5–3.5 µm, only 0–5-septate,

**Description:** Leaf spots amphigenous, subcircular to angular-irregular, 1–3 mm diam, somewhat raised, greyish brown to greyish white, with a narrow purplish margin. *Caespituli* hypophyllous, scattered, fine, dark brown. *Mycelium* internal. *Stromata* lacking or small, formed by a few swollen hyphal cells, brown, substomatal. *Conidiophores* in small, divergent to dense fascicles, arising from substomatal hyphae or small stromatic hyphal aggregations, through stomata, erect, subcylindrical, conical, straight to somewhat curved or slightly geniculate-sinuous, unbranched, 5–30 × 3–5 µm, 0(–1)-septate, pale olivaceous to olivaceous-brown, thin-walled, smooth; conidiophores usually aseptate, i.e. reduced to conidiogenous cells, conidiogenous cells occasionally integrated, terminal, 5–25 µm long, conidiogenous loci conspicuous, thickened and darkened, 1–2 µm diam. *Conidia* solitary, narrowly obclavate-cylindrical, straight to slightly curved, 25–35 × 2.5–3.5 µm, 0–5-septate, hyaline, thin-walled, smooth, apex acute to subobtuse, base truncate in cylindrical conidia to short obconically truncate in obclavate ones, 1–2 µm wide, hila slightly thickened and darkened.


**Host range and distribution:** Only known from the type collection.

**Notes:** Bagyanarayana et al. (1991) identified the type collection of this species as *Cercospora celosiae* which they considered a morphologically rather variable fungus. However, *C. celosiae* belongs to the *C. apii* s. lat. complex. The conidia are consistently acicular, and the conidiophores are much longer and septate.

*Cercospora gomphrenae* W.W. Ray, *Mycologia* 36: 172 (1944). (Fig. 71)
Description: Leaf spots amphigenous, circular to somewhat angular-irregular, 1–10 mm diam, at first brownish, greyish brown, later dingy grey to greyish white, margin darker, brown, yellowish brown, reddish brown to purplish brown. Caespituli amphigenous, punctiform, dark brown, scattered. Mycelium internal; hyphae branched, septate, subhyaline or pale, 2–3.5 µm wide. Stromata almost lacking or 10–35 µm diam, substomatal to immersed, brown, cells 2–5 µm diam. Conidiophores in small, divergent fascicles, 3–15, arising from internal swollen hyphae or stromata, through stomata or erumpent, erect, straight, subcylindrical to distinctly geniculate-sinuous, unbranched, 30–300 × 3–7 µm, usually 3–10-septate, pale to medium brown, paler towards the tip, thin-walled, smooth; conidiogenous cells intergrated, terminal and intercalary, 10–30 µm long, conidiogenous loci conspicuously thickened and darkened, 2–3.5 µm diam. Conidia solitary, acicular, straight to curved, 30–300(–450) × 2–5 µm, 3–20-septate, hyaline, thin-walled, smooth, apex acute to subobtuse, base truncate, 2–4 µm wide, hila thickened and darkened.


Host range and distribution: On Gomphrena (globosa, serrata [decumbens]). Amaranthaceae, Africa (Sudan), Asia (China; India, Andhra Pradesh; Korea), North America (USA, Georgia, Oklahoma, Texas), West Indies (Cuba).

Notes: This species is part of the Cercospora apii s. lat. complex. Due to confusion with the name Cercospora gomphrenae Sawada, several records of C. gomphrenae W.W. Ray are undoubtedly wrong or doubtful, e.g. those from Iran, Nepal and Taiwan (Crous & Braun 2003).


(Fig. 72)


Illustrations: Rai & Kamal (1987: 125, fig. 1), Bhartiya et al. (2003: 271, fig. 2).

Description: Leaf spots amphigenous, circular to angular-irregular, sometimes vein-limited, 2–8 mm diam or confluent and larger, light brown to dingy grey. Caespituli amphigenous,
finely punctiform, effuse, dark dingy olivaceous to brown. Mycelium internal. Stromata developed, substomatal to immersed, 10–40 µm diam, medium to dark olivaceous. Conidiophores fasciculate, divergent, 2–12 or occasionally solitary, arising from stromata, through stomata or erumpent, erect, straight, subcylindrical to geniculate-sinuous, usually unbranched, rarely branched, 20–150 × 3–6 µm, 1–8-septate, pale, subhyaline to pale olivaceous, thin-walled, smooth; conidiogenous cells integrated, terminal or occasionally intercalary, conidiogenous loci conspicuous, 1.5–2 µm diam, thickened and darkened. Conidia solitary, cylindrical, somewhat cylindrical-obclavate to subcircular, straight to curved, 15–150 × 1.5–4.5 µm, (0–)1–14-septate, hyaline, thin-walled, smooth, apex subacute or subobtuse, base truncate to somewhat obconically truncate, 1.5–3 µm wide, hila thickened and darkened.


Host range and distribution: On Celosia sp., Amaranthaceae, Asia (India, Uttar Pradesh).

Note: A true Cercospora s. str. well characterised by having cylindrical to somewhat obclavate or subacicular conidia.

Doubtful, excluded and insufficiently known species


Illustration: Rao et al. (1999: 63, fig. 3).

Description: Leaf spots amphigenous, circular to subcircular, 0.5–4 mm diam, olivaceous to olivaceous-brown on the upper leaf surface, pale olivaceous with brown margin below. Caespituli amphigenous, effuse. Mycelium internal; hyphae branched, septate, subhyaline to light olivaceous, about 2.5 µm wide. Stromata well-developed, substomatal, about 25 µm diam, pale olivaceous to olivaceous. Conidiophores solitary or in fascicles of 2–6, arising from stromata, through stomata, about 15–130 × 2–4.5 µm, cylindrical, straight to slightly curved, unbranched, 1–4 times geniculate, pale to dark olivaceous, smooth; conidiogenous cells integrated, terminal and intercalary, with conspicuous conidiogenous cells, thickened and darkened. Conidia solitary, acicular, straight to curved, about 35–210 × 2–4 µm, 5–27-septate, hyaline, thin-walled, smooth, apex subacute to obtuse, base truncate, hila thickened and darkened.


Host range and distribution: On Althernanthera sessilis, Amaranthaceae, Asia (India).

Notes: This invalid species name pertains to the Cercospora apii s. lat. complex. The conidia were described to be “cylindrical” although obviously acicular conidia were depicted in the original drawing.


Illustration: Simmons (1995: 147, fig. 117).


**Illustration**: *Rao et al. (1999: 70, fig. 6).*

**Description**: Leaf spots amphigenous, circular or subcicular, 1–10 mm diam, later to 30 mm diam, with whitish brown above, rusty brown below, with margin. *Caespituli* amphigenous, effuse. *Mycelium* internal; hyphae branched, septate, light olivaceous. *Stromata* well-developed, compact, subepidermal, about 10–30 µm diam, light olivaceous to brown. *Conidiophores* solitary or in small fascicles, 3–7, arising from stromata, erect, straight or almost so, unbranched, cylindrical, 1–4 times geniculate, about 45–155 × 3.5–5 µm, 2–7-septate, olivaceous to light brown, thin-walled, smooth; conidiogenous cells integrated, terminal and intercalary, conidigenous loci conspicuous, thickened and darkened. *Conidia* solitary, subcylindrical to subbiclaric (somewhat attenuated towards the tip), straight to somewhat curved, about 16–270 × 2–3 µm, usually 7–18-septate, hyaline, thin-walled, smooth, apex subacute to obtuse, base truncate, thickened and darkened.

**Syntypes** (holotype not clearly indicated): **India**: Uttar Pradesh: Gorakhpur, on *Gomphrena globosa*, Nov. 1993/1994, S. Narayan (GPU 5051, 5061; HCIO 41968, 41978).

**Host range and distribution**: On *Gomphrena globosa*, *Amaranthaceae*, Asia (India, Uttar Pradesh).

**Notes**: This species is well-characterised by having subcylindrical conidia, but a validation is not made since type material was not available. The authors cited collections from two years, 1993 and 1994, but a holotype was not clearly designated. Furthermore, the name *C. gomphrena-globosae* is invalid since it was published without any Latin diagnosis or description.


**Illustration**: *Patil (1975: 220, fig. 9).*

**Description**: Leaf spots circular 1–2 mm diam, centre depressed, whitish grey, margin red. *Caespituli* amphigenous. *Mycelium* internal. Stromata lacking. *Conidiophores* solitary or in small, loose groups, 2–3, erumpent, erect, straight to flexuous, non-geniculate, unbranched or occasionally branched, basal cells swollen, 20–70 × 5–5.5 µm, 1–2-septate, olivaceous-brown, paler towards the tip; conidigenous loci conspicuous, prominent. *Conidia* solitary, broadly obclavate with attenuated, almost rostrate apex, straight to slightly curved, 20–70 × 5–6.5 µm, 1–5-septate, constricted at the septa, pale olivaceous-brown, apex subobtuse, base obconically truncate, with a prominent hilum, darkened and thickened.


**Host range and distribution**: Only known from the type collections.

**Notes**: The general characters of this fungus are not cercosporoid. It seems to be a species of *Alternaria*, but it was not possible to confirm this assumption. Type material was not available.

Host range and distribution: On Gomphrena (celosioides, serrata [decumbens], globosa), Amaranthaceae, South Africa.

Notes: Cercospora pretoriensis is a cercosporoid species that cannot be unequivocally assigned to any of the currently recognised cercosporoid genera just based on morphology. The structure of the conidiogenous loci does not agree with scars of Cercospora s. str. and Passalora s. lat. Colourless conidia are also not in favour of the latter genus. The conidiogenous cells and conidiogenous loci are reminiscent of Cercosporella species, but the conidiophores are pigmented, often even throughout, which would be very unusual for Cercosporella. Moreover, relations to the complex around Paracerкосpora and Pseudocercosporella can also not be excluded with certainty. Phylogenetic data are required to elucidate the correct generic affinity of this species. For the interim, we prefer to maintain this species in Cercospora sensu latissimo.


MycoBank MB814568


Misapplied name: Cercospora achyranthis sensu Solheim & Stevens (1931: 378).


Illustration: Castañeda & Braun (1989: 44, pl. 1, fig. 1).

Description: Leaf spots amphigenous, subcircular to somewhat irregular, 0.5–4 mm diam, yellowish, ochraceous to brownish, margin indefinite or narrow and darker. Caespituli hypophyllous, punctiform-effuse, greyish white. Mycelium internal. Stromata well-developed, substomatal, subglobose, 20–40 µm diam, brownish, composed of swollen hyphal cells, 2–8 µm diam, subglobose-angular. Conidiophores in small to large fascicles (to 50 or even more), divergent to dense, arising from stromata, emerging through stomata, erect, straight to flexuous, curved, in the upper part slightly to mostly distinctly geniculate-sinuous, simple, rarely branched, (40–)50–200(–220) × (2–)3–5(–6) µm, septe, longer conidiophores pluriseptate, hyaline or subhyaline throughout to pale olivaceous below, occasionally slightly darker near the base, thin-walled, smooth; conidiogenous cells integrated, terminal, 10–40 µm long, conidiogenous loci conspicuous, terminal or formed on lateral shoulders caused by sympodial proliferation, subtruncate to usually convex (light microscopy), slightly thickened, not darkened, but often somewhat refractive, 1.5–2.5 µm diam. Conidia solitary, obclavate-cylindrical, straight to slightly curved, 20–80 × 3.5–7 µm, 1–8-septate, hyaline or subhyaline, thin-walled, smooth, apex obtuse, base obconically truncate to rounded, 1.5–2 µm wide, hila almost unthickened or only very slightly thickened, not darkened, but somewhat refractive.

Holotype: Cuba: San Miguel de los Baños, on Achyranthes...
Host range and distribution: On Achyranthes (aspera var. aspera, aspera var. indica [indica, Centrostachys indica], bidentata, Achryranthes sp.), Amaranthaceae, ?Asia (Pakistan), West Indies (Cuba, Puerto Rico, Virgin Islands).

Notes: The morphological characters of this species have previously been misinterpreted. Due to colourless conidia and conspicuous conidiogenous loci, this species was originally assigned to Cercospora (Castañeda & Braun 1989), but later reallocated to Passalora (Braun 1992). However, the structure of the conidiogenous loci does not coincide with the concepts of the two genera, and colourless conidia are not consistent with the current concept of Passalora. Type material of this species has been re-examined and revealed that the conidia and the structure of conidiogenous cells and conidiogenous loci are in agreement with Cercosporella (Braun 1995). The conidiophores are not quite colourless, above all in the lower half, but this kind of pigmentation is known in some tropical-subtropical species of Cercosporella (Braun 1995).

Chupp (1954) introduced the new species name Cercospora centrostachydis with reference to Solheim & Stevens (1931: 378, as C. achyranthis), but failed to add a Latin description or diagnosis which was necessary for a valid publication in 1954. The type material cited in Chupp (1954), not agreeing with the collections cited in Solheim & Stevens (1931), was not traced in Chupp’s herbarium in CUP. A record of C. centrostachydis from Pakistan (Ahmad et al. 1997) is unclear and unproven.


Description: Leaf spots circular to irregular, grey to dirty brown, margin brown to black, mostly epiphyllous. Stromata 10–30 µm diam, dark brown. Conidiophores in fascicles of 4–10, arising from stromata, divergent, unbranched, 18.5–96.6 × 3.7 µm, darker brown below, paler or subhyaline above. Conidia solitary, obclavate, straight, 51.8–70 × 3.7 µm, septate, hyaline to olivaceous.


Host range and distribution: Only known from the type collection.

Notes: Type material of this species was not available. The original description is meagre. Kamal (2010) alluded that he had examined type material of C. pupaliae. He emphasized that that this species is distinctly different from C. api, but he did not provide any description or illustration and did not specify these differences, above all he did not refer to the original description of conidia (hyaline to olivaceous), although olivaceous conidia could be an indication for Passalora s. lat., i.e. based on the original description and illustration, this species might belong to the latter genus. Furthermore, the conidia were described to be “acicular” but the illustration shows them to be obclavate. Hence, the generic affinity of C. pupaliae is not quite clear and this species is in urgent need of revision.


Description (based on Pavgh & Singh 1964 and examination of type material): Leaf spots amphigenous, greenish brown, on the upper surface becoming chlorotic, 4–8 mm diam,
confluent. Stromata substomatal, 10–25 µm diam, pale olivaceous-brown. Conidiophores in small to moderately large fascicles, divergent to moderately dense, arising from stromata, erect, straight, subcylindrical to somewhat geniculate-sinuous, unbranched, 25–90 × 3–4 µm, continuous to septate, light brown, thin-walled, smooth; conidiogenous cells integrated, terminal. Conidia solitary, obclavate, 30–120 × 1.5–3 µm, 3–11-septate, hyaline or subhyaline, thin-walled, smooth, apex subacute, base short obconically truncate.


**Passalora**

---

**Key to Passalora species on Amaranthaceae**

1 Conidia formed singly, obclavate-cylindrical, 30–85 × 3.5–6 µm, (1–)3–7-septate, pale olivaceous; mycelium internal; conidiophores fasciculate, arising from well-developed stromata, 25–75 µm diam; on Pfaffia sericea

Conidia at least partly catenate and/or superficial mycelium with solitary conidiophores formed and stromata lacking

---

2 (1) Superficial hyphae with solitary conidiophores formed; stromata lacking (mycovellosiella-like species) ........................................................... 3

Superficial hyphae and solitary conidiophores lacking; conidiophores fasciculate; stromata developed (phaeoramularia-like species) ................................................................. 4

3 (2) Conidiophores relatively long, 10–200 × 3–7 µm, longer conidiophores pluriseptate; on Cyathula achyranthoides

Conidiophores shorter, 5–30 × 3–5 µm, 0–1-septate; on Iresine spp. .................................................................................... P. cyathulae

---

4 (2) Conidia usually cylindrical or subcylindrical, hyaline or subhyaline, 20–60 × 2–5 µm, 0–5-septate; conidiophores narrow, 1.5–5 µm wide; on Iresine diffusa .......................................................... P. gilbertii

Conidial shape variable, cylindrical, fusiform to obclavate, olivaceous to olivaceous-brown, 3–8 µm wide; conidiophores broader, 2–8 µm ........................................................................ 5

5 (4) Conidiophores occasionally branched; on Iresine diffusa .......................................................... P. iresines

Conidiophores unbranched; on Gomphrena and Pfaffia spp. .............................................................. P. gomphricola

---

**Tabular key to Passalora species on Amaranthaceae according to host genera**

**Cyathula**

A single species .......................................................................................................................... P. cyathulae

**Gomphrena**

A single species .......................................................................................................................... P. gomphricola

**Iresine**

1 Stromata lacking; conidiophores solitary, arising from superficial hyphae, 5–30 µm long, 0–1-septate

Stromata developed; superficial hyphae and solitary conidiophores absent; conidiophores fasciculate, 20–60(–110) µm long, 0–5-septate .......................................................................................... 2

2 (1) Conidia usually cylindrical or subcylindrical, hyaline or subhyaline, 20–60 × 3.5–6 µm; conidiophores narrow, 1.5–5 µm wide, unbranched .......................................................... P. gilbertii

Conidial shape variable, cylindrical, fusiform to obclavate, olivaceous to olivaceous-brown, 3–8 µm wide; conidiophores broader, 4–6(–8) µm, occasionally branched .......................................................... P. iresines

---

Notes: Status and generic affinity unclear, type material deposited at K(M) has been examined, but it was too meagre for a final conclusion. According to the original publication, syntype material has been deposited in HCIO, but an accession number was not cited and the material concerned was not available. Some conidiophores were found, all without any conspicuous conidiogenous loci, and a single conidium which seemed to have a slightly thickened and darkened hilum.
Passalora species on Amaranthaceae

Passalora cyathulae (F. Stevens & Solheim) U. Braun & Crous, Mycosphaerella and Anam.: 148 (2003) (Fig. 75)

Literature: Chupp (1954: 33).

Illustration: Solheim & Stevens (1931: 403, fig. 12).

Description: Leaf spots lacking or indistinct, yellowish discolorations or small circular, subcircular to somewhat irregular spots, 0.5–2 mm diam, brown above, olivaceous-brown below, margin indefinite. Caespituli hypophyllous, effuse, dark olivaceous. Mycelium internal and external; superficial hyphae emerging through stomata, branched, septate, 2–5 µm wide, subhyaline to pale brown, thin-walled, smooth. Stromata lacking. Conidiophores solitary, arising from superficial hyphae, lateral or terminal, erect to decumbent (differentiation between solitary conidiophores arising from superficial hyphae and long decumbent conidiophores difficult), straight, subcylindrical to geniculate-sinuous, unbranched to branched, length variable, 10–200 × 3–7 µm, aseptate to pluriseptate throughout, subhyaline to pale brown, thin-walled, smooth; conidiogenous cells integrated, terminal or intercalary, sometimes conidiophores reduced to conidiogenous cells, 10–30 µm long, conidiogenous loci conspicuous, thickened and darkened, (1.5–)2–(2.5) µm diam. Conidia in simple or sometimes branched chains, straight to somewhat curved, ellipsoid to cylindrical, 20–65 × 3–7.5 µm, 0–4-septate, subhyaline to pale brownish, thin-walled, smooth, ends short obconically truncate to rounded, about 2 µm wide, hila somewhat thickened and darkened.

Holotype: Guyana: Coverden, on Cyathula achyranthoides, Amaranthaceae, 4 Aug. 1922, F. L. Stevens 743 (ILL 11981).

Host range and distribution: Only known from the type collection.

Notes: This species is a typical mycovelllosiella-like Passalora species with superficial hyphae with solitary conidiophores, thickened and darkened conidiogenous loci and conidal hila, and conidia formed in chains. Chupp (1954) confused Ragnhildiana cyathulae and the Indian Cercospora cyathulae described by Sydow (1937). Furthermore, he cited “Cercospora cyathulae (F. Stevens & Solheim) Syd.” which is, however, incorrect. Sydow (in Sydow et al. 1937) described a new Indian species but did not introduced a new species based on Ragnhildiana cyathaeae. Moreover, Chupp’s (1954) description of C. cyathulae was based on characters of both species, although the two species are readily distinguishable by obvious differences in the conidial length and septation.

Fig. 75. Passalora cyathulae (ILL 11981, holotype). A. Superficial hyphae. B. Solitary conidiophores arising from superficial hyphae. C. Conidiophore. D. Conidia. Bar = 10 µm.

(Fig. 76)


Illustration: Braun (1999: 12, fig. 14).


Description: Leaf spots amphigenous, subcircular to irregular, 1–10 mm diam, oblong patches to 20 mm in length, brown to dingy grey, zonate, sometimes with narrow brown margin. Caespituli amphigenous, punctiform, dark brown to blackish. Mycelium internal. Stromata well-developed, 20–60 µm diam, subglobose, immersed, brown. Conidiophores numerous, in loose to very dense fascicles, arising from stromata, erumpent, erect, filiform, flexuous, somewhat geniculate-sinuous, unbranched, 20–60 × 1.5–5 µm, aseptate to pluriseptate throughout, subhyaline to pale olivaceous, thin-walled, smooth; conidiogenous cells integrated, terminal, 10–30 µm long, conidiogenous loci subconspicuous, minute, 0.75–1.5 µm diam, slightly thickened and darkened-refractive. Conidia solitary or catenate, in short chains, subcylindrical, occasionally narrowly obclavate to fusiform, 20–60 × 2–5 µm, (0–)1–4-septate, hyaline or subhyaline, thin-walled, smooth, apex obtuse, subacute or truncate in catenate conidia, base short to long obconically truncate, about 1 µm wide, hila barely thickened, colourless to slightly darkened-refractive.


Host range and distribution: On Iresine diffusa [celosia, celosioides, paniculata], Amaranthaceae, Africa (São Tomé e Príncipe), North America (Mexico), South America (Colombia, Ecuador, Uruguay), West Indies (Puerto Rico, Virgin Islands).

Note: The orthographic variant “gibertii” sometimes used for this species is incorrect. The epithet was derived from the name of the collector, G. Gilbert. The phylogenetic affinity of Cercospora gilbertii, characterised by an unusual combination of morphological traits, is quite uncertain. Colourless conidia are in favour of Cercospora in its current circumscription, but catenate conidia and the structure of the conidiogenous loci argue against it. Molecular analyses are necessary to elucidate the true generic affinity of this species. For the interim we prefer to maintain this species in Passlora s. lat.

Passalora gomphrenicola (Speg.) U. Braun, Schlechtendalia 5: 64 (2000).

(Fig. 77)


Description: Leaf spots lacking or only visible as yellowish disolorations, turning dark olivaceous by abundant formation of caespituli, patches to 12 mm diam. Caespituli
hypophyllous, effuse, olivaceous, forming small patches or confluent. *Mycelium* internal, hyphae colourless, branched, septate, 2–4 µm wide. **Stromata** absent or small, about 10–70 µm diam, substomatal, pale to brown, composed of swollen hyphal cells, circular to somewhat angular-irregular in outline, 2–6 µm diam. **Conidiophores** in small to large, loose to dense fascicles, larger fascicles composed of 50 or even more conidiophores, arising from internal hyphae or from stromata, through stomata or erumpent, erect to decumbent, straight, subcylindrical-conical to sinuous or somewhat geniculate-sinuous, unbranched or branched, decumbent branched conidiophores reminiscent of and confusable with superficial hyphae giving rise to solitary conidiophores, 20–70(–90) × 2–7 µm, often irregular in width, 1–5-septate, often somewhat constricted at the septa, pale olivaceous to olivaceous-brown, darker in mass, thin-walled, smooth; conidiogenous cells integrated, terminal, intercalary and occasionally pleurogenous, 5–30 µm long, proliferation sympodial, occasionally percurrent, conidiogenous loci conspicuous, sometimes subdenticulate, thickened and darkened, 1.5–2.5 µm wide. **Conidia** in simple or branched chains, ellipsoid, ovoid, broadly fusiform, subcylindrical, straight to slightly curved, (10–)15–60 × 3–8 µm, (0–)1–4(–5)-septate, occasionally somewhat constricted at the septa, pale olivaceous to olivaceous-brown, thin-walled, smooth, occasionally faintly rough, apex rounded to truncate in catenate conidia, base short obconically truncate, (1.5–)2(–2.5) µm wide, hila somewhat thickened and darkened.


![Fig. 77. Passalora gomphrenicola (LPS 914, holotype). A. Conidiophore fascicles. B. Solitary conidiophores arising from superficial hypha. C. Conidiophores. D. Conidia. Bar = 10 µm.](image-url)
Host range and distribution: On Gomphrena globosa, Paffia (glomerata [glauc], stenophylla; Gomphrena glauca, resinosoides), Amaranthaceae, Africa (South Africa, Transvaal), South America (Argentina, Brazil, Venezuela).


(Fig. 78)


Illustration: Muntañola (1960: 208, fig. 15 A).

Description: Leaf spots indistinct, formed as yellowish discolorations on the upper leaf surface. Colonies hypophyllous, effuse, brownish. Mycelium internal and external; superficial hyphae solitary, occasionally intertwined, branched, septate, subhyaline to olivaceous-brown, 2–8 µm wide (sterile hyphae paler and narrow, fertile hyphae with conidiophores broader and darker), thin-walled, smooth. Stromata lacking. Conidiophores solitary, arising from superficial hyphae, lateral or terminal, erect to decumbent, 5–30 × 3–10 µm, 0–1-septate (decumbent threats with terminal conidiophores may be much longer and pluriseptate), sometimes with intercalary cells giving rise to minute peg-like protuberances with a single terminal scar, only about 2–5 µm long and wide, subhyaline to pale olivaceous or olivaceous-brown, thin-walled, smooth; conidiophores mostly reduced to conidiogenous cells, conidiogenous loci conspicuous, somewhat thickened and darkened, 1.5–2 µm diam. Conidia catenate, in simple or branched chains, ellipsoid-ovoid to cylindrical, 12–60 × 3.5–8.5 µm, (0–)1–7-septate, subhyaline to pale olivaceous-brown or brownish, thin-walled, smooth, apex rounded in terminal primary conidia or conically truncate in secondary (catenate) conidia, subdenticulate when in branched chains, base short obconically truncate, 1.5–2.5 µm wide, hila thickened and darkened.

Lectotype (designated here, MycoBank, MBT202803):

Isolectotypes: Syd., Fungi Exot. Exs. 930, e.g., BPI 436742, CUP, HBG, K(M) IMI 7704, MICH 15303, NY 937030.
Former syntypes (type locality but from 5 Jan. 1925 [H. Sydow, Fungi itin. Costaricensi Coll. 12]): E 417850, ILL 10671.


(Fig. 79)

Illustrations: Muntañola (1960: 217, fig. 20, 219, fig. 21).

Description: Leaf spots formed as epiphyllous chlorotic discolorations, scattered to confluent, circular, elliptical, diffuse, violent, finally ochraceous to brown. *Caespituli* hypophyllous, velutinous, brownish to brown-olivaceous, patches subcircular or sometimes vein-limited. Mycelium internal. Stromata moderately large, substomatal, olivaceous. Conidiophores in divergent to dense fascicles, arising from stromata, through stomata, erect, flexuous, geniculate-sinuous, simple or occasionally branched, 40–
60(–110) × 4–6(–8) µm, branchlets 15–20(–50) µm long, base sometimes swollen, aseptate to sparingly septate, often somewhat constricted at septa, olivaceous, paler towards the tip, subhyaline, thin-walled, smooth; conidiogenous cells integrated, terminal or intercalary, sometimes lateral, or conidiophores reduced to conidiogenous cells, conidiogenous loci conspicuous, thickened and darkened. Conidia catenate, in simple or branched chains, variable in shape and size, cylindrical, fusiform, obclavate, straight to curved, rarely sigmoid, 20–60 × 3.5–6 µm, 0–3-septate, olivaceous, thin-walled, smooth, base obconically truncate, with a single somewhat thickened and darkened hilum, apex rounded or with 1–3 hila.

Holotype: Argentina: Tucumán: El Cerro San Javier, on Iresine diffusa, 1 Aug. 1959, M. Muntañola (not traced).

Host range and distribution: On Iresine diffusa [polymorpha], Amaranthaceae, South America (Argentina).

Note: Muntañola (1960) designated a collection in her private herbarium (no. 600) as type. Muntañola-Cvetković died in 2011. The fate of her herbarium could not yet be clarified. After her time in South America, she worked in Serbia (University of Belgrade, Faculty of Biology), and after her retirement she moved to Portugal (University of Barcelona, Faculty of Biology), but Muntañola’s herbarium is not preserved in Belgrade (J. Vukojevic, pers. comm.). A part of her herbarium, mainly fungi collected in Portugal, are preserved in BCN, but South American collections are not included (according to the curator of BCN, Barcelona).


(Fig. 80)

Literature: Chupp (1954: 35).

Description: Leaf spots amphigenous, circular to somewhat angular-irregular, 1–6 mm diam, pale greyish brown to dingy grey, with narrow marginal line, somewhat raised. Caespituli amphigenous, punctiform, scattered, dark brown to blackish. Mycelium internal. Stromata substomatal to immersed, subglobose to oblong, 25–75 µm diam, dark
brown, composed of subglobose cells, 2.5–5 µm diam. **Conidiophores** in moderately large fascicles, divergent to mostly dense, arising from stromata, erect, subcylindrical, sinuous or slightly geniculate, unbranched, 10–70 × 3.5–6 µm, 0–3-septate, pale olivaceous to brown, thin-walled, smooth; conidiogenous cells integrated, terminal, occasionally conidiophores reduced to conidiogenous cells, 10–30 µm long, proliferation sympodial, occasionally percurrent, with fine anellations, conidiogenous loci conspicuous, small, 1–1.5 µm diam, somewhat thickened and darkened. **Conidia** solitary, obclavate-cylindrical, straight to somewhat curved, 30–85 × 3.5–6 µm, (1–)3–7-septate, pale olivaceous, thin-walled, smooth, apex obtuse, base short obconically truncate, 1.5–2 µm wide, hila somewhat thickened and darkened. **Holotype:** Brazil: Rio Grande do Sul: Taquari, Parque Apicola, on *Pfaffia sericea*, Amaranthaceae, 31 Dec. 1946, J. P. da Costa Neto 2224 (CUP 40533).

**Host range and distribution:** Only known from the type collection.

**Note:** Based on a combination of conspicuous conidiogenous loci, slightly thickened and darkened, 1–1.5 µm wide, and obclavate-cylindrical, pale olivaceous conidia, Crous & Braun (2003) reallocated this species to *Passalora*.

### Pseudocercospora

#### Key to Pseudocercospora species on Amaranthaceae

1. Superficial hyphae with solitary conidiophores developed
   - Superficial hyphae with solitary conidiophores lacking; conidiophores consistently fasciculate

2. (1) Conidia broad, 4–8 µm; on *Cyatha tomentosa* ................................................................. *P. cyathulae*
   - Conidia narrower, 2–4 µm ........................................................................................................... 3

3. (2) Conidia obclavate-cylindrical, pale olivaceous-brown; on *Alternanthera* spp. .................. *P. alternantherae*
   - Conidia cylindrical, subacicular to obclavate-cylindrical, subhyaline to pale olivaceous;
     on *Celosia* spp. .......................................................................................................................... 4

4. (1) Conidiophores 10–100 µm long; conidia narrowly filiform-subcylindrical, subacicular or narrowly obclavate-cylindrical, (40–)60–130(–150) × 1.5–3.5 µm, 2–12-septate, hila 1–1.5 µm wide; on *Gomphrena* spp. .................. *P. globosae*
   - Conidiophores much shorter, 10–35 µm long; conidia obclavate-cylindrical, 10–90 µm long, 0–10-septate or, if conidiophores longer, conidia 2.5–6 µm wide; on other hosts ................................................... 5

5. (4) Conidiophores 10–70 × 3–5.5 µm, 0–5-septate; conidia 25–135 × 2.5–6 µm, 3–16-septate, pale olivaceous-brown;
   - Conidiophores shorter, 5–35 µm long, only 0–1(–2)-septate; conidia shorter and narrower, 15–90 × 2–4 µm or
     subhyaline if broader; on other hosts ............................................................................. 6

6. (5) Stromata lacking; conidiophores in small fascicles; on *Gomphrena* spp. ......................... *P. gomphrenae*
   - Stromata developed, 10–100 µm diam; conidiophores mostly in larger fascicles arising from stromata ........................................ 7

7. (6) Stromata large, 10–100 µm diam; conidia (2–)3–5(–6) µm wide, hila 1.5–3 µm wide, subhyaline; on *Gomphrena pulchella* ......................................................................... *P. gomphrenae-pulchellaefi*
   - Stromata smaller, 10–65 µm; conidia 2–3.5 µm wide, hila 1–2 µm wide, subhyaline to pale olivaceous-brown;
     on other hosts .................................................................................................................. 8

8. (7) Conidia mostly narrowly cylindrical with truncate base, pale olivaceous-brown;
   - Conidia obclavate-cylindrical, base short obconically truncate, subhyaline to pale olivaceous; on other hosts ................................................................. 9

9. (8) Forming distinct leaf spots; caespituli hypophyllous, punctiform; stromata substomatal; on *Chamissoa altissima*
   - Leaf spots lacking or only with indistinct discolorations; caespituli epiphyllous, forming sooty patches; stromata immersed; on *Froelichia* sp. ......................................................... *P. froelichiae*

---

**Volume 6 · No. 2**
Tabular key to Pseudocercospora species on Amaranthaceae according to host genera

**Alternanthera**

1. Superficial hyphae with solitary conidiophores developed ............................................... *P. alternantherae*

Superficial hyphae and solitary conidiophores absent; conidiophores only in fascicles ....... *P. alternantherae-nodosae*

**Amaranthus**

A single species ................................................................. *P. amaranthicola*

**Celosia**

A single species ................................................................. *P. celosiarum*

**Chamissoa**

A single species ................................................................. *P. chamissoana*

**Cyathula**

A single species ................................................................. *P. cyathulae*

**Froelichia**

A single species ................................................................. *P. froelichiae*

**Gomphrena**

1. Stromata lacking, conidiophores in small fascicles ................................................................. *P. gomphrenae*

Stromata developed, 10–100 µm diam; conidiophores in small to large fascicles, arising from stromata ............. 2

2 (1) Stromata 10–100 µm diam; conidiophores 10–30 × 2.5–6 µm; conidia obclavate-cylindrical,
(2–)3–5(–6) µm wide, subhyaline; on *Gomphrena pulchella* ........................................ *P. gomphrenae-pulchellae*

Stromata 10–40 µm diam; conidiophores 10–100 × 1.5–5.5 µm; conidia filiform-subcylindrical, subacicular to
narrowly obclavate-cylindrical, narrower, 1.5–3.5 µm, subhyaline to pale olivaceous-brown; on *Gomphrena
globosa* ................................................................. *P. globosae*

Pseudocercospora species on Amaranthaceae


(Fig. 81)

**Literature:** Kamal (2010: 147).

**Illustration:** Yen et al. (1982: 40, fig. 3).

**Description:** Leaf spots amphigenous, scattered, oval to fusiform, 3–12 × 2–4 mm, often confluent, greyish brown, margin indistinct. *Caespitulus* amphigenous, mostly hypophyllous, not very conspicuous. *Mycelium* internal and external, superficial; hyphae branched, septate, olivaceous-brown, thin-walled, smooth, 2–5 µm wide, internal hyphae 2–5 µm wide, external hyphae 2–3.5 µm wide. *Stromata* globose, substomatal, 20–40 µm diam, brown to dark brown. *Conidiophores* in small to well-developed fascicles, about 5–60, divergent to dense, arising from stromata, through stomata, and solitary, arising from superficial hyphae, lateral, erect, straight, subcylindrical to sinuous, slightly geniculate, unbranched, 15–55 × 3–4.5 µm, 0–3-septate, pale olivaceous-brown, thin-walled, smooth; conidiogenous cells integrated, terminal or conidiophores reduced to conidiogenous cells, conidiogenous loci inconspicuous, unthickened, not darkened. *Conidia* solitary, obclavate-cylindrical, straight to slightly curved, 30–90 × 2.5–4 µm, 3–10-septate, pale olivaceous-brown, thin-walled, smooth, apex obtuse, base short obconically truncate, hila unthickened, not darkened.


**Host range and distribution:** Only known from the type collection.

**Notes:** Yen et al. (1982) cited “LAM, Yen #10583” as holotype. The mycological LAM collections are now housed at UC, but the type material concerned could currently not be traced. It is possible that this material is still among numerous unincorporated Yen collections in UC (unnamed, only provided with a collection number).


(Fig. 82)


Illustrations: Pavgi & Singh (1965: 94, pl. 1, figs 16–18), Hsieh & Goh (1990: 18, fig. 7), Guo & Hsieh (1995: 9, fig. 8), Guo et al. (1998: 19, fig. 8).

Description: Leaf spots at first indistinct or visible as small brown spots, about 2–4 mm diam, later lower leaf surface gradually turning brown, upper leaf surface also discoloured, leaves finally disfigured, faded, necrotic. Caespituli amphigenous, forming effuse, dark olivaceous patches, finally covering the whole leaf blade. Mycelium internal. Stromata small, substomatal, 10–20 µm diam, brown. Conidiophores in small to well-developed fascicles, 6–25, loose to moderately dense, arising from stromata, through stomata, erect, straight, subcylindrical to curved or distinctly geniculate-sinuous, unbranched, 10–70 × 3–5.5 µm, 0–5-septate, uniformly pale olivaceous-brown to brown, thin-walled, smooth; conidiogenous cells integrated, terminal, occasionally conidiophores reduced to conidiogenous cells, conidiogenous loci inconspicuous to visible as truncate or conically truncate tips or shoulders formed by sympodial proliferation, unthickened, not darkened. Conidia solitary obclavate-subcylindrical, straight to curved, 25–135 × 2.5–6 µm, 3–15-septate, pale olivaceous, thin-walled, smooth, apex obtuse, rounded to subacute, base oboconically truncate, 2–2.5 µm wide, hila unthickened, not darkened.

Lectotype (designated here, MycoBank, MBT202804): Taiwan: Taipei, on Alternanthera sessilis, 9 May 1924, K.

Host range and distribution: On Alternanthera sessilis [nodiflora], Alternanthera sp., Amaranthaceae, Asia (China; India, Uttar Pradesh; Taiwan).

Notes: Three duplicates of an additional syntype collection from Taiwan have been examined [Taipei, on Alternanthera sessilis, 5 Aug. 1907, K. Kawakami (BPI 432466, 432467; K(M) IMI 31945). Cercospora alternathericola, described from India on Alternanthera sessilis is barely distinct from Pseudocercospora alternantherae-nodiflorae and is therefore reduced to synonymy.


(Fig. 83)


Illustrations: Yen (1977: 146, fig. 1A–C), Guo et al. (1998: 377, fig. 307).

Description: Leaf spots circular or subcircular, 0.5–4 mm diam, pale brown, margin brown. Caespituli epiphyllous, rarely amphigenous, punctiform, dark brown to blackish. Mycelium internal. Stromata immersed, globose or subglobose, 25–65 µm diam, dark brown. Conidiophores in well-developed, dense fascicles, arising from stromata, erumpent, erect, straight, flexuous, subcylindrical to geniculate-sinuous, unbranched or occasionally branched, 8–30 × 2.5–3.5 µm, 0–3-septate, pale olivaceous-brown, thin-walled, smooth; conidiogenous cells integrated, terminal or conidiophores reduced to conidiogenous cells, conidiogenous loci inconspicuous or visible as truncate tips or lateral shoulders caused by sympodial proliferation. Conidia solitary, narrowly cylindrical or only slightly obclavate-cylindrical, straight to curved, 15–80 × 2–3 µm, 3–10-septate, pale olivaceous-brown, apex obtuse or subobtuse, base truncate to somewhat obconically truncate, hila neither thickened nor darkened.

Holotype: Taiwan: Peikuoshan, Yuanlin, Changhua Hsien, on Amaranthus tricolor [mangostanus], Amaranthaceae, 30 Oct. 1971, J. M. Yen 71282.

Host range and distribution: Only known from the type collection.

Notes: Type material of this species could not be traced, neither in PC nor UC. It is possible that the material concerned is still among the numerous untreated Yen collection in the latter herbarium.


(Fig. 84)


Illustrations: Kar & Mandal (1970: 423, fig. 1), Guo & Hsieh (1995: 10, fig. 9), Guo et al. (1998: 20, fig. 9).

Description: Leaf spots amphigenous, subcircular to angular-
irregular or diffuse, size variable, 1.5–30 mm diam, brown, dull brown to dark greyish brown, margin narrow, darker brown to reddish brown. *Caespituli* amphigenous, punctiform, dark brown or more greyish by abundant conidiation. *Myceium* internal and external; superficial hyphae, if present, emerging through stomata, branched, septate, 2–3 µm diam, subhyaline, thin-walled, smooth. *Stromata* almost lacking to well-developed, substomatal, subglobose, sometimes oblong, 5–60 µm diam, pale olivaceous to brown. *Conidiophores* in small to large fascicles, divergent to very dense, arising from stromata, through stomata or solitary, arising from superficial hyphae, lateral, erect, straight, subcylindrical-conical, not to strongly geniculate-sinuous, unbranched, 5–75 × 2–4 µm, 0–5-septate, pale olivaceous to olivaceous-brown, thin-walled, smooth; conidiogenous cells integrated, terminal or conidiophores reduced to conidiogenous cells, 5–20 µm long, conidiogenous loci inconspicuous, neither thickened nor darkened. *Conidia* solitary, cylindrical, subbiculic, long acicular to obclavate-cylindrical, 10–105 × 2–4 µm, straight to curved, 0–10-septate, subhyaline to pale olivaceous, thin-walled, smooth, apex obtuse to subacute, base truncate to short obconically truncate, 1.5–2 µm wide, hila unthickened, not darkened.

**Holotype:** *India*: West Bengal: Calcutta, Presidency College, on *Celosia argentea*, 17 Feb. 1967, A. K. Kar & M. Mandal (K(M) IMI 135869).

**Host range and distribution:** On *Celosia argentea* [cristata], *Amaranthaceae*, Asia (China; India, West Bengal; Japan).

**Notes:** The conidial width in this species is uniform, but the length rather variable, and relatively short in the type material. The length of conidiophores is also rather variable. Japanese collections of this species were originally wrongly identified and published as *Cercospora celosiae* (Katsuki 1965), which was clarified in Nakashima *et al.* (2002).


(Fig. 85)

**Illustration:** Castañeda Ruiz & Braun (1989: 47, pl. 3, fig. 18).

**Description:** Leaf spots amphigenous, subcircular to irregular, 1–10 mm diam, brownish, later pale, margin indefinite or narrow, light to dark brown, formed as marginal line. *Caespituli* hypophyllous, punctiform, dark brown to blackish, scattered. *Myceium* internal. *Stromata* substomatal, 20–45 µm diam, brown, composed of swollen hyphal cells, subglobose to angular in outline, 2–5 µm diam. *Conidiophores* in small to moderately large fascicles, divergent to dense, arising from stromata, through stomata, erect, straight, subcylindrical-conical to geniculate-sinuous, unbranched, 5–25 × 2–5 µm, 0–1-septate, pale brown, thin-walled, smooth; conidiogenous cells integrated, terminal or conidiophores reduced to conidiogenous cells, 5–20 µm long, conidiogenous loci inconspicuous to subdenticulate, but always unthickened and not darkened. *Conidia* solitary, obclavate-cylindrical to somewhat fusoid, straight to slightly curved, 40–65 × 2–3 µm, indistinctly 3–6-septate, subhyaline to pale olivaceous, thin-walled, smooth, apex obtuse to subacute, base obconically truncate, 1–2 µm wide, hila neither thickened nor darkened.

**Holotype:** *Cuba*: Granma: Guisa, Los Corrales, on *Chamissoa altissima*, *Amaranthaceae*, 15 Jun. 1987, R. F. Castañeda Ruiz (INIFAT, C87/167). *Isotype*: HAL 1651 F.

**Host range and distribution:** Only known from the type collection.
Pseudocercospora cyathulae (Syd.) U. Braun, comb. nov.
MycoBank MB814580
(Fig. 86)


Description: Leaf spots lacking or almost so, on the upper leaf surface only formed as yellowish discolorations, 5–10 mm diam or larger, diffuse, below visible as subcircular brownish patches caused by colonies of the fungus, margin indefinite.

Caespituli hypophyllous, effuse, brownish or dark olivaceous-brown. Mycelium internal and external; superficial hyphae plagiotropous or climbing leaf hairs, forming a dense net or cover, branched, sometimes anastomosing, septate, sterile hyphae narrow, 1–3.5 µm, subhyaline to pale olivaceous or olivaceous-brown, mostly without constrictions at septa, fertile hyphae with conidiophores or conidiogenous cells broader and darker, 3–8 µm wide, swollen cells occasionally to 12 µm diam, olivaceous to medium olivaceous-brown, often with constrictions at septa, thin-walled, smooth. Stromata lacking. Conidiophores solitary, arising from superficial hyphae,
lateral or terminal, length indefinite (forming decumbent fertile threats, but differentiation between individual conidiophores and hyphal portions difficult or even impossible), shorter lateral conidiophores arising from decumbent threats about 5–30 µm long, width about 3–8 µm, erect to decumbent, straight, subcylindrical to geniculate-sinuous, unbranched or branched, aseptate to prurisepitate, olivaceous, olivaceous-brown to light brown, thin-walled, smooth; conidiogenous cells integrated, terminal, 10–25 µm long, conidiogenous loci inconspicuous to subconspicuous by being slightly refractive, but unthickened, occasionally visible in front view as minute circle (paracercosporoid) or subdenticulate, 1.5–2 µm diam. Conidia solitary, rarely in short chains, cylindrical to verriform, shorter conidia sometimes ovoid, obovoid, 0–7-septate, occasionally somewhat constricted at the septa, pale brown, thin-walled, smooth, apex broadly rounded, rarely subtruncate or somewhat attenuated, base short obconically truncate, 1–2 µm wide, hila unthickened, not darkened, sometimes somewhat darker by being refractive.


Host range and distribution: Only known from the type collection.

Notes: The CUP collection is the only material of this species that could be traced and examined. Chupp (1954) confused this species with Ragnhildiana cyathulae (≡ Passalora cyathulae) and used the wrong citation “Cercospora cyathulae” although Sydow (1937) did not intend to introduce a new combination based on Ragnhildiana cyathulae. He undoubtedly published a new species without any reference to R. cyathulae. The two species are neither conspecific nor congeneric.

Pseudocercospora froelichiae U. Braun & F.O. Freire, Cryptog. Mycol. 25: 230 (2004). (Fig. 87)

Illustrations: Braun & Freire (2004: 231, fig. 7).

Description: Leaf spots lacking or only with inconspicuous discolorations, yellowish ochraceous, brownish or occasionally purplish, 1–5 mm diam. Colonies formed on the upper leaf surface as sooty patches caused by dense fructification. Mycelium internal. Stromata immersed or somewhat erumpent, 10–50 µm diam, olivaceous-brown, composed of swollen hyphal cells, 2–7 µm diam, walls somewhat thickened. Conidiophores in small to moderately large fascicles, loose to dense, arising from stromata, erumpent, erect, straight, subcylindrical-conical, slightly geniculate-sinuous, unbranched, 10–35 × 2–5 µm, 0–1(–2)-septate, pale olivaceous to olivaceous-brown, thin-walled, smooth; conidiogenous cells integrated, terminal or conidiophores reduced to conidiogenous cells, 10–25 µm long, conidiogenous loci inconspicuous. Conidia solitary, obclavate-cylindrical, 10–80 × 2–3.5 µm, 1–7-septate, pale olivaceous, thin-walled, smooth, apex obtuse or subacute, base short obconically truncate, 1–2 µm wide, hila unthickened, not darkened.


Host range and distribution: On Froelichia sp., Amaranthaceae, South America (Brazil, State of Ceará, Rio Grande do Norte State).

Note: Pseudocercospora froelichiae on Froelichia sp. (Amaranthaceae, Gomphrenoideae) is morphologically close to P. chamissoana, described from Cuba on Chamissoa altissima (Amaranthaceae, Amaranthoideae), which differs, however, in forming distinct leaf spots, hypophyllous caespituli and substomatal stromata.

Pseudocercospora globosae (J.M. Yen) Deighton, Mycol. Pap. 140: 144 (1976). (Fig. 88)


Illustrations: Yen (1964: 227, fig. 7), Yen & Lim (1980: 239, fig. 42).

Description: Leaf spots at first inconspicuous, later subcircular to somewhat irregular, greyish white to dark grey by abundant fungal colonies. Caespituli amphigenous, effuse to dense, velvety, greyish white. Mycelium internal. Stromata subglobose to somewhat irregular, substomatal to immersed, 10–40 µm diam, yellowish brown. Conidiophores in small to moderately large fascicles, loose to moderately dense, arising from stromata, through stomata or erumpent, straight and subcylindrical-conical to distinctly geniculate-sinuous, unbranched to branched, 10–100 × 1.5–5.5 µm, 0–6-septate, subhyaline to pale yellowish or olivaceous-brown, thin-walled, smooth; conidiogenous cells integrated, terminal, occasionally intercalary, 10–25 µm long, conidiogenous loci inconspicuous to distinctly denticle-like, subcylindrical-conical, apex truncate, 1–2 µm wide, always unthickened and not darkened. Conidia solitary, filiform-subcylindrical, subacicular to narrowly obclavate-subcylindrical, (40–)60–130(-150) × 1.5–3.5 µm, 2–12-septate, subhyaline to very pale olivaceous or olivaceous-brown, thin-walled, smooth, apex subacute to subobtuse, base truncate to short or long obconically truncate, 1–1.5 µm wide, hila unthickened, not darkened.


Host range and distribution: On Gomphrena (globosa, Gomphrena sp.), Amaranthaceae, Asia (Brunei, Singapore).

(Fig. 89)

Cercosporoid fungi 4


**Illustrations:** Hsieh & Goh (1990: 19, fig. 8), Guo & Hsieh (1995: 11, fig. 10), Guo et al. (1998: 21, fig. 10).

**Description:** Leaf spots amphigenous, subcircular to elliptical, 2–10 mm diam, centre greyish white, margin reddish, on the upper surface with yellowish halo, below olivaceous with dark olivaceous to brown border. Caespituli hypophyllous. Mycelium internal. Stromata lacking. Conidiophores in small fascicles, not more than six, arising from internal hyphae, erect, straight, subcylindrical to somewhat attenuated towards the tip, slightly geniculate-sinuous, unbranched, 20–30 × 3.5–4 μm, 0–2-septate, olivaceous, thin-walled, smooth; conidiogenous cells integrated, terminal or conidiophores reduced to conidiogenous cells, conidiogenous loci inconspicuous, unthickened, not darkened. Conidia solitary, cylindrical, obclavate-cylindrical to subcircular, straight to curved, 30–90 × 2.5–4 μm, 4–7-septate, subhyaline to very pale olivaceous, thin-walled, smooth, apex obtuse to subacute, base subtruncate to obconically truncate, about 1.5–2 μm wide, hila unthickened, not darkened.

**Lectotype (designated here, MycoBank, MBT202805):**
**Taiwan:** Taipei, on Gomphrena globosa, 15 Nov. 1925, K. Sawada (NTU-PPE, hb. Sawada). **Isolectotype:** TNS-F-220432

**Host range and distribution:** On Gomphrena globosa, Amaranthaceae, Asia (Brunei, China, Iran, Singapore, Taiwan).

**Notes:** Records of this species from Iran are based on Hedjaroude (1976) and Bakshi et al. (2012).


(Fig. 90)

**Illustration:** Braun et al. (2001: 29, fig. 8).

**Description:** Leaf spots amphigenous, subcircular to irregular, 1–5(–8) mm diam, centre pale, yellowish to ochraceous or greyish white, margin narrow, dark reddish brown to blackish brown. Caespituli amphigenous, punctiform, loose to dense, blackish, later greyish white by abundant

---

**Fig. 90.** *Pseudocercospora gomphrenae-pulchellae* (HAL 1635 F, isotype). A. Conidiophores fascicles. B. Conidiophores. C. Conidia. Bar = 10 μm.
conidial formation. Mycelium internal; hyphae branched, 1.5–6 μm diam, septate, subhyaline to pale brown, smooth. Stromata substomatal to intraepidermal, 10–100 μm diam, olivaceous-brown. Conidiophores in small to large fascicles, moderately dense, arising from stromata, through stomata or erumpent. erect, straight, subcylindrical or attenuated towards the tip, geniculate-sinuous, unbranched, 10–30 × 2.5–6 μm, 0–1-septate, subhyaline to pale brown, thin-walled, smooth; conidigenous cells integrated, terminal or conidiophores reduced to conidiogenous cells, 10–25 μm long, conidiogenous loci inconspicuous, occasionally subconspicuous, paracercosporoid, i.e. rim very slightly darkened or thickened, in front view visible as minute circle. Conidia solitary, obclavate-cylindrical, 20–90 × (2–)3–5(–6) μm, (0–)1–8-septate, subhyaline (with a very pale greenish tinge), smooth, apex obtuse, occasionally subacute, base rounded, truncate to obconically truncate, 1.5–3 μm wide, hila unthickened, not darkened.


Host range and distribution: Only known from the type collection.

Note: Pseudocercospora gomphrenicola differs from P. gomphrenae-pulchellae in lacking stromata, and P. globosa has smaller stromata, much longer, septate conidiophores and narrower conidia, only 1.5–3.5 μm wide.

**Doubtful, excluded and insufficiently known species**


Type: India: Maharashtra: Pune, on Gomphrena globosa, P. P. Chiddarwar (otherwise not specified).

Note: Type material of this species was not indicated, but based on the original description and illustration of a species with cicatrized conidiogenous cells and rostrate conidia, Kamal (2010) reduced P. gomphrenicola to synonymy with Alternaria gomphrenae.

**ACKNOWLEDGEMENTS**

We are very grateful to the directors and curators of B, BPI, CUP, DACM, FH, HBG, HMAS, ILL, ILS, IUM, K, L, LE, LEP, LPS, MA, NIAES, NTU, NY, NYS, PAD, PC, PDD, RREM, S, TFM, TNS and WSP for loaning type material and other collections in their keeping during the course of monographic studies of cercosporoid fungi on the hosts of the families treated in part 4.

**REFERENCES**


Braun U, Sivapalan A (1999) *Cercosporoid hyphomycetes from...
Ramula S. Casciano: Stabilimento tipografico Cappalli.
ARTICLE

Cercosporoid fungi 4

**Knoxdaviesia proteae is not the only Knoxdaviesia-symbiont of Protea repens**

Janneke Aylward¹, Léanne L. Dreyer¹, Emma T. Steenkamp², Michael J. Wingfield², and Francois Roets³

¹Department of Botany and Zoology, Stellenbosch University, Private Bag X1, Matieland 7602, South Africa; corresponding author e-mail: janneke@sun.ac.za
²Department of Microbiology and Plant Pathology, University of Pretoria, Pretoria 0002, South Africa
³Department of Conservation Ecology and Entomology, Stellenbosch University, Private Bag X1, Matieland 7602, South Africa

Abstract: Two polyphyletic genera of ophiostomatoid fungi are symbionts of Proteaceae in southern Africa. One of these, *Knoxdaviesia*, includes two closely related species, *K. proteae* and *K. capensis*, that have overlapping geographical distributions, but are not known to share *Protea* host species. *Knoxdaviesia capensis* appears to be a generalist that occupies numerous hosts, but has never been found in *P. repens*, the only known host of *K. proteae*. In this study, extensive collections were made from *P. repens* and isolates were identified using DNA sequence comparisons. This led to the surprising discovery of *K. capensis* from *P. repens* for the first time. The fungus was encountered at a low frequency, suggesting that *P. repens* is not its preferred host, which may explain why it has not previously been found on this plant. The basis for the specialisation of *K. proteae* and *K. capensis* on different *Protea* species remains unknown.

Article info: Submitted: 9 December 2014; Accepted: 28 September 2015; Published: 10 November 2015.

INTRODUCTION

Ophiostomatoid fungi are a polyphyletic assemblage (Spatafora & Blackwell 1994, Wingfield et al. 1999) that share morphological characters such as flask-shaped ascomata with long necks bearing sticky spore droplets, that make them ideally suited for arthropod-mediated dispersal (Wingfield et al. 1993). Species in two genera, *Ophiostoma* and *Knoxdaviesia* (Wingfield et al. 1999), occur in the flower heads (infructescences) of serotinous *Protea* species in southern Africa (Fig. 1). They are not associated with disease symptoms on their hosts but could benefit the plant by excluding harmful fungi from the infructescences that must protect viable seeds for long periods of time (Roets et al. 2013).

The dispersal biology of *Protea*-associated ophiostomatoid fungi is intriguing. The primary vectors are mites (Roets et al. 2011b) that have a mutualistic association with some of the fungi they carry (Roets et al. 2007). These mites can self-disperse to other infructescences on a *Protea* tree, but most often they use beetles as long-distance vectors to reach other *Protea* trees (Aylward et al. 2014a, Roets et al. 2009a). Although the vectors of the *Protea*-associated ophiostomatoid species are the same, the various fungal species display distinct patterns of affinity for their host *Protea* species (Roets et al. 2005, 2011b). For example, the closely related species *K. capensis* and *K. proteae* have overlapping geographic distributions and similar vectors, yet they have never been encountered together on the same *Protea* host (Wingfield et al. 1988, 1999, Wingfield & van Wyk 1993). *Knoxdaviesia proteae* consistently inhabits *P. repens* infructescences and it has not been found in other *Protea* species. In contrast, *K. capensis* occurs in at least eight different *Protea* species including *P. burchelli*, *P. coronata*, *P. laurifolia*, *P. lepidocarpospondron*, *P. longifolia*, *P. magnifica*, *P. nervifolia* and *P. obtusifolia*, but has never been found in *P. repens* (Marais & Wingfield 1994, Roets et al. 2005, 2011a, Wingfield & van Wyk 1993).

The reason for the difference in host specificity between *K. capensis* and *K. proteae* is unknown. One possibility is that this separation prevents inter-specific competition between these fungi, given that they appear to rely on similar nutritional resources and occupy similar niches. Separation through host-exclusivity could, therefore, have been key to reduce competition and promote speciation (Giraud et al. 2008). Inter-species competition could also be avoided through temporal separation (succession) of colonization by ophiostomatoid species (Roets et al. 2013), although there is no evidence to support this view. The apparent host separation in the *Knoxdaviesia* species stands in contrast to some *Protea*-associated *Ophiostoma* species, which often co-occur with *K. capensis* or *K. proteae* in a single infructescence (Roets et al. 2006, 2013).

The host specificities of these *Knoxdaviesia* species are based on numerous randomly made collections of these fungi for taxonomic and biological studies. There has, however, never been a large-scale and systematic survey that would provide confidence in the hypothesis that *K. proteae* is the...
only *Knoxdaviesia* species occurring in *P. repens*. Isolations of *Knoxdaviesia*-like sporing structures were made from infructescences in two natural populations of *P. repens*. These were then used to test the hypothesis that *K. proteae* is the only *Knoxdaviesia* species that colonizes *P. repens* infructescences.

**MATERIALS AND METHODS**

During November 2012 and January 2013, infructescences were sampled from two *Protea repens* populations in the Western Cape Province of South Africa (Table 1) in order to isolate *K. proteae* individuals as part of a previous study (Aylward et al. 2014a, 2015). In the Gouritz population (34.2062°S 21.6812°E), 220 infructescences from the current and 220 from the previous flowering seasons were sampled from 11 different *P. repens* trees (Aylward et al. 2014a). The site at Franschhoek (33.9044°S 19.1566°E) had been burnt in 2007, and was sampled just after the new *P. repens* recruits had flowered for the first time. Some *P. repens* trees at this site (ca 15-yr-old) had escaped the fire and were included in our surveys. At this site, 20 infructescences were collected from 11 plots (20 m diam) in the burnt area and
19 plots in the unburnt area (Aylward et al. 2015). Since the initial aim of the sampling was to collect *K. proteae*, only one *Knoxdaviesia* isolate was maintained per infructescence to prevent repeated isolation of the same individual. Because the sexual morphs of both *K. proteae* and *K. capensis* species are indistinguishable under x30 magnification (Fig. 1), fungal isolations were made from randomly selected sporing structures. *Knoxdaviesia* isolation methods and culturing techniques were as given in Aylward et al. (2014b). Isolates were identified by sequencing the ITS regions of the rDNA (White et al. 1990) as detailed by Aylward et al. (2014b).

Statistical analyses were conducted in R v. 3.1.0 (R Core Team 2014). The number of fungal isolates obtained from infructescences at each sampling site (Gouritz or Franschhoek) and for each subdivision (flowering season or burnt/unburnt area) was recorded and tested for normality with Shapiro-Wilk’s *W* test. Subsequently, a Mann-Whitney U test for independent groups and a Pearson’s Chi-square test was applied to test for significant differences between the numbers of isolates obtained from each infructescence age class (Gouritz population) and between the burnt and unburnt sampling plots (Franschhoek). These tests were chosen because the Mann-Whitney U test takes into account only the number of positive hits (i.e. the presence of the fungus) whereas the Chi-square test also includes the total number of observations (i.e. number of infructescences sampled) (McKillup 2006).

A Maximum Likelihood (ML) phylogenetic tree was constructed in order to illustrate the difference between the species identified in this study. MAFFT 7 (Katoh & Standley 2013) was used to align the ITS sequences of a subset of the isolated individuals to those of previously characterized species of *Gondwanamycetaceae* obtained from GenBank®. The ML tree was computed in MEGA6 (Tamura et al. 2013) under the Tamura-Nei substitution model (Tamura & Nei 1993) and reliability was calculated with 1 000 bootstrap replications.

**RESULTS**

The intensive sampling effort yielded 224 *Knoxdaviesia* isolates – 103 from the Gouritz and 121 from the Franschhoek population. Surprisingly, the ITS data used to identify the isolates (Aylward et al. 2014b) revealed that not all fungal strains collected were *K. proteae*, the only *Knoxdaviesia* species previously known to occur in *P. repens* (Fig. 2). The
closely related *K. capensis* was also encountered, although at a low frequency. Ten *K. capensis* strains were isolated from four of the 11 different *P. repens* plants in the Gouritz population. In Franschhoek, 15 *K. capensis* isolates were found in 10 of the 30 sampling plots, including six from a single plot in which *K. proteae* was not encountered (Table 1). Isolate R7 (CBS 140644) was deposited at the CBS-KNAW Fungal Biodiversity Centre as a representative of *K. capensis* on *P. repens*. The sampling strategy did not enable co-occurrence of the two *Knoxdaviesia* species in one infructescence to be detected.

The Shapiro-Wilk’s W tests for normality rejected the null hypothesis that the number of *K. capensis* isolates sampled from Gouritz (W = 0.60; p = 1.49 × 10^{-4}) and Franschhoek (W = 0.46; p = 2.39 × 10^{-4}) follows a normal distribution. Additionally, the combined dataset of *K. proteae* and *K. capensis* isolates in each population did not conform to a normal distribution (Gouritz: W = 0.84, p = 3.15 × 10^{-6}; Franschhoek: W = 0.74, p = 5.78 × 10^{-9}). Neither the Mann-Whitney U test for independent groups nor the Pearson’s Chi-square test could detect significant differences between the number of *K. capensis* individuals isolated from the burnt and unburnt areas (U = 93, p = 0.56; χ^2 = 0.73, df = 1, p = 0.79). The Pearson’s Chi-square test suggested a marginally significant difference between the number of isolates in the current and previous flowering season’s infructescences (χ^2 = 3.68, df = 1, p = 0.05), but this was not supported by the Mann-Whitney U test (U = 81.5, p = 0.09). Both tests indicated that the total number of *K. capensis* isolates was significantly lower than the number of *K. proteae* isolates obtained from each population (Gouritz: U = 455.5, p = 2.44 × 10^{-7}, χ^2 = 75.75, df = 1, p = 2.2 × 10^{-16}; Franschhoek: U = 732.5, p = 1.02 × 10^{-5}, χ^2 = 75.97, df = 1, p = 0.79, 2.2 × 10^{-16}).

---

**Fig. 2.** Maximum Likelihood phylogenetic tree depicting the position of the two *Knoxdaviesia* species sampled from *Protea repens* infructescences. The final dataset consists of 474 characters. *Knoxdaviesia proteae* sequences are from the studies of Aylward et al. (2014a, 2015) and *K. capensis* sequences were generated in this study. “T” and “ET” indicate type and ex-type strains, respectively.
**Knoxdaviesia symbionts of Protea repens**

**DISCUSSION**

*Knoxdaviesia capensis* has been isolated from numerous serotinous *Protea* species in South Africa (Wingfield & Van Wyk 1993, Roets et al. 2005, 2011a). The geographic distributions of the known *Protea* hosts of *K. capensis* often overlap with that of *P. repens*, the host of *K. proteae* (Wingfield et al. 1988), yet this study presents the first account of *K. capensis* also occurring in *P. repens*. Given that *K. capensis* is a generalist that occupies numerous *Protea* species (Wingfield & van Wyk 1993, Marais & Wingfield 1994, Roets et al. 2005, 2011a), the ability to live in the infructescences of *P. repens* is perhaps not surprising.

The low frequency of *K. capensis* individuals isolated from *P. repens* (9.7 % in Gouritz and 12.4 % in Franschhoek) illustrates the dominance of *K. proteae* in this niche. It also offers an explanation for the previous oversight of *K. capensis* in *P. repens*. This low frequency is also congruent with the suggestion that *P. repens* is not a preferred host of *K. capensis*. In vitro host exclusivity experiments conducted by Roets et al. (2011a) showed that *K. capensis* produces significantly more aerial hyphae on 1.5 % Water Agar (WA) supplemented with *P. repens* material than on WA alone. However, these authors also found that when supplementing nutrient-rich 1.5 % Malt Extract Agar (MEA), *K. capensis* grew significantly better on its natural host, *P. neriifolia*, than on *P. repens*. Indeed, compared to MEA alone, *P. repens* supplemented media “slightly inhibited” the growth of *K. capensis*. These results suggest that although *K. capensis* is able to utilize *P. repens* as a substrate, it is not the preferred host of this species. However, the low level of occurrence of *K. capensis* in *P. repens* is unlikely to be due to inadequate nutrition, but more likely to be attributable to competition between *K. capensis* and other ophiostomatoid species, specifically the most prevalent species, *K. proteae*. Inter-species competition is known to occur between Northern Hemisphere ophiostomatoid fungi associated with the southern pine beetle, where *Ophiostoma minus* consistently out-competes *Ceratocystis ranaculus* (Klepzig & Wilkens 1997). Further investigation of the interactions between *Knoxdaviesia* species in *Protea* are, however, necessary to resolve this question.

An alternative explanation for the dominance of *K. proteae* over *K. capensis* in *P. repens* could be the success of these fungi during initial colonization. The infructescences sampled from the burnt area in the Franschhoek population represent the first flowering season of those plants. Because of the absence of older infructescences, fungi in these new infructescences must have originated from sources outside the population of burnt *P. repens* trees. *Protea neriifolia* trees observed in the vicinity of the burnt area were most likely to be the source of the *K. capensis* colonizers. Where *K. capensis* spores from *P. neriifolia* reach new, uncolonized *P. repens* infructescences, this species is able to grow and sporulate. This is illustrated by our results from the Franschhoek sampling plot that exclusively yielded *K. capensis* (Table 1). However, once *K. proteae* is introduced, it apparently dominates *K. capensis* and reduces the prevalence of that species. However, *K. capensis* individuals were also isolated from mature *P. repens* plants in the unburnt area as well as from new and old infructescences in the Gouritz population. This implies that *K. capensis* can survive in a *P. repens* population even though *K. proteae* is dominant. Statistically, however, this study does not offer support for the premise of succession, since there was no difference in the number of *K. capensis* individuals isolated from infructescences of different ages (Gouritz) or burnt and unburnt areas (Franschhoek). However, the low numbers of *K. capensis* individuals found in this study, preclude us from completely disregarding the possibility that a succession of species could occur.

Roets et al. (2009b) hypothesized that the specificity of ophiostomatoid fungi to different *Protea* species may be more dependent on the vectors associated with the fungi than the specificity of the fungus to the *Protea* host. Results of recent studies (Roets et al. 2011a), including those of the present investigation, suggest that vectors are not a primary factor underlying specificity. *Knoxdaviesia capensis* is clearly capable of growing in *P. repens* infructescences and has the opportunity of being vectored to this suitable habitat. The apparent difference in prevalence of the two *Knoxdaviesia* species in *P. repens* must, therefore, be determined by other factors, the most plausible being interspecies competition. Future studies should consider the timing of colonization, and the interaction between and the potential effects that these *Knoxdaviesia* species may have on each other’s growth.

**ACKNOWLEDGEMENTS**

We thank the Western Cape Nature Conservation Board for supplying the necessary collection permits and the National Research Foundation (NRF) and the Department of Science and Technology (DST)-NRF Centre of Excellence in Tree Health Biotechnology (CTHB) for financial support.

**REFERENCES**


Microcyclic rusts of hollyhock (*Alcea rosea*)

Jill E. Demers1, Megan K. Romberg2, and Lisa A. Castlebury*

1USDA-ARS, Systematic Mycology and Microbiology Laboratory, Beltsville, MD 20705, USA; corresponding author e-mail: Jill.Demers@ARS.USDA.GOV

2USDA-APHIS-PPQ-National Identification Services, Beltsville, MD 20705, USA

Abstract: Rust fungi infecting hollyhock and other plants in *Malvaceae* are frequently intercepted at ports of entry to the USA, particularly *Puccinia malvacearum* and *P. heterogenea*. These two species can be difficult to distinguish and can be further confused with other, less common species of microcyclic rust fungi infecting hollyhock: *P. heterospora*, *P. lobata*, *P. platyspora*, and *P. sherardiana*. Molecular phylogenetic analysis revealed that *P. malvacearum* and *P. heterogenea* are closely related, along with *P. sherardiana* and *P. platyspora*. A key to the six microcyclic *Puccinia* species infecting hollyhock is presented.

**Key words:** *Pucciniales*  
*Puccinia* pathogen interceptions  
identification  
taxonomy

**Article info:** Submitted: 29 September 2015; Accepted: 16 November 2015; Published: 20 November 2015.

**INTRODUCTION**

Plants and plant products imported into the USA are inspected at ports of entry for pests including insects, mollusks, nematodes, bacteria, and fungi (McCullough et al. 2006). Plant pests that are found during inspections may be barred from entering the USA depending on their risk to agriculture and natural ecosystems and whether the pests are already established within the country. Some frequently imported plants include species of *Malvaceae*, the mallow subfamily that includes economically important plants such as cotton (*Gossypium hirsutum*), kenaf (*Hibiscus cannabinus*), okra (*Abelmoschus esculentus*), and many ornamental plants including hollyhock (*Alcea rosea*) (Baum et al. 2004). *A. rosea*, a plant grown worldwide, is often intercepted at ports of entry infected with rust fungi.

Many rust fungi can infect *A. rosea*. Six species of *Puccinia* have been reported to produce telia on *A. rosea*: *P. heterogenea*, *P. heterospora*, *P. lobata*, *P. malvacearum*, *P. platyspora*, and *P. sherardiana* (Arthur 1922, Lindquist 1982, Farr & Rossman 2015). All of these species are microcyclic and do not produce uredinia or aecia, have no known alternate hosts, and produce both one-celled and two-celled teliospores with different spore morphologies predominating in different species (Arthur 1922, Lindquist 1982). Another two species of *Puccinia*, *P. interveniens* and *P. schenedonardi*, are reported to produce aecia on *A. rosea*, and *A. rosea* is also reported to be infected by two other rust fungi, *Endophytium tuberculatum* and *Kuehneola malvicola* (Arthur 1934, Farr & Rossman 2015). Of these fungi, *P. malvacearum* is the most widely reported (Farr & Rossman 2015) and the most frequently intercepted at US borders. *Puccinia malvacearum* has been reported worldwide on a large number of hosts in *Malvaceae*, primarily in tribe *Malveae*, including the genera *Abutilon*, *Alcea*, *Anoda*, *Hibiscus*, *Lavatera*, *Malva*, *Malvastrum*, *Pavonia*, *Sida*, and *Sidalcea* (Farr & Rossman 2015). *Puccinia heterogenea*, although less commonly reported than *P. malvacearum*, has also been intercepted at US ports of entry multiple times. *Puccinia heterogenea* has only been reported from Ecuador and Peru on hosts in *Alcea*, *Malva*, and *Fuertesimalva* (Farr & Rossman 2015) but is morphologically similar to *P. malvacearum* and frequently misidentified.

In this study, *P. malvacearum*, *P. heterogenea*, and the other microcyclic rusts on *A. rosea*, *P. heterospora*, *P. lobata*, *P. platyspora*, and *P. sherardiana*, were characterized morphologically and the LSU rDNA region was sequenced. These data are presented as a guide to identification of the rusts of *A. rosea*. In addition, the phylogenetic placement of these six *Puccinia* species was determined.

**MATERIALS AND METHODS**

Specimens for this study were obtained from interceptions at US borders and from reference material, from the years 1890 to 2015. Morphological features of all specimens were characterized, and measurements were made using Zeiss AxioVision SE64 4.9.1 software (Carl Zeiss Microscopy, Jena). A minimum of 20 spores was examined from each specimen, with multiple specimens examined per species. DNA was extracted from small (approximately 0.5–1 cm²) leaf fragments containing telia using the Qiagen DNeasy Plant Mini kit (Gaithersburg, MD). PCRs were done using the KAPA2G Robust PCR kit (Wilmington,
### Key to the microcyclic rusts of hollyhock

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
<th>Option</th>
<th>Key to the microcyclic rusts of hollyhock</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Teliospores predominately one-celled</td>
<td>2</td>
<td>Puccinia heterospora</td>
</tr>
<tr>
<td></td>
<td>Teliospores predominately two-celled</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Teliospores with apical walls to 13 µm thick</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Teliospores with apical walls to 5 µm thick</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Telia light reddish brown, scattered</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Telia dark brown, in clusters</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Telia light reddish brown, scattered</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Telia dark brown, in clusters</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(Figs 2–3)

**Description:** Telia dark brown, mainly hypophyllous, in tight clusters. Teliospores light brown to reddish-brown, predominantly one-celled. One-celled teliospores globose, highly variable in size, 17–30 × 15–29 µm, on average 21 × 19 µm. Two-celled teliospores globose to oblong, frequently have septa perpendicular to the pedicel attachment point; length of the two-celled teliospores parallel to pedicel attachment point (17–35 µm, on average 22 µm) often shorter than the length of the spores perpendicular to the pedicel attachment point (16–34 µm, on average 20 µm). Side walls of teliospores 1.5–3 µm thick, apical walls 3–7 µm thick. Pedicels hyaline, to 100 µm long.


(Figs 2–3)

**Description:** Telia reddish brown, hypophyllous, scattered. Teliospores yellowish brown, often with apical pores or split walls at the apices, predominately one-celled; some two-celled teliospores with very thin septa or partial septa. **One-celled teliospores** 34–53 × 15–22 μm, on average 42 × 17 μm. **Two-celled teliospores** 34–57 × 13–21 μm, on average 46 × 16 μm. **Side walls of teliospores** 1.5–2 μm thick, apical walls 3–13 μm thick. **Pedicels** hyaline, to 70 μm long.

**Specimens examined:** Argentina: La Falda, on *Sphaeralcea sp.*, 21 Aug. 1922, E. W. D. & M. M. Holway (BPI 091376); Jujuy Province: Tilcara, on *Alcea rosea*, 3 Feb. 1996, J. R. Hernandez (BPI 841116).

**Puccinia heterogenea** Lagerh., *J. Mycol.* **7**: 44 (1891).

(Figs 2–3)

**Description:** Telia dark reddish brown, mainly hypophyllous, scattered. Teliospores yellowish brown, often with apical pores or split walls at the apices; one- and two-celled
spores mixed, one-celled spores predominating in most samples. One-celled teliospores 24–57 × 15–34 µm, on average 32 × 21 µm. Two-celled teliospores 28–57 × 15–34 µm, on average 42 × 24 µm. Side walls of teliospores 2–3 µm thick, apical walls 2–5 µm thick. Pedicels hyaline, to 140 µm long.


**Puccinia malvacearum** Bertero ex Mont., *Hist. fis. y polit.* Chile 8: 43 (1852). (Figs 2–3)

Puccinia lobata Berk. & M. A. Curtis, *Grevillea* 3: 54 (1874). (Figs 2–3)
Microcyclic rusts of hollyhock

**ARTICLE**

**481**

**volume 6 · no. 2**

**Description:** Telia dark brown, hypophyllous, in tight clusters. Teliospores yellowish brown, often with apical pores or split walls at the apices, predominately two-celled. One-celled teliospores 26–31 × 21–25 µm, on average 28 × 24 µm. Two-celled teliospores 25–40 × 16–27 µm, on average 32 × 22 µm. Side walls of teliospores 1.5–3 µm thick, apical walls 5–10 µm thick in one-celled spores and 4–9 µm thick in two-celled spores. Pedicels hyaline, to 120 µm long.

**Specimens examined:** Intercepted specimens (origin unknown):

**Puccinia sherardiana** Körn., *Hedwigia* 16: 19 (1877). (Figs 2–3)

**Description:** Telia dark brown, hypophyllous, in tight clusters. Teliospores light brown, often with apical pores or split walls at the apices, predominately two-celled. One-celled teliospores 34–36 × 18–30 µm, on average 35 × 24 µm. Two-celled teliospores 34–60 × 17–34 µm, on average 47 × 25 µm. Side walls of teliospores 1.5–3 µm thick, apical walls 3–7 µm thick. Pedicels hyaline, to 200 µm long.

**Specimens examined:** Intercepted specimens (origin unknown):

**DISCUSSION**

Multiple rust fungi can infect plants in Malveae, and plants from this tribe imported into the USA are often intercepted with visible rust disease, generally caused by *Puccinia malvacearum* and *P. heterogenea*. *Puccinia malvacearum* has been reported to have a much wider geographical distribution than *P. heterogenea* (Farr & Rossman 2015). The exact origin
of many of the specimens in this study is, however, uncertain because they were intercepted from luggage and cargo and may have been extensively moved during travel and trade. The reported origins of the intercepted \( P. \text{malvacearum} \) specimens include Israel, Mexico, The Netherlands, and Portugal, with additional specimens with verified origins from the USA, agreeing with the reported worldwide distribution of \( P. \text{malvacearum} \). In contrast, all intercepted \( P. \text{heterogenea} \) specimens were reported as originating from Ecuador, except for one reported as from Jamaica, which was possibly not its true origin. \( P. \text{heterogenea} \) has only been previously reported from Ecuador and Peru (Farr & Rossman 2015), and the samples collected in this study suggest that it has not spread further.

\( P. \text{malvacearum} \) has been intercepted on many species of plants and is reported to have a very large host range in \textit{Malvoideae} (Farr & Rossman 2015). This study focused on intercepted rust fungi of \textit{Malveae}, especially \textit{Alcea rosea}, so a comprehensive survey of other possible hosts of \( P. \text{malvacearum} \) was not completed. In this limited sampling, \( P. \text{malvacearum} \) was detected on \textit{A. rosea} and several \textit{Malva} species. \( P. \text{heterogenea} \) is reported on a much smaller host range than \( P. \text{malvacearum} \), only \textit{A. rosea} and various \textit{Malva} species (Farr & Rossman 2015). In this study, \( P. \text{heterogenea} \) was mainly detected on plants identified based on ITS sequences as \textit{Malva arborea}, tree mallow. Tree mallow is considered medicinal (Germplasm Resources Information Network; http://www.ars-grin.gov/cgi-bin/npgs/html/taxon.pl?455281), so it is more likely to be imported and intercepted than other, less ethnobotanically useful, hosts of \( P. \text{heterogenea} \).

The other four microcyclic \textit{Puccinia} species reported on \textit{A. rosea} are \( P. \text{heterospora} \), \( P. \text{lobata} \), \( P. \text{platyspora} \), and \( P. \text{sherardiana} \). Sequencing a representative sample of each confirmed that they are different from both \( P. \text{malvacearum} \) and \( P. \text{heterogenea} \). These rusts are morphologically similar and may be confused with each other, as well as with other \textit{Puccinia} species on mallows that resemble \textit{P. anodae}, \textit{P. exilis}, \textit{P. modiolae}, \textit{P. sidalcae}, and \textit{P. sphaeralceae}. \( P. \text{heterospora} \), \( P. \text{lobata} \), \( P. \text{platyspora} \), and \( P. \text{sherardiana} \) appear to have wide host ranges like \( P. \text{malvacearum} \), as all four were found on hosts not closely related to \textit{A. rosea} (Escobar Garcia et al. 2009): \textit{Sphaeralcea} for \( P. \text{platyspora} \) and \( P. \text{sherardiana} \); \textit{Malvella} for \( P. \text{lobata} \); and \textit{Abutilon}, \textit{Anoda}, and \textit{Sida} for \( P. \text{platyspora} \) – although the high degree of intraspecific variation in \( P. \text{heterospora} \) raises the possibility that it is composed of cryptic species with smaller, more specific host ranges. As with \( P. \text{heterogenea} \), \( P. \text{heterospora} \), \( P. \text{lobata} \), \( P. \text{platyspora} \), and \( P. \text{sherardiana} \) have a smaller reported geographic distribution than \( P. \text{malvacearum} \). \( P. \text{heterospora} \) has been reported worldwide except for Europe, \( P. \text{lobata} \) and \( P. \text{sherardiana} \) have been reported in North and South America, and \( P. \text{platyspora} \) has been reported only in South America (Farr & Rossman 2015). Although \( P. \text{platyspora} \) and \( P. \text{heterogenea} \) have not been reported in the US, and \( P. \text{lobata} \) and \( P. \text{sherardiana} \) have only been reported in the western US, their similarity to \( P. \text{malvacearum} \) suggests that they would not cause more damage than \( P. \text{malvacearum} \) if spread to new areas and that they could be similarly managed. The study of intercepted fungi can provide useful information about their biology, systematics, and long-distance movement in trade.

ACKNOWLEDGEMENTS

We thank Cathie Aime for the use of DNA sequences, the US National Fungus Collections for access to reference material, and Tunesha Phipps for technical support.

REFERENCES


\textit{Malvatheca} (Bombacoideae and Malvaceae; \textit{Malvaceae sensu lato}) as inferred from plastid DNA sequences. \textit{American Journal of Botany} \textbf{91}: 1863–1871.


**Baobabopsis**, a new genus of graminicolous downy mildews from tropical Australia, with an updated key to the genera of downy mildews

Marco Thines¹,²,³, Sabine Telle¹,², Young-Joon Choi¹,²,³, Yu Pei Tan⁵, and Roger G. Shivas⁴

¹Integrative Fungal Research (IPF), Georg-Voigt-Str. 14-16, D-60325 Frankfurt am Main, Germany; corresponding author e-mail: marco.thines@sankenbergn.de
²Biodiversity and Climate Research Centre (BiK-F), Georg-Voigt-Str. 14-16, D-60325 Frankfurt am Main, Germany
³Senckenberg Gesellschaft für Naturkunde, Senckenberganlage 25, D-60325 Frankfurt am Main, Germany
⁴Goethe University, Faculty of Biosciences, Institute of Ecology, Evolution and Diversity, May-von-Laue-Str. 9, D-60483 Frankfurt am Main, Germany
⁵Plant Pathology Herbarium, Department of Agriculture and Fisheries, Ecosciences Precinct, GPO Box 267, Brisbane, Qld 4001, Australia

**Abstract:** So far 19 genera of downy mildews have been described, of which seven are parasitic to grasses. Here, we introduce a new genus, Baobabopsis, to accommodate two distinctive downy mildews, *B. donbarrettii* sp. nov., collected on *Perotis rara* in northern Australia, and *B. enneapogonis* sp. nov., collected on *Enneapogon* spp. in western and central Australia. Baobabopsis donbarrettii produced both oospores and sporangiopores that are morphologically distinct from other downy mildews on grasses. Molecular phylogenetic analyses showed that the two species of *Baobabopsis* occupied an isolated position among the known genera of graminicolous downy mildews. The importance of the *Poaceae* for the evolution of downy mildews is highlighted by the observation that more than a third of the known genera of downy mildews occur on grasses, while more than 90 % of the known species of downy mildews infect eudicots.

**Key words:**
- cox2
- genus key
- nLSU
- phylogeny
- Peronosporaceae
- Poaceae

**INTRODUCTION**

Graminicolous downy mildews (GDM) occur on diverse wild and cultivated grasses, particularly in the semi-arid tropics and subtropics. Seven genera of GDM, namely, *Eraphthora* (Telle & Thines 2012), *Graminivora* (Thines et al. 2006), *Peronosclerospora* (Shirai & Hara 1927), *Poakasthesia* (Thines et al. 2007), *Scleropththora* (Thirumalachar et al. 1953), *Sclerospora* (Schröter 1886), and *Viennotia* (Göker et al. 2003), have been described to date. Only three of these genera, *Peronosclerospora*, *Scleropththora*, and *Sclerospora*, cause significant and widespread economic losses (Kenneth 1981, Jeger et al. 1998, Spencer & Dick 2002). The other GDM genera are known only from a single host plant genus, and, with the exception of *Eraphthora*, seemingly have limited geographical distributions. The genera *Poakasthesia* and *Viennotia* are only known from their type specimens. Several unusual and unidentified downy mildews have been collected on native tropical grasses, in particular on species of *Perotis* and *Enneapogon* that also extend into New Guinea and Malesia (Simon & Alfonso 2011). The aims of this study were to identify the downy mildews on specimens of *Perotis* and *Enneapogon* in BRIP (Plant Pathology Herbarium, Brisbane), and further, to investigate their phylogenetic relationships with other GDM.

**MATERIALS AND METHODS**

**Microscopy**

For morphological characterization, downy mildews were examined directly from infected plant tissue preserved in the fungarium at BRIP. Collection details are provided in the taxonomy section of this manuscript. Specimens were mounted on glass slides in 100 % lactic acid, stained with aniline blue (sporangia and sporangiopores only), and gently heated prior to microscopic examination. Ranges were expressed as either min. – max. or as (min.–) mean minus SD – mean – mean plus SD (–max.), depending on the amount of measurements done, with values rounded to 0.5 μm. Images were captured with a Leica DFC 500 camera attached to a Leica DM5500B compound microscope with Nomarski differential interference contrast.

**DNA extraction, PCR-amplification, sequencing and phylogenetic analysis**

Specimens for which new sequence data were obtained in this study are listed in Table 1. DNA was extracted and PCR for *cox2* mtDNA was performed as described in Telle et al. (2011). PCR for the D1-D3 region of the LSU nrDNA was done as described in Choi et al. (2011). Amplicons were sequenced by the sequencing laboratory at the Biodiversity and Climate Research Centre (BiK-F), Frankfurt am Main, for any reuse or distribution, you must make clear to others the license terms of this work, which can be found at http://creativecommons.org/licenses/by-nc-nd/3.0/legalcode. Any of the above conditions can be waived if you get permission from the copyright holder. Nothing in this license impairs or restricts the author’s moral rights.
Germany, with the primers used for PCR. Sequences were edited for bad quality bases and trimmed and assembled in contigs using Geneious v. 5.0 (Biomatters, Newark, NJ). Sequences were aligned using MAFFT (Katoh & Standley 2013), employing the Q-INS-i algorithm (Katoh & Toh 2008). Leading and trailing gaps were removed, and no manual adjustments of the alignment were made in order to prevent bias. Phylogenetic analyses were performed with MEGA v. 6.0 (Tamura et al. 2013) for Minimum Evolution inference, with all parameters set to default, except for using the Tamura-Nei substitution model and performing 1000 bootstrap replicates. For Bayesian Inference MrBayes (Huelsenbeck et al. 2001) as implemented in the graphical user interface siMBa (Mishra & Thines 2014) was used with default settings, except for running 10 M generations, sampling every 10 000th tree of which the first 25 % were discarded before calculating posterior probabilities. Maximum Likelihood analyses were done using RAxML (Stamatakis 2006, Stamatakis et al. 2008) v. 7, using the GTR+GAMMA algorithm and performing 1000 bootstrap replicates. Alignments are deposited in TreeBASE, study accession number S11476.

RESULTS

Morphology
The members of the new genus *Baobabopsis* exhibit several characteristics typical for GDM with evanescent sporangia (Fig. 1), including thin-walled sporangiophores and sporangia and thick-walled oospores. Unlike any known downy mildew genus, the ultimate branchlets are ampulliform in *Baobabopsis*. *Baobabopsis* further differs from other GDM in having stout unbranched sporangiophores.

Molecular phylogeny
Alignments contained 495 sites in case of cox2 (no gaps) and 879 sites in case of nrLSU (4 sites with gaps). Molecular phylogenetic analyses based on LSU nrDNA (Fig. 2) and cox2 mtDNA (Fig. 3) sequences place the new genus, *Baobabopsis*, among the GDM with evanescent sporangiophores. In the cox2-based tree *Baobabopsis* is sister to the genus *Sclerospora*, and in the nrLSU-based tree *Baobabopsis* is sister to a clade containing *Sclerospora* and *Peronosclerospora*, although with weak support in both cases. Further, the monophyly of all genera of GDM is inferred with high to maximum support. Apart from the high support for the grouping of *Eraphthora* and *Sclerophthora*, there was only weak support for other GDM relationships. As the trees from Minimum Evolution, Maximum Likelihood, and Bayesian phylogenetic reconstructions revealed no conflicting topologies with high support, only the Minimum Evolution trees are given (Figs 2 and 3), with support values from the other analyses.

TAXONOMY

*Baobabopsis* R.G. Shivas, Y.P. Tan, Telle & Thines, gen. nov.
MycoBank MB813445 (Fig. 1E)

*Etymology* – Named after the Australian baobab (also termed boab) tree (*Adansonia gregorii*), which has a trunk and branches that magnify the gross morphology of the sporangiophores. Coincidently, this fungus was found on its host plant, *Perotis rara*, in the shade of a baobab tree.

*Diagnosis*: Differs from all other genera of *Peronosporaceae* in having broad club-shaped to cylindrical sporangiophores with a cluster of terminal ampulliform projections bearing sporangia.
**Type species:** *Baobabopsis donbarrettii*

**Description:** Straminipila, Oomycota, Peronosporales, Peronosporaceae. Sporangiophores cover lower leaf surfaces, evanescent, aseptate, hyaline, cylindrical, 75–120 µm long, 20–28 µm wide, unbranched, with 5–20 ampulliform to lageniform ultimate branchlets. Sporangia hyaline, deciduous. Oogonia and oospores in leaves. Oogonia golden yellow, subglobose, 27–45 × 25–39 µm; wall (exosporium includes warts) 3–11 µm thick, uneven, verrucose with rounded warts. Oospores one per oogonium, pale to golden yellow, globose to broadly ellipsoidal, 19–29 × 18–28 µm; wall (endosporium) 1–3 µm thick, even, smooth.

**Baobabopsis donbarrettii** R.G. Shivas, Y.P. Tan & Thines, sp. nov.
MycoBank MB813446
(Fig. 1A–D)

**Etymology:** Named after Donald Barrett, former Dean of the Faculty of Arts, University of Queensland and scholar in Classics and Ancient History, in appreciation for generously providing many Latin translations for new Australian fungal species.

**Diagnosis:** Differs from *Baobabopsis enneapogonis* in parasitizing *Perotis rara*, and has densely verrucose oogonial walls.

**Type:** Australia: Western Australia: Kununurra, truck stop near Lake Kununurra, alt. about 45 m, on *Perotis rara*, 19 Apr. 2011, R.G. Shivas & T.Y. Chi (BRIP 54675 – holotype; sequences ex-type GenBank KT248945, cox2 mtDNA, KT248948, nrLSU).

**Description:** Sporangiophores cover lower leaf surfaces, evanescent, aseptate, hyaline, cylindrical, 75–120 × 20–28 (n = 5) µm, with 5–20 terminal ampulliform to lageniform branches with a narrow neck 7–14 × 3–7 (n = 10) µm. Sporangia hyaline, deciduous, broadly ellipsoid, 16–20 × 11–18 (n = 10) µm. Oogonia golden yellow, subglobose, (27–)32.5–36.0–39.5(–45) × (25–) 28–31.7–36 (–39) (n = 22) µm diam; wall (exosporium includes warts) 3–9 (n = 20) µm thick, even, densely verrucose with rounded warts. Oospores one per oogonium, subhyaline to golden yellow, globose to broadly ellipsoidal, (19–)22–24.1–
Fig. 2. Phylogenetic tree (Minimum Evolution), based on nrLSU sequences. Numbers on branches denote support from Minimum Evolution, Maximum Likelihood and Bayesian analyses, in the respective order. - = no support for the displayed or an alternate topology.

27(–29) × (18–) 20–22.5–25(–28) (n = 22) μm diam; wall (endosporium) 1–3 μm thick, even, smooth.

**Habitat**: In living plants of *Perotis rara*, Australia.

*Baobabopsis enneapogonis* R.G. Shivas, Y.P. Tan, Telle & Thines, sp. nov.

Mycobank MB813447 (Fig. 1E)

**Etymology**: Named after the host genus *Enneapogon* (*Poaceae*).

**Diagnosis**: Differs from *Baobabopsis donbarrettii* in parasitizing *Enneapogon* spp., and in having slightly less prominent warts and moderately verrucose oogonial walls.

**Type**: Australia: Northern Territory; East MacDonnell Ranges, near turnoff to Corroboree Rock, on *Enneapogon*...
Fig. 3. Phylogenetic tree (Minimum Evolution), based on cox2 sequences. Numbers on branches denote support from Minimum Evolution, Maximum Likelihood and Bayesian analyses, in the respective order. - = no support for the displayed or an alternate topology, x = support for an alternate topology.
Key to the genera of downy mildews

1. Sporangiophores unbranched (hyphal, club-shaped or cylindrical) .............................................................. 2
   Sporangiophores branched multiple times .............................................................. 6

2 (1). Sporangiophores hyphal, undifferentiated; oospores 30–80 µm diam ...................................................... Sclerophthora

3 (2). Sporangiophores persistent .............................................................................................................. 4
   Sporangiophores evanescent .............................................................................................................. 5

4 (3). Sporangiophores stout, immersed in the stomata, ultimate branchlets short, stout, and hardly differentiated

        Sporangiophores slender, with an enlarged apex bearing sporangia on well-differentiated ultimate branchlets

        .................................................................................................................................................. 7
   Benua

5 (3). Sporangiophores less than 60 µm in length, club-shaped, sporangia irregular lemon-shaped, tapering towards

        apex and pedicel ......................................................................................................................... Eraphthora
   Sporangiophores more than 60 µm in length, broadly club-shaped to cylindrical, sporangia obovoid to oval

        .................................................................................................................................................. 8
   Baobabopsis

6 (1). Sporangiophores evanescent .............................................................................................................. 7
   Sporangiophores persistent .............................................................................................................. 8

7 (6). Sporangia produce zoospores; with an operculum ............................................................................. Sclerospora
   Sporangia germinate with a germ-tube; without an operculum ....................................................... Peronosclerospora

8 (6). Haustoria small, globose to pyriform .............................................................................................. 9
   Haustoria large, digitate, globose, lobate or irregular ....................................................................... 15

9 (8). Sporangiophores stout and irregularly branched, pedicels wider than 2 µm, broadening widely toward the apex

       Sporangiophores slender, mostly regularly branched, pedicels less than 2 µm wide, not or only slightly

       broadening toward the apex ....................................................................................................... 10
   Plasmoverna

10 (9). Branching monopodial; branches arising at almost rectangular angles ................................................ Plasmopara
   Branching sub-monopodial; branches not arising at rectangular angles ........................................ 11

11 (10). Sporangia aggregated at slightly to conspicuously widened terminal branches ................................ 12
   Sporangia not aggregated, usually 2–3 on the pedicels at the terminal branches ........................... 14

12 (11). Branching of the sporangiophores often subdichotomous, aggregation regular, end of terminal branches strongly

       widened, bearing 4–6 sporangia ......................................................................................... Bremia
   Branching of the sporangiophores usually sub-monopodial to monopodial, end of branches not strongly

       widened, aggregation of the sporangia irregular ........................................................................ 13

Description: Asexual morph not seen. Oogonia and oospores in leaves that split into tangled vascular strands up to 10 cm long. Oogonia golden yellow, subglobose, (30–)32.5–36.3–40(–42) × (29–)30–33.1–36(–39) (n = 20) µm diam; wall (exosporium includes warts) 3–11 (n = 20) µm thick, uneven, moderately verrucose with rounded warts, often with remnants of antheridium attached. Oospores one per oogonium, pale to golden yellow, globose to broadly ellipsoidal, (20–)21.3–23.0–24.7(–26) × (19–)20.5–21.9–23.5(–24) (n = 20) µm diam; wall (endosporium) 1–1.5 (–2) µm thick, even, smooth.

Habitat: In living plants of Enneapogon avenaceus and E. cylindricus, Australia.

DISCUSSION

The majority of downy mildews are pathogenic to eudicots, encompassing more than 700 described species in 19 genera (Dick 2002, Thines 2014). Six of these genera are host-specific to a single eudicot plant family, namely, Basidiophora, Benua, Brevia, and Paraperonospora on Asteraceae; Plasmovema on Ranunculaceae; and Perofascia on Brassicaceae. Other eudicot-infecting genera have hosts in more than one plant family. During the past decade it has been established that none of the eudicot-infecting genera contain species that infect monocots, the exceptions being two Peronospora species (Constantinescu 1991, Dick 2002, Göker et al. 2003, Voglmayr 2003). Alliaceae and Poaceae are the only monocot families infected by downy mildews. The GDM contain less than 30 species, but exhibit a high genetic and morphological diversity (Göker et al. 2003, Thines et al. 2006, 2007, 2008, Telle & Thines 2012). Baobabopsis is the eighth known genus of GDM.

In multigene phylogenetic reconstructions, GDM generally occupy basal positions (Göker et al. 2003, 2007, Thines et al. 2009). However the phylogenetic relationships amongst the GDM or with the three major eudicot monophyletic groups that contain all eudicot downy mildews remains unclear (Thines et al. 2009, Thines et al. 2014). Phylogenetic studies have shown that the oomycete genus Phytophthora is paraphyletic with respect to the downy mildews (Cooke et al. 2000, Göker et al. 2007, Thines et al. 2009, Runge et al. 2011). However, recent phylogenomic studies inferred a monophyly for the four species of Phytophthora that were studied (Matari & Blair 2014, or a polyphyly of the downy mildews (Sharma et al. 2015). Further studies with more taxa of downy mildew and Phytophthora are warranted to ascertain the degree of paraphyly for Phytophthora. Morphological studies also support a close link between the downy mildews and Phytophthora, as the basal GDM genera exhibit traits that are reminiscent of Phytophthora species (Thines 2009), for example: repeated outgrowth after sporangium dehiscence in Viennotia (Göker et al. 2003, Thines et al. 2009); the presence of intracellular mycelium in Poakatesthia (Thines et al. 2007); and the hyphal sporangiophores with limoniform sporangia in Sclerophthora (Thirumalachar et al. 1953, Telle & Thines 2012). This led Thines et al. (2009) to speculate that the root of downy mildew evolution might be in hosts of Poaceae. Interestingly, some monocot-infecting Phytophthora species are not easily cultivated and the Phytophthora species on Cyperaceae, which have not yet been included in detailed phylogenetic analyses, seem to be obligate biotrophs (Erwin & Ribeiro 1996). For the latter, the genus Kawakamia was introduced (Miyabe & Kawakami 1903), but not widely accepted, especially as the genus became heterogeneous after some additional species of Phytophthora were transferred to it (Sawada 1942, Sawada 1943). It is unclear if Kawakamia should be considered a member of the downy mildews and thereby represents a link between Phytophthora and the GDM. The eight genera of GDM exhibit a morphological diversity greater than that of the eudicot-infecting genera. Baobabopsis is morphologically unlike any other downy mildew, with broad cylindrical sporangiophores and ampulliform ultimate branchlets. Although the highest diversity of GDM seems to be in eastern Asia and Australia, two of the three genera of GDM with persistent sporangiophores are only known from Africa. More detailed investigations including multigene phylogenies of Phytophthora and downy mildews on native grasses, particularly from Australia and Africa, are needed to clarify if the evolutionary origins of the downy mildews are in Poaceae and to provide insights into how and from where these pathogens diversified.
ACKNOWLEDGEMENTS

The present study was financially supported by the research funding programme ‘LOEWE – Landes-Offensive zur Entwicklung Wissenschaftlich-ökonomischer Exzellenz’ of the Ministry of Higher Education, Research, and the Arts of Hesse in the framework of IPF.

Author contributions: conceived the study RGS and MT; performed microscopy RGS; performed sequencing ST and YPT; analysed the data MT and YPT; wrote the manuscript MT and RGS, with contributions from all other authors.

REFERENCES


Baobabopsis, a new genus of downy mildews

based on cox2 sequence data. *Mycological Research* **112**: 345–351.


IMA Genome-F 5

Draft genome sequences of Ceratocystis eucalypticola, Chrysoporthe cubensis, C. deuterocubensis, Davidsoniella virescens, Fusarium temperatum, Graphilbum fragrans, Penicillium nordicum, and Thielaviopsis musarum

Brenda D. Wingfield1, Irene Barnes1, Z. Wilhelm de Beer2, Lieschen De Vos1, Tuan A. Duong1, Aquillah M. Kanzi1, Kershney Naidoo1, Hai D.T. Nguyen3, Quentin C. Santana1, Mohammad Sayari1, Keith A. Seifert1, Emma T. Steenkamp2, Conrad Trollip1, Nicolaas A. van der Merwe1, Magriet A. van der Nest1, P. Markus Wilken1, and Michael J. Wingfield1

1Department of Genetics, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Private Bag X20, Pretoria, 0028 South Africa; corresponding author e-mail: brenda.wingfield@fabi.up.ac.za
2Department of Microbiology and Plant Pathology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Private Bag X20, Pretoria, 0028 South Africa
3Biodiversity (Mycology), Ottawa Research and Development Centre, Agriculture and Agri-Food Canada, 960 Carling Avenue, Ottawa, Ontario, K1A 0C6, Canada

Abstract: The genomes of Ceratocystis eucalypticola, Chrysoporthe cubensis, Chrysoporthe deuterocubensis, Davidsoniella virescens, Fusarium temperatum, Graphilbum fragrans, Penicillium nordicum and Thielaviopsis musarum are presented in this genome announcement. These seven genomes are from plant pathogens and otherwise economically important fungal species. The genome sizes range from 28 Mb in the case of T. musarum to 45 Mb for Fusarium temperatum. These genomes include the first reports of genomes for the genera Davidsoniella, Graphilbum and Thielaviopsis. The availability of these genome data will provide opportunities to resolve longstanding questions regarding the taxonomy of species in these genera. In addition these genome sequences through comparative studies with closely related organisms will increase our understanding of how these pathogens cause disease.

Key words: Ceratocystis wilt, Chrysoporthe canker, conifer-infesting beetles, Davidsoniella sapstreak disease, mycotoxins in maize, mycotoxin ochratoxin A, stem-end rot of banana

Article info: Submitted: 13 August 2015; Accepted: 23 November 2015; Published: 1 December 2015.

IMA Genome-F 5A

Draft genome sequence of Ceratocystis eucalypticola

Many species of Ceratocystidaceae have been studied extensively due to their significance as pathogens of agricultural forestry crops (Roux & Wingfield 2009), as well as their impact on natural woody ecosystems (Roux et al. 2007, Lee et al. 2015). The family includes eight genera accommodating more than 80 phylogenetically closely related but often morphologically similar species (van Wyk et al. 2013, de Beer et al. 2014, Mayers et al. 2015). These genera, as defined by de Beer et al. (2014), are clearly delimited based on a combination of phylogenetic inference, morphology, and in some cases distinct ecological partitioning. For example, the genus Huntiella accommodates species that are saprobes, whereas most species of Ceratocystis are pathogens of angiosperms.

Species of Ceratocystis include important pathogens of trees propagated as non-natives in plantations in the tropics and Southern Hemisphere (Roux & Wingfield 2013, Wingfield et al. 2013), including Eucalyptus (Laia et al. 1999, Roux et al. 2000, Roux et al. 2001, Barnes et al. 2003). Isolates of Ceratocystis from Eucalyptus in South Africa, related to those known to kill trees in these plantations, were described as the new species C. eucalypticola (van Wyk et al. 2012). The taxonomy of this species and some of its relatives remains open to debate (Fourie et al. 2015, Oliveira et al. 2015) and there is a clear need to gain a deeper understanding of species boundaries as well as issues relating to its biology and ecology.

The aim of this study was to sequence the genome of C. eucalypticola in order to allow for genomic analysis and comparisons with already available genomes from other Ceratocystidaceae (Wilken et al. 2013, van der Nest et al. 2014a, b). These comparisons, coupled with phylogenomic studies, will be useful in resolving the taxonomic debates ongoing in Ceratocystis. Additionally, these resources will provide a platform to characterise factors associated with pathogenicity and fungal ecological strategy, as well as provide an opportunity to study the evolution of these traits within a family of closely related fungi.
**NUCLEOTIDE SEQUENCE ACCESSION NUMBER**

The draft genome sequence of *Ceratocystis eucalypticola* (CMW9998) has been deposited at DDBJ/EMBL/GenBank with the accession number LJOA00000000. Here we describe version LJOA01000000.

**METHODS**

Genomic DNA of *Ceratocystis eucalypticola* isolate CMW 9998 was sequenced using the Illumina HiSeq 2000 platform at the UC Davis Genome Centre, University of California, Davis (CA). Two libraries with medium insert sizes of 350 bp and 530 bp were used to generate pair-end sequences with read lengths of approximately 100 bases. CLC Genomics Workbench v. 7.5.1 (CLCBio, Aarhus, Denmark) was used to analyse the NGS-data, as well as to perform a de novo assembly. Reads of low quality (P error limit of 0.05) and/or terminal nucleotides were trimmed, with the remaining reads being retained for assembly. De novo genome assembly was performed with a word size of 64, and a bubble size of 100 bp. The raw reads were mapped back to the contigs in order to perform scaffolding, with an estimated paired distance ranging from 147 to 654 bp. The completeness of the assembled genome was assessed using the Benchmarking Universal Single-Copy Orthologs tool, BUSCO (Software v. 1.1b1 of May 2015) (Simão et al. 2015). BUSCO was performed on all contigs >1 kb, making use of the fungal lineage dataset. Lastly, the assembly was submitted to AUGUSTUS (Stanke et al. 2004) in order to predict putative open reading frames (ORFs) using the gene models of *Fusarium graminearum*.

**RESULTS AND DISCUSSION**

The draft genome of *Ceratocystis eucalypticola* had an estimated size of 31,260,284 bases, with an N50 of 116,489 and an average coverage of 80x. The de novo assembly generated a total of 2129 contigs, of which 961 were longer than 1 kb. The average scaffold length was 14,676 bases, with the largest scaffold being 726,305 bases in size. The GC content of the assembly was 47.9%. BUSCO analysis defined the genome as 97% complete with 1408 single-copy orthologs present, while 92 BUSCO orthologs were found to be duplicated. Only 30 BUSCO orthologs were missing or fragmented out of the possible 1438 groups searched from the fungal lineage dataset. Gene prediction resulted in a total of 7353 putative ORFs, at a gene density of approximately 235 ORFs/Mb.

The assembled genome of *C. eucalypticola*, with a size of approximately 31.2 Mb and 7353 ORFs, closely resembled those of other sequenced *Ceratocystis* spp. (Wilken et al. 2013, van der Nest et al. 2014a, b). The fungus had a genome size most similar to that of *C. fimbrata* (29.4 Mb, 7266 ORFs) and *C. manguineans* (31.7 Mb, 7494 ORFs), while the *C. abilundus* genome is slightly smaller (27.2 Mb) with only 6967 genes predicted. The genome size statistics for *Ceratocystis* are similar to those found in the genus *Huntiella*, with *H. omanensis* and *H. moniliformis* being 31.5 Mb and 25 Mb, respectively (van der Nest et al. 2014a, b). Gene predictions for *Huntiella* showed a slightly higher gene density when compared with those of *Ceratocystis* (243 ORFs/Mb on average), with *H. omanensis* having a density of 266 ORFs/Mb and *H. moniliformis* having 280 ORFs/Mb, respectively.

The availability of these resources will provide opportunities to answer questions regarding the similarities and differences seen in this genus. The genome data for *Ceratocystis* s. str. is particularly useful for exploring the species boundaries through phylogenomic analysis. This, in combination with genomic comparisons to other species within *Ceratocystidaceae*, will lead to a better understanding of the evolution of pathogenicity and other life history traits.

**Authors:** C. Trollip*, T.A. Duong, M.A. van der Nest, I. Barnes, M.J. Wingfield, and B.D. Wingfield

*Contact: Conrad.Trollip@fabi.up.ac.za*

**IMA Genome-F 5B**

Draft genome sequences of *Chrysoporthe cubensis* and *C. deuterocubensis*, causal agents of Eucalyptus canker

Fungi in the genus *Chrysoporthe* are economically important pathogens of plantation grown *Eucalyptus* spp. and other members of Myrtales (Gryzenhout et al. 2004). These fungi cause serious stem canker disease, referred to as Chrysoporthe canker (Gryzenhout et al. 2004), and are predominantly found in tropical and subtropical parts of the world where conditions favour their growth (Alfenas et al. 1982). Although Chrysoporthe canker has been successfully managed through propagation of disease resistant clones, it is still considered a threat since it can lead to substantial economic losses where resistance breeding is not in place (Wingfield 2003).

There are eight described species of *Chrysoporthe*, including *C. cubensis* (Hodges et al. 1976, 1979, Rodas et al. 2005), *C. doradensis* (Gryzenhout et al. 2005), *C. inopina* (Gryzenhout et al. 2006), and *C. hodgesiana* (Gryzenhout et al. 2004) which occur in South and Central America. *Chrysoporthe deuterocubensis* is primarily found in Southeast Asia, although introductions to Australia, China, Hawaii, and parts of East Africa have been suggested (Myburg et al. 2002, Nakabonge et al. 2006, van der Merwe et al. 2010). *Chrysoporthe zambiensis* and *C. syzygicola* are found in Zambia (Chungu et al. 2010), while *C. austroafricana* is found only in southern Africa (Wingfield et al. 1989, Gryzenhout et al. 2004).
Chrysoporthe cubensis, C. deuterocubensis, and C. austroafricana have been isolated from native trees, suggesting that these fungi might be native to the regions where the trees are found (Myburg et al. 2003, Rodas et al. 2005, Heath et al. 2006). Interestingly, despite the distinct geographical distribution, these species seem to be closely related (Chungu et al. 2010, van der Merwe et al. 2010). Unfortunately, there is limited available information regarding the evolution of Chrysoporthe species.

The genome of C. austroafricana was recently sequenced and released in the public domain (Wingfield et al. 2015). This is the only whole genome sequence resource available for the genus Chrysoporthe. Additional genomic resources could enhance further understanding of the biology of this assemblage of fungi, through genome-wide comparisons. The aim of this study was thus to sequence the genomes of C. cubensis (isolate CMW 10028) and C. deuterocubensis (isolate CMW 8650).

### Resequenced Strains


### Nucleotide Sequence Accession Number

The Chrysoporthe cubensis isolate number CMW 10028 and C. deuterocubensis isolate CMW 8650 Whole Genome Shotgun projects were deposited in GenBank with accession numbers LJCY00000000 and LJDD00000000, respectively. The version described here is LJCY000000000 and LJDD000000000 for C. cubensis and C. deuterocubensis, respectively.

### Materials and Methods

Genomic DNA was extracted using a modified protocol (Steenkamp et al. 1999) from isolate CMW 10028 (Chrysoporthe cubensis) and CMW 8650 (C. deuterocubensis) mycelium obtained from 7-d-old fungal cultures. The Illumina MiSeq paired-end sequencing protocol at the Agricultural Research Council (ARC, South Africa) was used to obtain whole genome sequence data. To assemble the paired-end MiSeq sequences, CLC Genomics Workbench v. 7.5.1 (CLCBio, Aarhus, Denmark) was used. The assemblies were subsequently scaffolded using SSPACE v. 2.0 (Boetzer et al. 2011), which included unused MiSeq reads from the CLC Genomics Workbench assembly. The AUGUSTUS (Stanke & Morgenstern 2005) protein coding gene prediction software was used for de novo annotation of protein coding gene models using Neurospora crassa and Fusarium graminearum as references. Genome completeness was assed using BUSCO (Benchmarking Universal Single-Copy Orthologs) which utilizes single-copy orthologs to predict genome completeness (Simão et al. 2015).

### Results and Discussion

The approximate size of the Chrysoporthe cubensis genome was 42 624 564 base pairs (bp) including gaps, while the C. deuterocubensis assembly was 43 969 123 bp in size. These figures were calculated from 989 and 2 599 scaffolds for C. cubensis and C. deuterocubensis, respectively. From the AUGUSTUS analysis, 12 435 gene models were predicted from the C. cubensis genome, while 13 098 gene models where predicted in the C. deuterocubensis genome. Despite the differences observed in the assembly statistics, the CEGMA analysis for genome completeness in both C. cubensis and C. deuterocubensis was predicted at 95.16 %.

Compared to the closely related C. austroafricana genome, that of C. cubensis was slightly smaller, while the C. deuterocubensis genome was slightly larger. Similarly, C. cubensis had fewer predicted gene models than either C. austroafricana or C. deuterocubensis (Table 1). In terms of gene content, the Chrysoporthe spp. genomes were slightly larger than that of the distantly related Cryphonectria parasitica (43.9 Mb, 11 184 gene models) (http://genome.jgi.doe.gov/Crypa2/Crypa2.info.html) although the genome sizes were relatively close, and the model filamentous fungi, Neurospora crassa (39.9 Mb, 10 082 gene models) (Galagan et al. 2003) and Magnaporthe grisea (40.3 Mb, 11 109 gene models) (Dean et al. 2005).

The significance of differences observed in genome size and the number of predicted genes among the Chrysoporthe species is not known. However, it might be speculated that their geographical distribution could have played a role in the evolution of these genomes. The availability of these genomes will make it possible to answer such phylogeographic
Table 2. Summary of whole nuclear genome DNA sequence assembly statistics (Wilken et al. 2013, van der Nest et al. 2014a, b, Belbahri 2015, Wingfield et al. 2015).

<table>
<thead>
<tr>
<th>Species</th>
<th>Ceratocystis fimbriata</th>
<th>Huntiella moniliformis</th>
<th>Ceratocystis manginecans</th>
<th>Ceratocystis albifundus</th>
<th>Huntiella omanensis</th>
<th>Thielaviopsis punctulata</th>
<th>Ceratocystis platani</th>
<th>Thielaviopsis musarum</th>
<th>Ceratocystis eucalypticola</th>
<th>Davidsoniella virescens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accession Numbers</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Genome size</td>
<td>29 410 862</td>
<td>25 429 610</td>
<td>31 706 104</td>
<td>27 149 029</td>
<td>31 502 652</td>
<td>28 117 660</td>
<td>29 181 400</td>
<td>28 493 324</td>
<td>31 260 284</td>
<td>33 645 160</td>
</tr>
<tr>
<td>Genome Coverage</td>
<td>20x</td>
<td>38x</td>
<td>22x</td>
<td>24x</td>
<td>9x</td>
<td>75x</td>
<td>655x</td>
<td>95x</td>
<td>80x</td>
<td>82x</td>
</tr>
<tr>
<td>N50 contig size</td>
<td>42 879</td>
<td>191 280</td>
<td>77 070</td>
<td>58 335</td>
<td>41 324</td>
<td>18 915</td>
<td>77 580</td>
<td>103 017</td>
<td>116 489</td>
<td>118 189</td>
</tr>
<tr>
<td>GC content</td>
<td>48.065</td>
<td>48</td>
<td>47.9</td>
<td>48.6</td>
<td>47.6</td>
<td>48.3</td>
<td>48.2</td>
<td>49.17</td>
<td>47.9</td>
<td>44.50</td>
</tr>
<tr>
<td>Genome completeness (%)</td>
<td>96.77(^1)</td>
<td>96.4(^1)</td>
<td>96.4(^1)</td>
<td>90.83(^{completed genes})</td>
<td>98.38(^1)</td>
<td>97(^2)</td>
<td>96(^2)</td>
<td>97(^2)</td>
<td>97(^2)</td>
<td></td>
</tr>
<tr>
<td>Assembly software</td>
<td>Newbler</td>
<td>AbySS</td>
<td>Velvet</td>
<td>Velvet</td>
<td>SPAdes</td>
<td>CLC Genomics</td>
<td>Velvet</td>
<td>CLC Genomics</td>
<td>CLC Genomics</td>
<td></td>
</tr>
<tr>
<td>Number of Contigs</td>
<td>3688</td>
<td>680</td>
<td>2234</td>
<td>1958</td>
<td>8127</td>
<td>3751</td>
<td>1213</td>
<td>672</td>
<td>2129</td>
<td>1500</td>
</tr>
<tr>
<td>Retained contigs</td>
<td>2641(^a)</td>
<td>365(^a)</td>
<td>980(^a)</td>
<td>939(^b)</td>
<td>1638(^a)</td>
<td>2379(^a)</td>
<td>1213(^b)</td>
<td>541(^b)</td>
<td>96(^b)</td>
<td>561(^b)</td>
</tr>
<tr>
<td>ORFs</td>
<td>7266</td>
<td>6832</td>
<td>7494</td>
<td>6967</td>
<td>8359</td>
<td>5480</td>
<td>5963</td>
<td>6963</td>
<td>7353</td>
<td>6953</td>
</tr>
<tr>
<td>Gene density (ORFs/Mb)</td>
<td>246</td>
<td>269</td>
<td>236</td>
<td>257</td>
<td>266</td>
<td>195</td>
<td>204</td>
<td>244</td>
<td>235</td>
<td>207</td>
</tr>
</tbody>
</table>

\(^1\) CEGMA completeness score (Parra et al. 2007).

\(^2\) BUSCO completeness score.

\(^a\) contigs greater than 500 nucleotides.

\(^b\) contigs greater than 1000 nucleotide.
questions and will aid in addressing questions relating to the biology of Chrysoporthe species.

**IMA Genome – F5C**

**Draft nuclear genome sequence for Davidsoniella virescens, the causal agent of sapstreak disease in hardwood trees**

The newly recognized genus Davidsoniella (de Beer et al. 2014) includes species previously accommodated in the Ceratocystis coerulescens s. lat. clade. Davidsoniella virescens is a tree pathogen that infects hardwood trees such as Acer saccharum (sugar maple) in eastern North America (Davidson 1944). This fungus is highly pathogenic to sugar maple in plantations, where it feeds on the sugars and other carbohydrates in the wood of the trees (Bal et al. 2013). The disease caused by D. virescens is commonly referred to as sapstreak and the fungus affects the internal wood chemistry where it has been implicated in the production of volatiles that can enhance the growth of other fungi (Wargo & Harrington 1991).

The aim of this study was to assemble a draft nuclear genome sequence of D. virescens, which would ultimately allow for comparative studies with other sequenced genomes in Ceratocystidaceae. The genomes of seven other species of Ceratocystidaceae are publically available to aid with such a comparative analyses. These include Ceratocystis fimбриata, a pathogen affecting sweet potatoes (Wilken et al. 2013); the canker and wilt disease causing C. albifundus, occurring on Acacia mearnsii trees (van der Nest et al. 2014a); the mango wilt pathogen, C. manginecans (van der Nest et al. 2014b); species in the related genus Huntiella, H. moniliformis and H. omanensis, saprobic fungi usually found on freshly cut or wounded logs (van der Nest et al. 2014a), the causal agent of black scorch disease in date palms, Thielaviopsis punctulata (Wingfield et al. 2015) and the plane tree pathogen C. platani (Belbahri 2015). Two additional Ceratocystidaceae genomes, those for C. eucalypticola and Thielaviopsis musarum, are included in this issue and collectively, these will add value to the comparative analysis of the genomes across this family. Understanding the general biology of D. virescens will further assist in developing a deeper understanding of sap streak and potentially contribute to disease management strategies.

**SEQUENCED STRAIN**

**USA: New Hampshire: isol. ex Acer saccharum, Aug. 1987, D. Houston (CMW 17339 = CBS 130772; PREM 61293 – dried culture).**

**NUCLEOTIDE SEQUENCE ACCESSION NUMBER**

The Whole Genome Shotgun project of the Davidsoniella virescens genome has been deposited at DDBL/EMBL/GenBank under the accession no. LJZU00000000. The version described in this paper is version LJZU01000000.

**METHODS**

Davidsoniella virescens isolate CMW 17339 was used in this study. Cultures were grown at 25 °C on 2 % malt extract agar (MEA: 20 % w/v, Biolab, Midrand, South Africa) supplemented with 100 μg/L thiamine. Total genomic DNA was isolated from 2-wk-old cultures using a phenol-chloroform method previously described (Roux et al. 2004). Sequencing was carried out on the Genomics Analyzer Ix platform (Illumina) at the Genome Centre (University of California at Davis, CA). Paired-end libraries with insert fragments of 350 and 600 bases were used to generate the read lengths of 100 bases. CLCBio Genomics workbench software v. 7.5.1 (CLCBio, Aarhus, Denmark) was used for quality assessment and de novo assembly. Poor-quality reads (limit of 0.05) and/or terminal nucleotides were discarded. The remaining reads were assembled de novo using a word size of 64 with a bubble size of 100 base pairs. Scaffolding with an estimated pair distance of 99 to 562 base pairs was performed by mapping raw reads back to the contigs. Only contigs greater than 1000 bases were retained. Predictions of open reading frames (ORFs) based on the gene model for Fusarium graminearum (http://bioinf.uni-greifswald.de/augustus) were made using AUGUSTUS (Stanke et al. 2006). The quantitative assessment of the genome assembly completeness was assessed against the Benchmarking Universal Single-Copy Orthologs software program, BUSCO (Simão et al. 2015) using contigs greater than 1000 bases in length.

**RESULTS AND DISCUSSION**

Davidsoniella virescens had an estimated nuclear genome size of 33 645 160 bases. The N50 value was determined to be 118 189 bases generating a mean GC content of 44.50 %. A total of 563 contigs were produced from the CLCBio assembly, of which 561 were retained after excluding the mitochondrial scaffolds. The AUGUSTUS gene prediction pipeline estimated 6 953 ORFs. This draft genome assembly had a BUSCO completeness score of 97 % indicating that the core eukaryotic genes were present. From this analysis, 1404 single-copy genes were observed, of which 73 were duplicated genes. Of the 1 438 genes searched, only 2.2 % were classified as fragmented or missing. A gene density of 207 ORFs per Mb was observed for the 6 953 genes predicted.

Davidsoniella virescens has the largest estimated genome size (33 Mb) of all Ceratocystidaceae genomes sequenced thus far (Table 2). The dissimilarity in the coverage, N50 values and number of contigs can be attributed to the different sequencing and assembly
platforms used to generate the data (Table 2). The retention of the final number of contigs processed in gene prediction tools also differed because some researchers have chosen to retain smaller contigs (greater than 500 nucleotides). Davidsonella virensens had a similar GC content and genome completeness to the genomes sequenced of the other species in Ceratocystidaceae (Table 2). Adding to the growing number of sequenced and assembled genomes, the D. virensens genome provides a powerful resource to aid in its phylogenetic classification in Ceratocystidaceae. Similar or shared biological features can now be identified due to the availability of these genomes.

Authors: K. Naidoo*, C. Trollip, P.M. Wilken, M.J. Wingfield, and B.D. Wingfield
*Contact: Kershney.Naidoo@fabi.up.ac.za

IMA Genome-F 5D

Nuclear genome assembly for the maize pathogen Fusarium temperatum

Fusarium temperatum (formerly F. subglutinans group 1, de Vos et al. 2014) is an important mycotoxin-producing pathogen of maize (Scaufaire et al. 2011). This fungus is a member of the Fusarium fujikuroi complex which includes numerous pathogens responsible for destructive diseases of many plants (Kvas et al. 2009). Due to the economic importance of the complex, the whole genome sequences for several of its members have been determined and are publicly available. These include F. verticillioides (Fusarium Comparative Sequencing Project, Broad Institute of Harvard and MIT; http://www.broad.mit.edu), F. circinatum (Wingfield et al. 2012), F. fujikuroi and F. mangiferae (Wiemann et al. 2013), as well as F. nygamai (Wingfield et al. 2015).

To complement these genomic resources, genetic linkage maps for some of these fungi are also available (Jurgensen et al. 2002, de Vos et al. 2007). For example, a genetic linkage map available for a hybrid cross between F. circinatum and F. temperatum (de Vos et al. 2007) has been used as a framework in the analyses of certain loci and traits in these fungi (de Vos et al. 2011, 2013). Most recently, an analysis of the genomic architecture of species in this complex, allowed the anchoring of this genetic linkage map to the genomic sequence data for F. verticillioides and F. fujikuroi (de Vos et al. 2014). The aim of this study was therefore to determine the whole genome sequence for the other parent (F. temperatum) in this hybrid cross. The availability of genome data for this fungus would allow comparisons to the other sequenced members of the F. fujikuroi complex as well as contribute to improving our knowledge of the genetic processes and properties underlying the biology of these important fungi.

SEQUENCED STRAIN


NUCLEOTIDE SEQUENCE ACCESSION NUMBER

The Fusarium temperatum genomic sequence data has been deposited at DDBJ/EMBL/GenBank under the accession LJR00000000. The version described in this paper is version LJR01000000.

METHODS

DNA was extracted from Fusarium temperatum grown on ½ PDA (Iturritxa et al. 2011). One mate-pair (2 840 bp average insert size) and two paired-end (average insert sizes of 213 and 476 bp) libraries were prepared and subjected to 100 bp Illumina HiSeq 2000 sequencing at Fasteris (Geneva). After removing poor quality reads using CLC Genomics Workbench v. 6.5 (CLCbio, Aarhus, Denmark), sequences were assembled using ABYSS v. 1.3.7 (Simpson et al. 2009). Closing of gapped regions within the scaffolds was done using GapFiller v. 1.11 (Boetzer & Pirovano 2012). The completeness of the genome assembly was evaluated using CEGMA (Parra et al. 2008) and putative open reading frames (ORFs) were predicted using AUGUSTUS (Hoff & Stanke 2013) together with the gene models for F. graminearum and cDNA data from the closely related F. circinatum (Wingfield et al. 2012). By making use of MUMmer v. 3.22 (Kurtz et al. 2004), the F. temperatum scaffold sequences were compared to the chromosomes of two other sequenced members in the F. fujikuroi complex, F. fujikuroi (Wiemann et al. 2013) and F. verticillioides (Fusarium Comparative Sequencing Project) (De Vos et al. 2014).

RESULTS AND DISCUSSION

Assembly of 188 294 812 good quality reads yielded a draft genome for Fusarium temperatum that consisted of 458 781 bp with 414x coverage. This assembly consists of 43 scaffolds with an N50 of 4 506 647 bp and an average scaffold size of 1 057 181 bp. Based on the CEGMA analysis, this draft genome is 97.38 % complete (Parra et al. 2008). The GC content is 47 %. The assembly contains 14 284 putative ORFs with an average length of 1576 bp and an average density of 314 ORFs/Mb. These genome statistics for F. temperatum are comparable to those of the other sequenced Fusarium members (Fusarium Comparative Sequencing Project, Wingfield et al. 2012, 2015, Wiemann et al. 2013), which highlights the genomic similarities amongst the members in the F. fujikuroi complex.

Sequence comparisons of the sixteen largest scaffolds (which accounts for 99.56 % of the total genome size) to
The information for the *F. verticillioides* and *F. fujikuroi* genomes suggests that these scaffolds likely make up the 12 chromosomes predicted for species in the *F. fujikuroi* complex (Xu et al. 1995). This was further illustrated by the alignments of the *F. temperatum* scaffolds to the chromosome sequences for *F. verticillioides* and *F. fujikuroi* (Fig. 1). These alignments also confirmed the reciprocal translocation in *F. temperatum* and *F. circinatum* observed by de Vos et al. (2014) between chromosomes 8 and 11 (Fig. 1). The subtelomeric regions missing from chromosome 4 in *F. fujikuroi* (Wiemann et al. 2013) are present in *F. temperatum* (Fig. 1B), confirming that the shortened chromosome 4 is *F. fujikuroi*-specific.

Like *F. verticillioides, F. temperatum* also harboured the large inversion previously reported in chromosome 11 between *F. verticillioides* and *F. fujikuroi* (Wiemann et al. 2013, de Vos et al. 2014) (Fig. 1B), although *F. temperatum* appears to have an additional inversion in this chromosome when compared to *F. verticillioides* and *F. fujikuroi* (Fig. 1). Sequence comparisons also revealed that chromosome 12 is present in the *F. temperatum* assembly, albeit 1.42 times larger than its counterpart in *F. fujikuroi* (Wiemann et al. 2013). Within the *F. fujikuroi* complex, chromosome 12 has also been shown to be dispensable as well as strain-specific (Xu et al. 1995, Jurgenson et al. 2002, Ma et al. 2010, Wiemann et al. 2013, van der Nest et al. 2014a). Collectively, chromosome 11 and 12 therefore seems to be the most variable of the chromosomes in this complex. The addition of the whole genome sequence of *F. temperatum*, to the other sequenced members of the *F. fujikuroi* complex, would assist phylogenomic studies into the evolution and biology of these important fungi.

**Authors:** L. De Vos*, Q.C. Santana, B.D. Wingfield, M.A. van der Nest, M.J. Wingfield, and E.T. Steenkamp

*Contact:* Lieschen.devos@up.ac.za

**IMA Genome-F 5E**

**Draft genome sequence of Graphilbum fragrans**

*Graphilbum* is one of six currently recognized genera in *Ophiostomatales* (*Ascomycota, Sordariomycetes*) (de Beer & Wingfield 2013). The genus includes nine named species and some undescribed taxa (de Beer & Wingfield 2013). As with most other species of *Ophiostomatales*, species of *Graphilbum* are commonly found associated with coniferous hosts. *Graphilbum fragrans* was first described in 1954 from Sweden (Mathiesen-Käärik 1954), where it was initially treated in *Graphium* (as *G. fragrans*). This species was later reported from conifers or conifer-infesting beetles from various other countries including Australia, Canada, China, New Zealand, Korea, Poland, South Africa, Spain, and the USA (Harrington et al. 2001, Jacobs et al. 2003, Zhou et al. 2006, Kim et al. 2007, Romon et al. 2007, Paciura et al. 2010, Jankowiak & Bilański 2013).
The availability of whole genome sequences and recent advancements in genome analyses have contributed to a better understanding of the biology, pathogenicity and evolutionary processes in fungi. A number of genomes of species in Ophiostomatales have been sequenced and analysed, however, these include only species in Leptographium, Ophiostoma, and Sporothrix (DiGuistini et al. 2011, Haridas et al. 2013, Khoshafrtar et al. 2013, Teixeira et al. 2014, van der Nest et al. 2014, Wingfield et al. 2015). The aim of this study was to generate the genome sequence for G. fragrans, the first genome available for the genus Graphilbum and thus to provide a basis for comparison between the genera of Ophiostomatales.

SEQUENCED STRAIN


NUCLEOTIDE SEQUENCE ACCESSION NUMBER

The genomic sequence of Graphilbum fragrans (CMW 19357, CBS 138720) has been deposited at DDBJ/EMBL/GenBank under the accession LLKO00000000. The version described in this paper is version LLKO01000000.

METHODS

Methods for DNA extraction, genome sequencing, assembly and annotation were similar to those used for Leptographium lundbergii (Wingfield et al. 2015). Total genomic DNA was extracted following the protocol of Duong et al. (2013). Two pair-end libraries (350 bp and 530 bp average insert size) were prepared and sequenced using the Illumina HiSeq 2000 platform. Obtained reads were first subjected to quality filtering and trimming, followed by de novo assembly using CLC Genomics Workbench v. 8.0.1 (CLCBio, Aarhus, Denmark). Genome completeness was estimated using BUSCO (Simão et al. 2015). Total number of gene models was predicted using the MAKER genome annotation pipeline (Cantarel et al. 2008).

RESULTS AND DISCUSSION

Over 26.2 million reads were obtained after filtering and trimming. De novo assembly using CLC Genomic Workbench resulted in 80 scaffolds that were over 500 bp in size. The assembly had a N50 value of 973.6 kb and the longest scaffold was 2.66 Mb. The genome of Graphilbum fragrans was estimated to be 34.26 Mb, with the mean GC content of 55.7 %. We assessed the completeness of the obtained genome by running BUSCO on the resulting assembly using the fungal reference dataset and obtained BUSCO values of C: 97 % [D: 5.8 %], F: 1.8 %, M: 0.6 %, n: 1348 (C: complete, [D: duplicated], F: fragmented, M: missed, n: genes), indicating that the obtained genome sequence should cover most of the organism’s gene space. Genome annotation using MAKER resulted in 10 633 gene models filtered based on MAKER max build (8 942 gene models if MAKER standard build was applied) (Campbell et al. 2014). Of 10 633 gene models predicted using MAKER max build, 8102 were multi-exonic genes, mean intron length was 121.1 bp and mean exon length was 552.8 bp. The genome of G. fragrans, which is the first genome reported for Graphilbum, represents a useful resource for various comparative genomic and systematic studies in Ophiostomatales.

Authors: T.A. Duong*, M.J. Wingfield, Z.W. de Beer, and B.D. Wingfield

*Contact: Tuan.Duong@fabi.up.ac.za

IMA Genome-F 5F

Draft genome sequence of Penicillium nordicum DAOMC 185683

Penicillium nordicum is classified in the subgenus Penicillium section Fasciculata (Houbraken & Samson 2011) and is commonly isolated from cheese, nuts and other fat and protein rich substrates like salami and ham (Frisvad & Samson 2004). The importance of this fungus relates to its production of the regulated mycotoxin ochratoxin A (OTA), which is hepatoxic, nephrotoxic, teratogenic and immunotoxic in animals (Pitt et al. 2012), known to promote oxidative DNA damage by the production of reactive oxygen species and to generate DNA adducts (Hadjeba-Medjdoub et al. 2012), and is classified as a possible human renal carcinogen (group 2B) by the International Agency for Research on Cancer (IARC, Pitt et al. 2012).

OTA is also produced by P. verrucosum, the sister species to P. nordicum (Samson et al. 2004), and by several species of Aspergillus (Visagie et al. 2014a). Despite the importance of OTA in grain, coffee and grape products, its biosynthetic pathway has yet to be fully elucidated. However, there is evidence that a gene cluster including an alkaline serine protease, a polyketide synthase and a non-ribosomal peptide synthase may play a role in OTA production in P. nordicum (Karolewiez & Geisen 2005, Geisen et al. 2006). In this study, we sequenced and annotated a genome draft of P. nordicum DAOMC 185683, as part of our investigation of genes regulating OTA production in Penicillium species.

SEQUENCED STRAIN

NUCLEOTIDE SEQUENCE AND RAW READS ACCESSION NUMBERS

This Whole Genome Shotgun project was deposited at DDBJ/EMBL/GenBank under accession LHQQ00000000. The version described in this paper is version LHQQ01000000. Raw reads were deposited in NCBI SRA (http://www.ncbi.nlm.nih.gov/sra) accession number SRR2146067.

DNA EXTRACTION, WHOLE GENOME SEQUENCING AND ASSEMBLY

Penicillium nordicum DAOMC 185683 was grown on Blakeslee’s malt extract agar for 7 d at 25 °C (Visagie et al. 2014b). To make a spore suspension, the colonies were flooded with 5 mL of sterile distilled water. One mL of this spore suspension was inoculated in 100 mL of Blakeslee’s malt extract broth and was left shaking at 300 rpm at 25 °C for 6 d. To obtain fungal tissue for DNA extraction, cells were removed from the liquid culture by filtration. DNA was extracted with the OmniPrep kit for fungi (G-Biosciences) following the manufacturer’s protocol. Whole-genome sequencing (paired-end with 101 bp) was performed on an Illumina HiSeq 2500 with TrueSeq V3 chemistry at the National Research Council Canada in Saskatoon (Saskatchewan, Canada).

The quality of genomic reads was determined with FastQC v. 0.10.1 (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). Using fastx_trimmer (part of the FASTX-Toolkit v.0.0.13 (http://hannonlab.cshl.edu/fastx_toolkit/)), 10 bases from the 5’ end were trimmed to yield higher quality reads of 91 bp. Adaptor sequences were removed with Trimmomatic v. 0.33 (Bolger et al. 2014). Prior to genome assembly, the optimal k parameter was calculated with KmerGenie v. 1.6950 (Chikhi & Medvedev 2014). Error correction was performed on the trimmed reads with BayesHammer (Nikolenko et al. 2013).

De novo genome assembly was performed with SPAdes v. 3.5.0 (Bankevich et al. 2012) with the option to correct mismatches and short indels enabled. Scaffolds shorter than 91 bp. Adaptor sequences were removed with Trimmomatic v. 0.33 (Bolger et al. 2014). Prior to genome assembly, the optimal k parameter was calculated with KmerGenie v. 1.6950 (Chikhi & Medvedev 2014). Error correction was performed on the trimmed reads with BayesHammer (Nikolenko et al. 2013).

The assembly was assessed by alignment of the corrected reads onto the scaffolds using Bowtie2 v. 2.0.0 (Langmead & Salzberg 2012). Alignments produced by Bowtie2 in SAM format were converted to sorted BAM format by SAMtools v. 0.1.19 (Li et al. 2009) and statistics for nucleotide coverage were generated with Qualimap v. 2.1 (Garcia-Alcalde et al. 2012). To evaluate the completeness of our genome assembly, CEGMA v. 2.5 (Parra et al. 2007) was run on the scaffolds to detect the percentage of conserved eukaryotic genes (CEG’s) and BUSCO v. 1.1b1 (http://busco.ezlab.org/) was run on the scaffolds using the fungal profile (Dec. 19, 2014 release) to detect Universal Single-Copy Orthologs.

Genome annotation was carried out using webAugustus (Hoff & Stanke 2013) running Augustus v. 3.0.3 (Stanke et al. 2006). Predicted proteins were compared against UniProt/Swiss-Prot manually curated fungal protein data set by BLASTp v. 2.2.28+. The BLAST hits with e-values less than 1.0E-100 and similarity hits ≥ 90 % were assumed to be orthologs and were given protein names in the annotation set. Genome Annotation Generator (http://genomeannotation.github.io/GAG/) and tbl2asn (http://www.ncbi.nlm.nih.gov/genbank/tbl2asn2/) were used to validate annotations.

RESULTS AND DISCUSSION

Approximately 22 million reads, comprising 2.2 Gbp of data, were assembled into 996 scaffolds resulting in an assembly of 30.8 Mb with a GC content of 47.8 %. The N50 value was 92.3 Kb and the longest scaffold was 391 Kb. The median nucleotide coverage across the whole assembly was 57x. The assembled genome had a CEGMA score of 96.8 % when calculated from the complete gene set and 98.4 % when calculated from both partial and complete gene sets. Assessment of the completeness of the genome using BUSCO groups for fungi resulted in values of C: 99 %, [D: 6.8 %], F: 0.7 %, M: 0.1 %, n: 1438 (C: complete, [D: duplicated], F: fragmented, M: missed, n: genes). Therefore, the assembled genome covered most of the organism’s gene content. After annotation and validation, the genome contained 12 959 protein-coding genes. Of all suggested gene models, 12 448 were complete (96.0 %), but 511 gene models lacked a start codon, stop codon or both (4.0%). Mean gene length was 1388 bp, mean exon length was 437 bp and mean intron length was 85 bp. One other P. nordicum genome is accessioned in NCBI (JNNR), sequenced from a strain isolated from crop fields in Karlsruhe, Germany (UASWS BFE487). A comparative analysis has not yet been published, but as with our strain, the genome size was 30.4 Mb contained in 915 scaffolds, but the genome has less than half the coverage (at 20×) and only 46 genes were annotated.

This draft genome of a North American strain of P. nordicum, the first record of this species from Canada, represents a useful resource for biogeographical and comparative genomic studies of OTA (ochratoxin A) producing species of Penicillium, Aspergillus, and other related fungi. It will facilitate future gene knockout studies aiming to uncover the full OTA biosynthetic pathway in P. nordicum.

Authors: H.D.T. Nguyen* and K.A. Seifert
*Contact: hai.nguyen.1984@gmail.com

IMA Genome-F 5G

Draft genome sequence of the banana pathogen Thielaviopsis musarum

Thielaviopsis musarum is a pathogen of banana (Mitchell 1937, Riedl 1962) that typically infects banana fruits during maturation. This is especially true under conditions of high
Thielaviopsis musarum was previously treated as Ceratocystis musarum, but was transferred to Thielaviopsis as part of a major revision of the family Ceratocystidaceae by de Beer et al. (2014). The fungus was first reported as a new variety of C. paradoxa causing stem-end rot of banana in Australia (Mitchell 1937). Riedl (1962) isolated a similar fungus from banana stems in Vienna although the plant material probably originated elsewhere, and described it as a new species distinct from C. paradoxa. Although some authors regarded the species from banana as distinct from C. paradoxa (de Hoog 1974, Nag Raj & Kendrick 1975), others viewed C. musarum as a synonym of C. paradoxa (Upadhyay 1981). These disputes have, however, been settled with DNA-based studies (Harrington 2009, de Beer et al. 2013) and T. musarum is now recognised as a distinct species in Thielaviopsis. The aim of this study was to sequence and assemble the whole genome of an isolate of T. musarum. This was undertaken to provide information allowing for the recognition of fungal genes that are associated with pathogenicity and other important biological properties in members of Ceratocystidaceae.

SEQUENCED STRAIN


NUCLEOTIDE SEQUENCE ACCESSION NUMBERS

This Whole Genome Shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession LKBB00000000. The version described in this paper is version LKBB00000000.

METHODS

Isolate CMW1546 (CBS 139399) of Thielaviopsis musarum was grown in malt extract agar (MA). High quality DNA was isolated from harvested mycelium (Raeder & Broda 1985) and sequencing was performed using the Genomics Analyzer IIx platform (Illumina) using paired-end libraries with insert sizes of approximately 350 and 600 bases. Reads with an average length of 97 bases were quality-trimmed using the software package CLC Genomics Workbench v. 6.0.1 (CLCBio, Aarhus, Denmark). The quality-filtered reads were assembled using the Velvet de novo assembler (Zerbino & Birney 2008), with an optimized k-mer size of 77. We used SSPACE v2.0 (Boetzer et al. 2011) to assemble contigs into scaffolds and gaps were filled using GapFiller v. 2.2.1 (Boetzer & Pirovano 2012). The completeness of the assembled genome was assessed using the Benchmarking Universal Single-Copy Orthologs (BUSCO) tool, (Software v. 1.1b1 of May 2015; Simão et al. 2015). The BUSCO analysis was performed on all contigs >1 kb, making use of the fungal lineage dataset. AUGUSTUS (Hoff & Stanke 2013) and the gene models for Fusarium graminearum were used to identify putative open reading frames (ORFs).

RESULTS AND DISCUSSION

The Thielaviopsis musarum draft genome had an estimated size of 28 493 324 bases, a 95x coverage, N50 contig size of 103 017 bases and a mean GC content of 49.17%. The assembly was composed of 672 contigs, of which 541 were larger than 1 kb. Based on the BUSCO analysis, this assembly is 96 % complete. A total of 1392 single-copy BUSCO orthologs were present, of which 78 were duplicated. Out of a possible 1438 BUSCO groups searched, 11 BUSCO groups were missing or fragmented. The final assembly was predicted to encode 6 963 putative ORFs at a density of 244 ORFs/Mb.

The T. musarum genome appears to be relatively small and harbours fewer genes than other Sordariomycetes (e.g., Fusarium fujikuroi, 43.8 Mb with 14813 ORFs; Cryphonectria parasitica, 43.9Mb with 11184 ORFs) (Wiemann et al. 2013; http://genome.jgi.doe.gov/Crypa2/Crypa2.home.html). The genome size of T. musarum was, however, in the same range of some species of Ceratocystidaceae such as Ceratocystis manginecans (of 31.7 Mb with 7494 ORFs), C. albifundus albifundus (27.1 Mb with 6967 ORFs), C. fimbriata (29.4 Mb with 7266 ORFs), Huntiella omanensis (31.5 Mb with 8395 ORFs) and H. moniliformis (25.5 Mb with 6832 ORFs) (Wilken et al. 2013, van der Nest et al. 2014a, b).

The Thielaviopsis musarum genome was only marginally larger than that of T. puntulata (accession number: LAEV00000000) with its 28.1 Mb genome. However, T. puntulata was reported to encode 5480 ORFs (Wingfield et al. 2015) as opposed to the 6963 of T. musarum, suggesting a higher ORF density for the latter (i.e., 244 ORFs/Mb for T. musarum vs. 195 ORFs/Mb for T. puntulata). Future research should, therefore, consider whether these differences in genome size and ORF density could be ascribed to differences in the methodologies used to sequence and annotate the respective genomes. Overall, these genomes will provide interesting perspectives regarding the development and evolution of important biological traits in these fungi.

Authors: M.A Sayari*, C. Trollip, K. Naidoo, B.D. Wingfield, and M.J. Wingfield
*Contact: Mohammad.Sayari@fabi.up.ac.za

ACKNOWLEDGEMENTS

Genome sequencing of Penicillium nordicum DAOMC 185683 was funded by Growing Forward 2 funding from Agriculture & Agri-Food Canada, with additional support from Canadian Safety and Security Programme grant CRTI 09-462RD/CSSP 309v01. We thank Ekaterina Ponomareva for technical assistance.

For the Ceratocystis eucalypticola, Chrysoporthe cubensis, C. deuterocubensis, Fusarium temperatum, and Graphilbum fragrans genomes we recognise the co-funding by the Genomics Research Institute (University of Pretoria), the University of Pretoria Research
Development Programme, the DST/NRF Centre of Excellence in Tree Health Biotechnology (FABI, University of Pretoria), and the National Research Foundation (NRF) (Grant number 87332).

Studies on *Davidsoniella virescens* and *Thielaviopsis musarum* were funded by the University of Pretoria, members of the Tree Protection Cooperative Program (TPCP), the National Research Foundation (NRF)/Department of Science and Technology (DST), Centre of Excellence in Tree Health Biotechnology (CTHB), and the THRIP initiative of the Department of Trade and Industry (DTI) in South Africa for financial assistance. This work was based on the research supported in part by a number of grants from the National Research Foundation of South Africa (includes Grant specific unique reference numbers (UID) 83924 and 87332). The Grant holders acknowledge that opinions, findings and conclusions or recommendations expressed in any publication generated by the NRF supported research are that of the author(s), and that the NRF accepts no liability whatsoever in this regard.

REFERENCES


Gryzenhout M, Myburg H, van der Merwe NA, Wingfield

Gryzenhout M, Myburg H, Wingfield BD, Montenegro F, Wingfield
MJ (2005) *Chrysoporthe doradensis* sp. nov. pathogenic to

Gryzenhout M, Rodas CA, Portales MJ, Clegg P, Wingfield BD,
et al. (2006) Novel hosts of the Eucalyptus canker pathogen
*Chrysoporthe cubensis* and a new *Chrysoporthe* species from


Hadjieba-Medjdoub K, Tozlovanu M, Pfohl-Leszkwicz A, Frenette
different mechanisms of action for ochratoxin A-mediated

genome and transcriptome of the pine saprophyte *Ophiostoma
cube*nsae and a comparison with the bark beetle-associated pine
pathogen *Gromsmanxia clavigera*. *BMGenomics* **14**: 373.

Harrington TC (2009) The genus *Cerato*cytis*. Where does the oak
wilt fungus fit? In: *Proceedings of the 2nd National Oak Wilt
Symposium*, Billings RF, eds. Austin, TX: USDA Forest
Service.

Harrington TC, McNew D, Steimel J, Hofstra D, Farrell R (2001)

canker pathogen *Chrysoporthe austroafricana* on native

Hodges C, Reis M, Ferreira F, Henfling J (1976) O cancro do
cupuçu causado por *Diapothecium cubense* [*Eucalyptus* spp.;
Fungo; Brasil]. *Fitopatologia Brasileira* **1**: 129–170.

Hodges CS, Geary TF, Cordell CE (1979) The occurrence of

training AUGUSTUS and predicting genes in eukaryotes. *Nucleic
Acid Research* **41**: W123–W128.

Houbraken J, Samson RA (2011) *Phylogeny of C*hytho*porthe*
cubi*nsae* studies. *In Mycology* **70**: 1–51.

Iturritxa E, Garley RJ, Wright J, Happe E, Steenkamp ET, et al. (2011) A genetically homogenous population of *Fusarium cingu*natu*m causes pitch canker of *Pinus radiata* in the Basque

Jacobs K, Seifert KA, Harrison KJ, Kirisits T (2003) Identity and
phylogenetic relationships of ophiostomatoid fungi associated

associated with the large pine weevil, *Hylobius abietis*, and
infested Scots pine seedlings in Poland. *Annals of Forest

Jurgenson JE, Zeller KA, Leslie JF (2002) Expanded genetic map of

Karolewicz A, Geisen R (2005) Cloning a part of the ochratoxin A

Sequencing and annotation of the *Ophiostoma ulmi* genome.
*Inside Genomics* **14**: 162.


Versatile and open software for comparing large genomes.
*Genome Biology* **5**: R12.

Kvas M, Marasas WFO, Wingfield BD, Wingfield MJ, Steenkamp
ET (2009) Diversity and evolution of *Fusarium* species in the

Laia M, Allenas A, Harrington T (1999) Isolation, detection in soil, and inoculation of *Cerato*cytis*is* *eugeni*c*ae*, causal agent of wilting, die-
back and canker in *Eucalyptus*. In: *Proceedings of the 12th biennial conference of the Australasian Plant Pathology Society*.
eds.): 77.

Langmead B, Salzberg SL (2012) Fast gapped-read alignment with

Lee DH, Roux J, Wingfield BD, Wingfield MJ (2015) Variation in
growth rates and aggressiveness of naturally occurring self-
fertile and self-sterile isolates of the wilt pathogen *Cerato*cytis*is

Sequence Alignment/Map format and SAMtools. *Bioinformatics*
**25**: 2078–2079.

Ma L-J, van der Does HC, Borkovich KA, Coleman JJ, Daboussi M-J.,
et al. (2010) Comparative genomics reveals mobile pathogenicity

Mathiesen-Käärik A (1953) Eine Übersicht über die gewöhnlichsten
*Syzygium* spp.; [with invasive and native *Cyphomelid*
*Endothia eu*gen*iae* and *C*rypho*netria c*ubi*nsae*]. *Studies in Mycology*
**25**: W123–W128.

Mayers CG, McNew DL, Harrington TC, Roepper RA, Fraedrich
SW, et al. (2015) Three genera in the *C*erato*cytis*idaeae are the
respective symbionts of three independent lineages of
ambrosia beetles with large, complex mycangia. *Fungal Biology

Mitchell RS (1937) Stem end rot of bananas with special reference
to the physiological relationships of *Thielaviopsis paradoxa* (de
Seynes) von Hohn. *Journal of the Council for Scientific and
Industrial Research* **10**: 123–130.

β-Tubulin and histone H3 gene sequences distinguish
*Cyphomycteria cubensis* from South Africa, Asia, and South

Conspecificity of *Endothia eugeniae* and *Cyphomycteria cubensis*:
a re-evaluation based on morphology and DNA sequence data.

Nag Raj TR, Kendrick WB (1975) *A Monograph of Chalara and allied


Recommended names for pleomorphic genera in *Dothideomycetes*


1Department of Botany and Plant Pathology, Oregon State University, Corvallis, Oregon 97331, USA; corresponding author e-mail: amydiander@yahoo.com
2CBS-KNAW Fungal Biodiversity Institute, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands
3Department of Microbiology and Plant Pathology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria 0002, South Africa
4Center of Excellence in Fungal Research, School of Science, Mae Fah Luang University, Chiang Rai 57100, Thailand
5School of Science, Mae Fah Luang University, Chiang Rai 57100, Thailand
6Department of Life Sciences, The Natural History Museum, Cromwell Road, SW7 5BD London, UK
7Departamento de Biología Vegetal II, Facultad de Farmacia, Universidad Complutense de Madrid, Plaza Ramón y Cajal, Madrid 28040, Spain
8Comparative Plant and Fungal Biology, Jodrell Laboratory, Royal Botanic Gardens, Kew, Surrey, TW9 3DS, UK
9ABL Herbarium, G.v.d.Veenstraat 107, NL-3762 XK Soest, The Netherlands
10Departamento de Micología, Universidade Federal de Pernambuco Rua Nelson Chaves, s/n, Cidade Universitária, Recife, 50670-901, Brazil
11No. 128/1-J, Azad Housing Society, Curca, P.O. Goa Velha-403108, India; formerly, Department of Botany, Goa University, Goa, India
1242 Longacre Dr., Livingston, NJ, 07039, USA
13Martin-Luther-Universität, Institut für Biologie, Bereich Geobotanik und Botanischer Garten, Herbarium, Neuwerk 21, 06099 Halle (Saale), Germany
14A.M.B. Gruppo Micologico Forlivese “Antonio Cicognani”, Via Roma 18, Forli, Italy; A.M.B. Circolo Micologico “Giovanni Carini”, C.P. 314, Brescia, Italy; Società per gli Studi Naturalistici della Romagna, C.P. 144, Bagnacavallo (RA), Italy
15Institute of Plant and Environment Protection, Beijing Academy of Agriculture and Forestry Sciences, Beijing 100097, PR China
16College of Science, Botany and Microbiology Department, King Saud University, Riyadh 11455, Saudi Arabia
17Institute of Forest Entomology, Forest Pathology and Forest Protection, Dept. of Forest and Soil Sciences, BOKU-University of Natural Resources and Life Sciences, Hasenauerstraße 38, 1190 Vienna, Austria, and Division of Systematic and Evolutionary Botany, Department of Botany and Biodiversity Research, University of Vienna, Renngasse 14, 1030 Wien, Austria
18Jodrell Laboratory, Royal Botanic Gardens, Kew, Richmond, Surrey TW9 3DS, UK
19Department of Biology, George Mason University, 4400 University Drive, Fairfax, VA 22030-4444, USA
20Manaaki Whenua Landcare Research, Private Bag 92170, Auckland, New Zealand
21University of Lisbon, Faculty of Sciences, Biosystems and Integrative Sciences Institute (BioISI), University of Vienna, Rennweg 14, 1030 Wien, Austria, and Division of Systematic and Evolutionary Botany, Department of Botany and Biodiversity Research, University of Vienna, Renngasse 14, 1030 Wien, Austria
22Department of Botany and Plant Pathology, Oregon State University, Corvallis, Oregon 97331, USA
23Department of Plant and Soil Sciences, Utah State University, Logan, Utah, USA
24Fungal Biodiversity Laboratory (BFBD), BIOTEC, National Science and Technology Development Agency (NSTDA), 113 Thailand Science Park, Phahonyothin Road, Khlong Nueng, Amphoe Khlong Luang, Pathum Thani, 12120, Thailand
25Faculty of Agriculture and Life Science, Hirosaki University, 3 Bunkyo-cho, Hirosaki, Aomori 036-8561, Japan
26Royal Botanic Garden Edinburgh, 20A Inverleith Row, Edinburgh EH3 5LR, UK
27Guizhou Key Laboratory of Agricultural Biotechnology, Guizhou Academy of Agricultural Sciences, Guiyang, Guizhou 550006, PR China
28International Fungal Research and Development Centre, Key Laboratory of Resource Insect Cultivation & Utilization State Forestry Administration
29The Research Institute of Resource Insects, Chinese Academy of Forestry Kunming 650224, PR China
30Microbiology, Department of Biology, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands
31Institute of Microbiology, Beijing Forestry University, P.O. Box 61, Beijing 100083, PR China

© 2015 International Mycological Association

You are free to share - to copy, distribute and transmit the work, under the following conditions:

**Attribution:**
You must attribute the work in the manner specified by the author or licensor (but not in any way that suggests that they endorse you or your use of the work).

**Non-commercial:**
You may not use this work for commercial purposes.

**No derivative works:**
You may not alter, transform, or build upon this work.

For any reuse or distribution, you must make clear to others the license terms of this work, which can be found at http://creativecommons.org/licenses/by-nc-nd/3.0/legalcode. Any of the above conditions can be waived if you get permission from the copyright holder. Nothing in this license impairs or restricts the author's moral rights.
Abstract: This paper provides recommendations of one name for use among pleomorphic genera in Dothideomycetes by the Working Group on Dothideomycetes established under the auspices of the International Commission on the Taxonomy of Fungi (ICTF). A number of these generic names are proposed for protection because they do not have priority and/or the generic name selected for use is asexually typified. These include: Acrogenospora over Farlowiella; Alternaria over Allewia, Lewia, and Crivella; Botryosphaeria over Fusicoccum; Camarospora over Anthracostroma; Capnodium over Polychaeoton; Cladosporium over Davidiella; Corynespora over Corynesporasca; Curvularia over Pseudocochliobolus; Elsinóe over Sphaeloma; Excipulariopsis over Kentingia; Exserosperla over Anomalemma; Exserohilum over Setosphaeria; Gymmamyces over Megalosporiella; Kellermannia over Planistromella; Kirschsteiniothelia over Drechsler; Lecanosticta over Eruptio; Paraneictriella over Araneomyces; Phaeosphaeria over Phaeoseptoria; Phyllosticta over Guignardia; Podonectria over Tetracrium; Polythricnicum over Cymatodora; Prostemphyllum over Pleomassaria; Ramularia over Mycosphaerella; Sphaeroellipsospora over Eudarluca; Sphaeropsis over Phaeobotryosphaeria; Stemphyllium over Pleospora; Teratosphaeria over Kirramyces and Colletogloeospora; Tetraploa over Tetraplophaeria; Venturia over Fusidiadium and Pollaccia; and Zelospermiosporium over Neomicrothyrium. Twenty new combinations are made: Acrogenospora camichaeliana (Berk.) Rossman & Crous, Alternaria scrophulariae (Desm.) Rossman & Crous; Pyrenophora catenaria (Drehsler) Rossman & K.D. Hyde, P. dematioides (Bubák & Wróbl.) Rossman & K.D. Hyde, P. fugax (Wallr.) Rossman & K.D. Hyde, P. nobleae (McKenzie & D. Matthews) Rossman & K.D. Hyde, P. triseptata (Drehsler) Rossman & K.D. Hyde, Schizothyrium cryptogamum (Batzer & Crous) Crous & Batzer, S. cylindricum (G.Y. Sun et al.) Crous & Batzer, S. empororae (G.Y. Sun & L. Gao) Crous & Batzer, S. inequaale (G.Y. Sun & L. Gao) Crous & Batzer, S. musae (G.Y. Sun & L. Gao) Crous & Batzer, S. qiense (G.Y. Sun & Y.Q. Ma) Crous & Batzer, S. tardescens (Batzer & Crous) Crous & Batzer, S. wiscinsinense (Batzer & Crous) Crous & Batzer, Teratosphaeria epicoccoides (Cooke & Massee) Rossman & W.C. Allen, Venturia catenosphora (Butin) Rossman & Crous, V. convolvularum (Ondrej) Rossman & Crous, V. oleaginea (Cestagne) Rossman & Crous, and V. phyllelae (Nicolas & Aggy) Rossman & Crous, combs. nov. Three replacement names are also proposed: Pyrenophora grahamii Rossman & K.D. Hyde, Schizothyrium sunii Crous & Batzer, and Venturia bariab Rossman & Crous noms. nov.

INTRODUCTION

A comprehensive account of the genera of Dothideomycetes was provided by Hyde et al. (2013), and updated by Wijayawardene et al. (2014). These works serve as the basis for the move to one scientific name for pleomorphic genera of fungi in this class. Based on the latter publication, an account is presented for all pleomorphic genera in Dothideomycetes including the generic names recommended for use. This article is essentially abstracted from Wijayawardene et al. (2014) to present only competing pairs of genera for consideration by the Nomenclature Committee for Fungi (NCF), as well as including minor corrections. All but three of the recommendations listed here agree with those of Wijayawardene et al. (2014). For Acrogenospora-Farlowiella, a case is now made for protecting Acrogenospora based on the wider use and fewer name changes required rather than following the principle of priority. Similarly Camarospora was determined to be more widely used than the competing generic name Anthracostroma, which has equal priority; Camarospora is consequently recommended for use. Although Sydowia and Hormonema were considered distinct by Wijayawardene et al. (2014), a study by Hirooka et al. (2012) suggested that their type species were congeneric. Thus, Sydowia is now recommended for use based on the greater number of species, wider use, and priority. Four additional pairs of genera were discovered to be synonyms as explained below. In addition, three generic names with synonyms listed in Wijayawardene et al. (2014) are probably not, as explained under names not included. Generic names with synonyms that are not pleomorphic, i.e. all sexual or all asexually typified synonyms are not included.

A list of names of all pleomorphic genera, i.e. those having synonymous generic names for an alternate morph along with their type species and citations and the action required, if any, is presented in Table 1. A number of genera recommended for use require action by the NCF for two reasons. Generic names that do not have priority must be approved for protection by the NCF, equivalent to conservation. In addition, according to Article 57.2 of the International Code of Nomenclature for algae, fungi and plants (ICN; McNeill et al. 2012), generic names with type species typified by sexual morphs of species (S) must be suppressed or rejected before a generic name typified by a species with an asexual morph (A) can be used. We note, however, that the mycological community has proposed deletion of Art. 57.2 (Hawksworth 2015) so that names will in future compete on priority of publication regardless of the morph of their type species.

Clarifications of elements of the ICN relevant to this paper are as follows. One concerns the publication of two or more scientific names in the same publication. When this occurs, all names in that publication are considered to have equal priority. If names in that publication are determined to be synonyms, the first author to select one of them for use determines the priority. Secondly, if a generic name is protected for use because it is considered a synonym of another generic name but is later found not to be a synonym, that generic name remains available for use. This is similar to the concept of a genus that may initially be broadly circumscribed and later more narrowly defined. These and
many other nomenclatural situations related to moving to one scientific name for fungi are explained in Rossman (2014), as determined by the ICN. For an updated account of the scientific names of fungi associated with plants including those previously having two names, consult the USDA SMML Fungal Databases (http://nt.ars-grin.gov/fungaldatabases/), which includes the scientific names of plant-associated fungi that reflect the most recent literature along with the host and worldwide distribution of each species.

PLEOMORPHIC GENERIC NAMES OF DOTHIDEOMYCETES AND RECOMMENDATIONS FOR USE OF ONE NAME

Protect Acrogenospora M.B. Ellis 1971 (A) over Farlowiella Sacc. 1891 (S)
The generic name Acrogenospora, typified by *A. sphaerocephala*, includes two of the 11 species that have sexual morphs placed in *Farlowiella* typified by *F. repanda* (also considered to be *F. carmichaeliana*). Although no molecular data exist to support the synonymy of *Acrogenospora* with *Farlowiella*, the distinctive morphology of the asexual morph suggests this and has long been accepted (Ellis 1971, 1976, Schoch et al. 2009). Three names representing two species have been described in *Farlowiella*, both of which have earlier names in *Acrogenospora* and thus would require name changes if *Farlowiella* were retained. All names in *Acrogenospora* would need to be changed if *Farlowiella* were used. In addition, confusion exists with the fungal name *Farlowiella* because it has also been used for an algal genus of *Phaeophyta* for which a replacement name was published in 1975, and because there is also an insect genus named *Farlowiella*. If the generic name *Acrogenospora* is protected, only one name change would be required. *Acrogenospora* has been monographed (Goh et al. 1998), including especially those known from freshwater that may not all belong in that genus. *Acrogenospora* is commonly used by plant pathologists and ecologists, thus protection of the generic name *Acrogenospora* is favoured as it would contribute to nomenclatural stability of these species. This disagrees with our previous recommendation (Wijayawardene et al. 2014), which was supported by the argument that *Farlowiella* was adopted in the comprehensive account of *Dothideomycetes* by Schoch et al. (2009), a paper published prior to the shift to single nomenclature for pleomorphic fungi (Crous et al. 2015a).

Acrogenospora carmichaeliana (Berk.) Rossman & Crous, comb. nov.
MycoBank MB814513


Use Alternaria Nees 1816 (A) rather than Lewia M.E. Barr & E.G. Simmons 1986 (S), Allewia E.G. Simmons 1990 (S) and Crivellia Shoemaker & Inderb. 2006 (S)
The genus *Alternaria*, typified by *A. alternata*, is a well-known genus with over 700 names including the causal organisms of diseases such as leaf spot of crucifers (*A. brassicaceae*), citrus fruit black spot (*A. citri*), sunflower blight (*A. helianthi*), and early blight of potatoes (*A. solani*) among others. Simmons (1986) was the first to describe a sexual morph for *Alternaria* based on *Lewia scrophulariae* having the asexual morph *Alternaria conjuncta*. A second sexually typified genus *Allewia* based on *A. proteae* was described for species that Simmons (1990) placed in *Embellisia*, a segregate of *Alternaria*. These genera, as well as the monotypic genus *Crivellia* typified by *C. papaveracearum*, were shown to be monophyletic and recognized as a broadly circumscribed *Alternaria* by Woudenberg et al. (2013, 2104). Given its widespread use, the number of species, and its priority, the use of *Alternaria* is recommended.

Alternaria scrophulariae (Desm.) Rossman & W.C. Allen, comb. nov.
MycoBank MB815091
*Leptosphaeria scrophulariae* (Desm.) Sacc., *Syll. Fung.* 2: 57 (1883)
When Barr & Simmons (in Simmons 1986) introduced the new generic name *Lewia* for the sexual morph of species of *Alternaria*, they selected *L. scrophulariae* (based on *Sphaeria scrophulariae*) as the type species. In the same publication the asexual morph of *L. scrophulariae* was described as a new species, *A. conjuncta*, thus there is no doubt that these names represent the same species. However, the oldest epithet for this species should be placed in *Alternaria*. Most reports of this species are under the names *L. scrophulariae* or *Pleospora scrophulariae*.

Protect Bipolaris Shoemaker 1959 (A) over Cochliobolus Drechsler 1954 (S)
The generic names *Bipolaris* typified by *B. maydis* and *Cochliobolus* typified by *C. heterostrophus*, the sexual morph of *B. maydis*, are unquestionably synonyms (Manamgoda et al. 2014). These genera include a number of economically important plant pathogens causing diseases of cereal crops worldwide, especially southern corn leaf blight caused by *B. maydis*. At present 115 names have been included in *Bipolaris* while 54 names have been described in *Cochliobolus*. 
Table 1. Names of pleomorphic genera in *Dothideomycetes* that are proposed for protection or are asexually typified and recommended for use over sexually typified genera. For each genus the citation, type species and accepted name is given. NCF = Nomenclature Committee for Fungi.

<table>
<thead>
<tr>
<th>Generic name recommended for use, citation and type species</th>
<th>Suppressed generic name(s), citation, type species, and currently accepted name</th>
<th>Action required</th>
</tr>
</thead>
<tbody>
<tr>
<td>Typus: <em>A. tenuis</em> Nees 1816; now <em>A. alternata</em> (Fr.) Keissl. 1912 (<em>Torula alternata</em> Fr. 1832).</td>
<td>Typus: <em>A. tenuis</em> Nees 1816; now <em>A. alternata</em> (Fr.) Keissl. 1912 (<em>Torula alternata</em> Fr. 1832).</td>
<td></td>
</tr>
<tr>
<td>Typus: <em>B. dothidea</em> (Moug.) Ces. &amp; De Not. 1863.</td>
<td>Typus: <em>F. aesculi</em> Corda 1829; now <em>Botrysphaeria dothidea</em> (Moug.) Ces. &amp; De Not. 1863.</td>
<td></td>
</tr>
<tr>
<td><strong>Camarosporula</strong> Petr. in <em>Sydowia</em> 8: 99. 1954.</td>
<td><em>Anthracostroma</em> Petr. in <em>Sydowia</em> 8: 96. 1954.</td>
<td>Asexual type. Approval needed by NCF.</td>
</tr>
<tr>
<td>Typus: <em>C. persoonii</em> (Henn.) Petr. 1954 (<em>Hendersonia persoonii</em> Henn. 1901).</td>
<td>Typus: <em>A. persoonii</em> (Henn.) Petr. 1954 (<em>Mycosphaerella persoonii</em> Henn. 1903); now <em>Camarosporula persoonii</em> (Henn.) Petr. 1954.</td>
<td></td>
</tr>
<tr>
<td>Generic name recommended for use, citation and type species</td>
<td>Suppressed generic name(s), citation, type species, and currently accepted name</td>
<td>Action required</td>
</tr>
<tr>
<td>------------------------------------------------------------</td>
<td>---------------------------------------------------------------------------------</td>
<td>----------------</td>
</tr>
<tr>
<td>Typos: C. herbarum (Pers.) Link 1816 (Dermatium herbarum Pers. 1794).</td>
<td>Typos: D. tassiana (De Not.) Crous &amp; U. Braun 2003 (Sphaerella tassiana De Not. 1863); now Cladosporium herbarum (Pers.) Link 1816.</td>
<td>None.</td>
</tr>
<tr>
<td>Typos: E. canavaliace Racib. 1900.</td>
<td>Typos: S. ampelinum de Bary 1874; now Elsinoë ampelina Shear 1929.</td>
<td>None.</td>
</tr>
<tr>
<td>Typos: E. fungorum (Fr.) P. Karst. 1892 (Epochniun fungorum Fr. 1832).</td>
<td>Typos: A. epochnii (Berk. &amp; Broome) Sivan. 1983 (Sphaeria epochnii Berk. &amp; Broome 1866); now Exosporiella fungorum (Fr.) P. Karst. 1892.</td>
<td>None.</td>
</tr>
<tr>
<td><strong>Lecanosticta</strong> Syd. in Annls mycol. 20: 211. 1922.</td>
<td>Eruptio M.E. Barr in Mycotaxon 60: 437. 1996.</td>
<td>Asexual type. Approval needed by NCF.</td>
</tr>
<tr>
<td>Typos: L. pini Syd. 1922; now Lecanosticta acicola (Thüm.) Syd. 1924 (Cryptosporium acicola Thüm. 1878).</td>
<td>Typos: E. acicola (Dearn.) M.E. Barr 1996 (Oligostroma acicola Dearn. 1926); now Lecanosticta acicola (Thüm.) Syd. 1924.</td>
<td>None.</td>
</tr>
</tbody>
</table>
### Table 1. (Continued).

<table>
<thead>
<tr>
<th>Generic name recommended for use, citation and type species</th>
<th>Suppressed generic name(s), citation, type species, and currently accepted name</th>
<th>Action required</th>
</tr>
</thead>
<tbody>
<tr>
<td>Generic name recommended for use, citation and type species</td>
<td>Suppressed generic name(s), citation, type species, and currently accepted name</td>
<td>Action required</td>
</tr>
<tr>
<td>-----------------------------------------------------------</td>
<td>---------------------------------------------------------------------------------</td>
<td>-----------------</td>
</tr>
</tbody>
</table>
Although the sexually typified Cochliobolus is an older name than the asexually typified Bipolaris, asexual morphs are more commonly encountered in nature, thus the name Bipolaris has been used more frequently than Cochliobolus. In all but one case, the sexual morphs for these species were named at a later time than the asexual morphs. Three species of Cochliobolus have been studied as model organisms and their genomes sequenced with publications concerning their genomics and genetics using the name in Cochliobolus. Rossman et al. (2013) proposed that the generic name Bipolaris be conserved over Cochliobolus and that B. maydis (syn. Helminthosporium maydis), be conserved over the type species of Cochliobolus, C. heterostrophus (syn. Ophiobolus heterostrophus). If the generic name Bipolaris and the species name B. maydis are conserved, none of the names of Bipolaris will need to be changed. In all cases except that of Bipolaris maydis, the oldest epithet is already placed in Bipolaris. Use of the generic name Cochliobolus would result in the need to transfer 46 names from Bipolaris to Cochliobolus while another seven names in Bipolaris would replace names currently used in Cochliobolus. Given the frequency with which the name Bipolaris is used by plant pathologists, including a recent monograph (Manamgoda et al. 2014), and the number of name changes required if Cochliobolus were retained, protecting the generic name Bipolaris is recommended.

Use Botryohypoxylon Samuels & J.D. Rogers 1986 (S) rather than Iledon Samuels & J.D. Rogerson 1986 (A)
The monotypic genera Botryohypoxylon based on B. amazonense and Iledon based on L. versicolor were described in the same article and thus have equal priority. Neither name has been used later in the literature. Botryohypoxylon is recommended for use.

Protect Botryosphaeria Ces. & De Not. 1863 (S) over Fusicoccum Corda 1829 (A)
The type species of Botryosphaeria, B. dothidea, was shown to be a synonym of Fusicoccum aesculi, the type species of Fusicoccum, by Slippers et al. (2004). Although many species names have been placed in both genera, this complex has been divided into several genera with relatively few species remaining in Botryosphaeria. In the most recent account, Phillips et al. (2013) accepted only six species in Botryosphaeria, which has now been clearly defined and the type species epitypified. Additionally, most names in Fusicoccum have been redisplayed in other genera (Crous et al. 2006, Xu & Zhang 2006, Mohali et al. 2007, Phillips & Alves 2009, Phillips et al. 2013) and Botryosphaeria is the generic name used most commonly by plant pathologists. Given that the recent studies of this group have adopted the name Botryosphaeria while names in Fusicoccum have been placed in other genera, we recommend protection of Botryosphaeria.

Use Brooksia Hansf. 1956 (S) rather than Hiospira R.T. Moore 1962 (A)
Brooksia tropicalis, the type species of Brooksia, is a leaf parasite reported on diverse hosts throughout tropical regions (Farr & Rossman 2015). The asexual morph was described as Hiospira hendrickxii, the type species of Hiospira, by Moore (1962); there is no question that these types represent the same species. A second variety of Brooksia tropicalis was described as well as a second species of Hiospira, but the identities of these remain obscure. Given the widespread use of Brooksia and its priority, the use of Brooksia is recommended.

Use Camarosporula Petr. 1954 (A) rather than Anthracostroma Petr. 1954 (S)
The monotypic generic names Camarosporula typified by C. persooniae and Anthracostroma by A. persooniae were published in the same article as alternate morphs of the same species by Petrak (1954), and thus have equal priority. Because this fungus has been reported most frequently as Camarosporula persooniae (Farr & Rossman 2015), as used by Crous et al. (2011b), Camarosporula is recommended for use, contrary to the proposal of Wijayawardende et al. (2014).

Protect Capnodium Mont. 1849 (S) over Polychaeton (Pers.) Lév. 1846 (A)
The generic name Capnodium is typified by C. salicinum, a species now regarded as a synonym of C. citri (Reynolds 1999). This genus is relatively large with over 100 names and is used for many common tropical leaf-inhabiting “sooty moulds”. On the other hand, Polychaeton, typified by P. quercinum (Hughes 1976), includes only 16 names some of which have been placed in other genera. Crous et al. (2009a) and Chomnunti et al. (2011) suggested that these genera were congeneric although they did not include the type species of Polychaeton in their studies. It seems likely that these generic names are synonyms. Chomnunti et al. (2011) assumed this and suggested that, given the great number of epithets and its widespread use, the name Capnodium should be used and thus protected over Polychaeton. Protection of Capnodium will prevent an excessive number of name changes and is, by far, the most commonly used generic name. This case is cited as an example of good practice in the ICN (Art. 57.2 Ex 2).

Use Cladosporium Link 1816 (A) rather than Davidiella Crous & U. Braun 2003 (S)
Cladosporium, typified by C. herbarum, is a well-known genus including over 700 names and the ubiquitous air-borne species C. cladosporioides. A sexual morph of C. herbarum was discovered and described in Davidiella, typified by D. tassiana (Braun et al. 2003), thus these two generic names are synonyms. The monograph of Bensch et al. (2012) provided a reliable circumscription of the genus and included 169 species based on a multi-gene phylogeny. Most names in Davidiella have already been redisplayed in Cladosporium (Crous et al. 2007a). Given its widespread use, the importance of the genus in indoor air and buildings (Bensch et al. 2015), the greater number of species, and priority of publication, the name Cladosporium is recommended for use.

Use Comminutispora A.W. Ramaley 1996 (S) rather than Hyphospa A.W. Ramaley 1996 (A)
These monotypic generic names describe alternate morphs of the same species, Communitispora based on C.
agavacearum and Hyphospora agavacearum, thus these names have equal priority. Several reports of this species as C. agavacearum exist (Farr & Rosman 2015), thus Comminutispora is recommended for use.

**Use Corynespora Güßow 1906 (A) rather than Corynesporasca Sivan. 1996 (S)**
The monotypic genus Corynesporasca, typified by C. carotae, was described by Sivanesan (1996) for the sexual morph of a species of Corynespora on a tropical plant. Corynespora, typified by C. maezi, a synonym of C. cassicola, has been widely used and includes approximately 200 species names. The ubiquitous leaf spot fungus C. cassicola has been shown to occur on many plant hosts, especially in tropical regions (Smith et al. 2009). Given the extensive use of the name Corynespora for plant pathogenic fungi and its priority, this generic name is recommended for use.

**Use Curvularia Boedijn 1933 (A) rather than Pseudocochliobolus Tsuga et al. 1978 (S)**
The generic name Curvularia, typified by C. lunata, has been recently separated from the related genera Bipolaris, Exserohilum, and Pyrenophora and monographed by Manamgoda et al. (2015). Although the sexual morph is known and placed in Pseudocochliobolus based on P. nisikadii, that morph is rarely encountered. Species of Curvularia occur as both plant and animal pathogens with over 30 species described. Given its widespread use, priority, and number of species, the use of Curvularia is recommended.

**Protect Elsinoë Racib. 1900 (S) over Sphaceloma de Bary 1874 (A)**
The genus Elsinoë includes many species that cause a number of economically important leaf scab diseases, especially in tropical regions. The type species, E. canavaliae, occurs on Canavalia and is known from leguminous plants in the tropics (Sivanesan & Holliday 1971). Many species of Elsinoë have asexual morphs that are placed in Sphaceloma, a genus typified by S. ampelina, and now known as Elsinoë ampelina, causing grape scab. Although Sphaceloma has priority, both genera contain an equal number of names. Because Elsinoë is more commonly applied to these scab diseases and this name has been adopted in recent literature (Li et al. 2011, Crous et al. 2013), it is recommended that Elsinoë be protected.

The monotypic genus Kentingia, typified by K. corticola, was established for the sexual morph of another monotypic genus, Excipulariopsis based on E. narsapurensis (Sivanesan & Hsieh 1989); there is no question that these genera are synonyms. Following the principle of priority of publication for these genera would prevent a name change, thus Excipulariopsis is recommended for use.

**Use Exosporiella P. Karst. 1892 (A) rather than Anomalemma Sivan. 1983 (S)**
The monotypic genus Anomalemma, based on A. epochnii, was described for the sexual morph of the monotypic Exosporiella, typified by E. fungorum (Sivanesan 1983), thus these generic names are synonyms. Tian et al. (2015) found the asexual morph of an Exosporiella species when examining the isolate of Anomalemma epochnii. Even though molecular data for either the sexual or asexual morphs are lacking, following the principle of priority and use of the asexually typified name, Exosporiella is recommended for protection.

The generic names Exserohilum, typified by E. turcicum, and Setosphaeria, typified by S. turcicum, were described in the same paper and thus have equal priority. Despite the use of the same epithet, these names are based on different type specimens and so are nomenclaturally distinct; however, Leonard & Suggs (1974) demonstrated that they represent the same species and so Exserohilum and Setosphaeria are synonyms. Exserohilum includes 36 names of important plant pathogens, such as E. rostratum, the cause of leaf spot and rot of wheat and other grasses, while only nine names have been placed in Setosphaeria. Use of Exserohilum would prevent a number of name changes, and so the use of Exserohilum is recommended.

**Protect Gemmamyces Casagr. 1969 (S) over Megaloseptoria Naumov 1925 (A)**
The generic name Gemmamyces, typified by G. piceae, was established for the cause of spruce bud blight occurring in northern Europe and China for which the asexual morph is Megaloseptoria mirabilis, type of the monotypic genus Megaloseptoria (Casagrande 1969, Sivanesan 1984). The basionym of G. piceae, Cucurbitaria piceae, has also been used when referring to this species although Yuan & Wang (1995) suggest that Gemmamyces is distinct from Cucurbitaria based on both biological and morphological characteristics. The latter authors describe a second species, G. piceicola. Given that Gemmamyces is widely used for the causes of spruce bud blight diseases (Hansen & Lewis 1997) and includes two species, the protection of Gemmamyces is recommended.

**Use Kellermania Ellis & Everh. 1885 (A) rather than Planistromella A.W. Ramaley 1993 (S)**
The generic name Kellermania, typified by K. yuccigena, was monographed by Minnis et al. (2012) who showed that Planistromella, typified by P. yuccifoliorum, is a synonym; this was subsequently confirmed by Monkai et al. (2013). Ramaley (1993) established Planistromella for the sexual morph of K. yuccifoliorum. Kellermania includes 38 names while 13 names have been placed in Planistromella, all except one of which also have names in Kellermania. Given its priority, widespread use, and adoption in a recent monographic account, the use of Kellermania is recommended.

**Protect Kirschsteiniothelia D. Hawksw. 1985 (S) over Dendryphiopsis S. Hughes 1953 (A)**
The type species of Kirschsteiniothelia, K. aethiops, is congeneric with the type species of Dendryphiopsis, D. atra,
as demonstrated by the molecular phylogeny presented in Boonmee et al. (2012). Both species have been regarded as having various synonyms, but it now seems likely that this represents a species complex. Hughes (1958) treated D. atra as the asexual morph of *Amphishiaerella incurstans*, and the connection was confirmed by ascospore cultures (Hughes 1978), then using the generic name *Microthelia*, subsequently ruled as a *nomen rejiciendum* in favour of *Anisomeridium* nom. cons. Sequenced epitypes may be required to resolve the connections at the species level, as Boonmee et al. (2012) shows some material named as *K. aethiops* and *D. atra* to be distinct but congeneric species. *Kirschsteiniothelia* currently includes 17 species, with some recently added by Chen & Hsieh (2004), and Wang et al. (2004). *Dendryphiopsis* includes six names, one of which is recombined as *K. atra*. Given that *Kirschsteiniothelia* includes the most species and is now widely used, that name is proposed for protection.

**Use Lecanosticta Syd. 1922 (A) rather than Eruptio M.E. Barr 1996 (S)**

*Lecanosticta acicola*, an older name for the type species of *Lecanosticta*, *L. pini*, is now regarded as the name for the fungus that causes the widespread disease of pine known as brown spot needle blight. Previously this species had been referred to as *Scirrhia acicola* in the asexual morph and *Eruptio acicola* (the type species of *Eruptio*) and *Mycosphaerella dearnessii* in the sexual morph; all are now treated as synonyms of *L. acicola*, as evidenced by Crous et al. (2009b) and Quaedvlieg et al. (2012). Neither *Scirrhia* typified by *S. rimosa* (Crous et al. 2011a), nor *Mycosphaerella* now considered a synonym of *Ramularia* (see p. 518 below) are synonyms of *Lecanosticta*. The genus *Lecanosticta* includes eight names, while only the type species of the three names originally placed in *Eruptio* is currently retained in that genus. Given the widespread use of *Lecanosticta*, its priority, and the greater number of names, the use of *Lecanosticta* is recommended.

**Protect Paraneectria (Henn. ex Sacc.) Höhn. 1910 (S) over Araneomyces Höhn. 1909 (A)**

The type species of *Paraneectria*, *P. juruana*, is a relatively uncommon hyperparasite of stromatic leaf-inhabiting fungi in the tropics. Some authors have observed an associated asexual morph similar to the stauropsores of *Araneomyces*, possibly *A. acarifer*, the generic type (Rossman 1987, Kirschner et al. 2010). It appears likely that these generic names are synonyms. The two names in *Araneomyces* were moved to *Titaea* (Damon 1952), but Sutton (1984) considered *A. acarifer* and thus *Araneomyces* to be distinct from *Titaea*. The genus *Paraneectria* includes 10 names while only two names have been placed in *Araneomyces*. In addition, *Paraneectria* has been more widely reported than *Araneomyces* and no name changes would be required if it were used, thus *Paraneectria* is proposed for protection.

**Protect Phaeosphaeria I. Miyake 1909 (S) over Phaeoseptoria Spec. 1908 (A)**

The type species of *Phaeosphaeria*, *P. oryzae*, was shown to be congeneric with the type species of *Phaeoseptoria*, *P. papayae*, by Quaedvlieg et al. (2013). The latter authors reclassified a number of species in both genera placing some species of *Phaeosphaeria in Phaeoseptoria*. Over 200 names have been placed in *Phaeosphaeria* while *Phaeoseptoria* includes only 49 names. As *Phaeosphaeria* has a greater number of names and is more commonly used than *Phaeoseptoria*, *Phaeosphaeria* is proposed for protection.

**Use Phragmocapnias Theiss. & Syd. 1918 (S) rather than Conidiocarpus Woron. 1927 (A)**

The type species of *Phragmocapnias*, *P. betle*, was epitypified and classified as a member of *Capnodioaceae* by Chomnunti et al. (2011). Although molecular data were lacking, they followed Hughes (1976) who considered the type species of *Conidiocarpus*, *C. penzigii*, to be related to *Phragmocapnias* and transferred it to that genus; they therefore consider *Phragmocapnias* and *Conidiocarpus* to be synonyms. *Phragmocapnias* includes 13 names while only 10 names have been placed in *Conidiocarpus*. *Phragmocapnias* is more widely used for these species than *Conidiocarpus*, has priority, and includes the greatest number of species, so we recommend the use of *Phragmocapnias*.

**Use Phyllosticta Pers. 1818 (A) rather than Guignardia Vila & Ravaz 1892 (S)**

Both *Phyllosticta* and *Guignardia* have been widely used for ubiquitous leaf spot fungi on diverse hosts including black rot of grape (Farr & Rossman 2015). The relationship between the commonly encountered asexual morphs placed in *Phyllosticta* and the sexual morphs described in *Guignardia* is well known. Recent molecular research has also confirmed this relationship for such common species as *Phyllosticta macroliata* (syn. *Guignardia musae*) causing freckle disease of banana in Southeast Asia and Oceania (Wong et al. 2012) and *P. citricarpa* (syn. *G. citricarpa*) causing citrus black spot (Glienke et al. 2011). These species are also commonly encountered as endophytes in leaves of woody plants, especially *P. capillalensis* (Wikee et al. 2013b). The relationship between *Guignardia bidwellii*, conserved type of *Guignardia*, and *Phyllosticta amplexicida* has been known for several decades (Aa 1973) and has recently been confirmed using molecular data (Zhang et al. 2013). Placement of the type species of *Phyllosticta*, *P. convallarias*, which is now considered a synonym of *P. cruenta* (Aa 1973), in the same genus has also been shown using a multigene phylogeny (Motohashi et al. 2009). There is therefore no doubt that these two generic names are synonyms. Over 3000 names have been placed in *Phyllosticta* (Aa & Vanev 2002), while over 300 names have been placed in *Guignardia*. The asexual morph is most commonly encountered, and thus species of *Phyllosticta* are widely reported (Farr & Rossman 2015). Given the priority of *Phyllosticta*, the greater number of names, and its widespread use including a recent account (Wikee et al. 2013a), the use of *Phyllosticta* is recommended.

**Protect Podoneectria Petch 1921 (S) over Tetracrium Henne. 1902 (A)**

The generic name *Podoneectria* is typified by *P. coccicola*, a species that has been used for the biocontrol of scale insects on *Citrus* (Moore 2002). The asexual morph of *P. coccicola* is
Tetracrium coccicola, which appears morphologically similar to T. auranti, the type species of Tetracrium, a name with a sexual morph regarded as P. auranti (Rossman 1978, 1987). Although neither genus has been studied using molecular data, these generic names appear to be synonyms. At present 11 names exist in Podonectria, and nine in Tetracrium. Many of the species are, however, poorly known. Although Tetracrium is older, the name Podonectria has been widely used within the biocatalyst community and thus Podonectria is proposed for protection.

Use Polythrincium Kunze 1817 (A) rather than Cymadotheca F.A. Wolf 1935 (S)
The type species of Polythrincium, P. trifoli, is known as the cause of sooty blotch of clover occurring on leaves throughout temperate regions. The sexual morph of this species was described as Cymadotheca trifoli, the type of the monotypic genus Cymadotheca, thus these generic names are synonyms. Both names have been well-used in the literature, but Polythrincium features more commonly than Cymadotheca (in papers using these generic names). Given that there are five species names in Polythrincium, and just one in Cymadotheca, and priority, the use of Polythrincium is recommended.

Use Prillieuxina G. Arnaud 1918 (S) rather than Leprieurina G. Arnaud 1918 (A)
The generic name Leprieurina, typified by L. winteriana, was established for the asexual morph of Prillieuxina winteriana, the type species of Prillieuxina, in the same article; these names therefore have equal priority. Over 70 names have been placed in Prillieuxina, compared with just four in Leprieurina, so Prillieuxina is recommended for use. The type species have both been placed in Asterinella Theiss. 1912, typified by A. puiggarii (Speg.) Theiss. 1912, but the species in that genus have a distinct peridial morphology and Asterinella is not congeneric with Prillieuxina.

Use Prosthemium Kunze 1817 (A) rather than Pleomassaria Speg. 1880 (S)
The type species of Prosthemium, P. betulinum, is the asexual morph of the type species of Pleomassaria, P. siparia. The connection was initially based on morphology (Sivanesan 1984, Hantula et al. 1998) and later confirmed using molecular data (Tanaka et al. 2010). Although more names have been placed in Pleomassaria, a number of these have now been removed to other genera, and recent studies have used Prosthemium (Kamiyama et al. 2009). Based on its priority and recent use in the literature, the use of Prosthemium is recommended.

Use Pseudodidymella C.Z. Wei et al. 1997 (S) rather than Pycnopleiospora C.Z. Wei et al. 1997 (A)
The monotypic generic names, Pseudodidymella typified by P. fagi and Pycnopleiospora typified by P. fagi, were described in the same publication and so have equal priority. Both names remain obscure, and no subsequent reports were traced, so we recommend the use of Pseudodidymella.

Use Pyrenophora Fr. 1849 (S) rather than Drechslera S. Ito 1930 (A) or Marielliottia Shoemaker 1999 (A)
The type species of Pyrenophora, P. phaeocomes, has long been said to have a Drechslera asexual morph (Sivanesan 1987), although it remained unnamed. Recent studies place this species in a genus including the type of Drechslera, D. tritici-vulgaris, now regarded as P. tritici-repentis (Ariyawansa et al. 2014). Many previous authors had noted this relationship (e.g. Shoemaker 1959, 1962, Sivanesan 1987, Zhang & Berbee 2001, Crous et al. 2011b), but there is no doubt that Pyrenophora and Drechslera are generic synonyms. These fungi cause a number of important diseases on grasses, such as yellow leaf spot of wheat caused by P. tritici-repentis, and leaf blotch and head rot of oats caused by P. avenae. Both generic names are well known to plant pathologists. Many species of Drechslera, however, are now placed in the segregate genera Bipolaris, Curvularia, and Exserohilum (Sivanesan 1987, Manamgoda et al. 2012, 2014, 2015). More names have been placed in Pyrenophora than in Drechslera (199 vs 136 species epithets, respectively). Based on priority, the number of species, and recent usage, Pyrenophora is recommended for use.

The generic name Marielliottia, typified by M. biseptata, was established for three species segregated from Drechslera by Shoemaker (1999). Marielliottia biseptata (syn. Drechslera biseptata) as well as the two other species were shown to belong in Pyrenophora by Zhang & Berbee (2001) and Ariyawansa et al. (2014); Marielliottia is therefore considered a synonym of Pyrenophora.

The following new combinations into Pyrenophora are needed, based on the studies of Zhang & Berbee (2001), Crous et al. (2011b), and Ariyawansa et al. (2014):

Pyrenophora catenaria (Drechsler) Rossman & K.D. Hyde, comb. nov.
MycoBank MB8815092

Pyrenophora dematioidea (Bubák & Wróbl.) Rossman & K.D. Hyde, comb. nov.
MycoBank MB8815093

Pyrenophora fugax (Wallr.) Rossman & K.D. Hyde, comb. nov.
MycoBank MB8815094
Pyrenophora grahamii Rossman & K.D. Hyde, nom. nov.
MycoBank MB819095
Non P. phlei (E. Mull.) Crivelli 1983.

Pyrenophora nobleae (McKenzie & D. Matthews)
Rossman & K.D. Hyde, comb. nov.
MycoBank MB815096

Pyrenophora triseptata (Drechsler) Rossman & K.D. Hyde, comb. nov.
MycoBank MB815097

Use Ramularia Unger 1833 (A) rather than Mycosphaerella Johansen 1884 (S)
The very large genus Mycosphaerella, typified by M. punctiformis, has long been known to include a diverse range of relatively non-descript sexual morphs that cause leaf spots. This became more obvious as their asexual morphs were explored and determined to belong to numerous genera (Sivanesan 1984, Crous & Braun 2003). Crous et al. (2009b, 2011b) determined that M. punctiformis, now regarded as Ramularia endophylla (Videira et al. 2015a), belongs in Ramularia typified by R. pusilla, thus Mycosphaerella and Ramularia are synonyms. Crous et al. (2009b, 2011b) and others have also determined that most of the 1738 names placed in Mycosphaerella are not congeneric with the type of the genus such that many species have already been placed in segregate genera including Batcheloromyces, Delphinella, Passalora, Pseudocercospora, Sterellia, and Pseudocercospora amongst many others (Farr & Rossman 2015). Although Mycosphaerella includes many names, those that are congeneric with the type species are relatively few. Around 225 names in Mycosphaerella have been reported to be morphologically indistinguishable from M. punctiformis (Aptroot 2006) and are thus likely to belong to Ramularia. Given the extreme morphological convergence of the sexual morphs placed in Mycosphaerella and confusion associated with these names, the use of the generic name that has priority, Ramularia, for the species of Mycosphaerella sensu stricto as already reflected in the recent literature (Videira et al. 2015b), is recommended.

Use Schizothyrium Desm. 1849 (S) rather than Zygophiala E.W. Mason 1945 (A)
The type species of Zygophiala, Z. jamaicensis, was initially shown to be the asexual morph of Schizothyrium pomi by Batzer et al. (2005), although later Batzer et al. (2008) considered Z. jamaicensis to be distinct from S. pomi. Nevertheless Batzer et al. (2005, 2008), Gao et al. (2014), Li et al. (2010) and Ma et al. (2010) demonstrated that S. pomi and species of Zygophiala are congeneric. The type species of Schizothyrium, S. acerinum, is relatively unknown and has long been considered to be a synonym of S. pomi (Arx 1959). Schizothyrium includes over 50 names while Zygophiala includes only 11 names. These fungi cause sooty blotch and fly speck diseases such as S. pomi on the fruits of apple and pear. Based on priority, widespread use, and the greater number of names, the use of Schizothyrium is recommended. In the event that S. acerinum should eventually be typified and prove to not be congeneric with Z. jamaicensis, further disruption could be avoided by the conservation of Schizothyrium with S. pomi.

Based on the molecular phylogeny presented in Batzer et al. (2008), Li et al. (2010), Ma et al. (2010), and Gao et al. (2014), the following additional species are placed in Schizothyrium:

Schizothyrium cryptogamum (Batzer & Crous) Crous & Batzer, comb. nov.
MycoBank MB815098

Schizothyrium cylindricum (G.Y. Sun et al.) Crous & Batzer, comb. nov.
MycoBank MB815099

Schizothyrium emperorae (G.Y. Sun & L. Gao) Crous & Batzer, comb. nov.
MycoBank MB815100

Schizothyrium inaequale (G.Y. Sun & L. Gao) Crous & Batzer, comb. nov.
MycoBank MB815101

Schizothyrium musae (G.Y. Sun & L. Gao) Crous & Batzer, comb. nov.
MycoBank MB815103

Schizothyrium qianense (G.Y. Sun & Y.Q. Ma) Crous & Batzer, comb. nov.
MycoBank MB815104

Schizothyrium sunii Crous & Batzer, nom. nov.
MycoBank MB815102
Replaced name: Zygophiala longispora G.Y. Sun & L. Gao,


Use Sphaerellopsis Cooke 1883 (A) rather than Eudarluca Spreg. 1908 (S)
The confusion regarding the commonly encountered mycoparasitic species on rust fungi considered under the generic names Sphaerellopsis and Eudarluca has been clarified by Trakunyingcharoen et al. (2014). The type species of Sphaerellopsis, S. quercum, for which the oldest name is S. filum, was suggested to be congeneric with the type species of Eudarluca, E. australis, often considered a synonym of E. carici (Eriksson 1966). The name Sphaerellopsis was used in preference to Eudarluca by Trakunyingcharoen et al. (2014). Sphaerellopsis has the greater number of names, is most widely used, and has priority so the the use of the generic name Sphaerellopsis is recommended.

Use Sphaeropsis Sacc. 1880 (A) rather than Phaeobotryosphaeria Spreg. 1908 (S)
The generic name Sphaeropsis Sacc. 1880, typified by S. visci, has long been conserved against Phaeobotryosphaeria Lév. 1842 (Donk 1968) and over 600 names have been included in this conserved genus. Phillips et al. (2008, 2013) have now shown that S. visci has a sexual morph that they placed in Phaeobotryosphaeria within Botryosphaeriaceae. The type species of Phaeobotryosphaeria, P. yerbae, was examined by Phillips et al. (2008) but not placed within Sphaeropsis. Nevertheless these genera appear to be synonyms. Given its widespread use, the number of names, its priority, and the recent study of this genus, the use of Sphaeropsis is recommended.

Use Stemphylium Wallr. 1833 (A) rather than Pleospora Rabenh. ex Ces. & De Not. 1863 (S)
The type species of Stemphylium, S. botryosum, is considered the asexual morph of Pleospora tarda, cause of black mold rot and leaf blight on diverse hosts while the type species of Pleospora, P. herbarum, has an asexual morph referred to as Stemphylium herbarum, a widespread species (Ariyawansa et al. 2015). There is no question that these two generic names are synonyms. Although over 1000 names have been placed in Pleospora with only about 200 names in Stemphylium, many names initially placed in Pleospora have now been moved to other genera. Stemphylium is more widely used, especially by plant pathologists, and has priority, thus the use of Stemphylium is recommended.

Use Sydowia Bres. 1895 (S) rather than Hormonema Lagerb. & Melin 1927 (A)
The genus Sydowia, typified by S. gregaria, is congeneric with the type species of Hormonema, H. dematioides, now recognized as S. polymorpha by Hirooka et al. (2012). Twelve species are currently accepted in Sydowia while only seven are retained in Hormonema. Given the priority, the greater number of species, as well as the widespread use, Sydowia is recommended for use. This recommendation is contrary to that presented in Wijayaawardene et al. (2014) who considered these genera to be distinct. Protection of Sydowia would not, however, preclude the use of Hormonema by anyone wishing to follow an alternative taxonomy and recognize both genera.

Use Teratosphaeria Syd. & P. Syd. 1912 (S) rather than Kirramyces J. Walker et al. 1992 (A) and Colletogloboeopsis Crous & Wingfield 1997 (A)
The genus Teratosphaeria, typified by T. fibrillosa, has long been circumscribed by Crous et al. (2009b) to include species having asexual morphs placed in Kirramyces and Colletogloboeopsis. The type species of Colletogloboeopsis, C. nubilosum, was placed in Kirramyces by Andjic et al. (2007), which was supported by molecular data. The type species of Kirramyces, K. epicocoides (syn. T. suttonii), has also been sequenced and shown to be a species of Teratosphaeria (Crous et al. 2009b; Quaedvlieg et al. 2014). Teratosphaeria includes 90 names while Colletogloboeopsis includes only 9, and Kirramyces 19. As Teratosphaeria has priority and the most names, the use of Teratosphaeria is recommended.


This species causes a leaf spot and premature defoliation of Eucalyptus, as described by Taole et al. (2012) and Walker et al. (1992) who provide the synonyms listed here. Cercospora epicocoides provides the oldest epithet for this species, which is now placed in Teratosphaeria.
Use *Tetraploa* Berk. & Broome 1850 (A) rather than *Tetraplosphaeria* Kaz. Tanaka & K. Hiray. 2009 (S)

When Tanaka et al. (2009) proposed the generic name *Tetraplosphaeria*, they showed that the type species *T. sasicola* grouped closely with the type species of *Tetraploa*, *T. aristata*. This relationship was accepted by Hyde et al. (2013) and Wijayawardene et al. (2014), both of whom recommended the use of *Tetraploa* over *Tetraplosphaeria*. *Tetraploa* includes 20 names while *Tetraplosphaeria* has only four names, thus, having priority and the most names, use of *Tetraploa* is recommended.

Protect *Venturia* Sacc. 1882 (S) over *Fusicladium* Bonord. 1851 (A) and *Pollaccia* E. Bald. & Cif. 1947 (A)

The generic name *Venturia* is well known because of the ubiquitous disease of apple known as apple scab, caused by *V. inaequalis*, and for species causing other diseases especially on Rosaceae. *Venturia* is typified by *V. inaequalis*, which has an asexual morph referred to as *Fusicladium poni*, while the type of *Fusicladium, F. virescens*, has a sexual morph known as *Venturia pyrina*, the cause of pear scab (Sivanesan & Waller 1974, Schubert et al. 2003). The genus *Pollaccia*, typified by *P. radiosa*, was established for the asexual morph of *Venturia radiospora*. Using a molecular phylogeny Crous et al. (2007b) showed that *V. inaequalis*, *V. pyrina* and *V. radiosa* were congeneric, thus *Venturia, Fusicladium*, and *Pollaccia* are synonymous generic names. All three generic names have been used in reports of the diseases. The names in *Venturia*, however, are more widely known than those in *Fusicladium or Pollaccia* so *Venturia* is recommended for protection.

Based on the molecular phylogeny presented by Crous et al. (2007b) and Zhang et al. (2011), the following species should also be placed in *Venturia*:

**Venturia barriæ** Rossman & Crous, nom. nov.
MycoBank MB815108


Non *V. fagi* M.E. Barr 1968.

**Venturia catenospora** (Butin) Rossman & Crous, comb. nov.
MycoBank MB815110


**Venturia convolvularum** (Ondřej) Rossman & Crous, comb. nov.
MycoBank MB815111


**Venturia oleaginae** (Castagne) Rossman & Crous, comb. nov.
MycoBank MB815112

Basionym: *Cyclopeltis oleagineum* Castagne, Cat. Pl. Mars.: 220 (1845).

**Venturia phyllyraeae** (Nicolas & Aggény) Rossman & Crous, comb. nov.
MycoBank MB815113


Use *Zeloasperisporium* R.F. Castañeda 1996 (A) rather than *Neomicrothyrium* Boonme et al. 2011 (S)

The type species of *Zeloasperisporium*, *Z. hyphopodioides*, was recently show to be congeneric with the monotype species of *Neomicrothyrium, N. siamense*, by Crous et al. (2015b) and Hongsanan et al. (2015), thus these generic names are synonyms. Crous et al. (2015b) added another species to *Zeloasperisporium* while Hongsanan et al. (2015) described two further new species of *Zeloasperisporium* and placed *N. siamense* in *Zeloasperisporium*. Given its priority and greater number of species, *Zeloasperisporium* is recommended for use here.

**Genera Not Competing for Use**

Notes are provided below on generic names that were considered synonyms by Wijayawardene et al. (2014), but upon closer examination may not be. Until questions about the relationships between the type species involved are answered, no recommendations are made.

**Antennariella** Woron. 1915 (S) and **Antennariella** Bat. & Cif. 1963 (A) may not be synonyms

The type species of *Antennariella, A. fulignosa*, has been placed in *Wentiomyces*, a genus that includes 22 names while the type species of *Antennariella, A. unedonis*, is now considered a synonym of *Polychaeton brasilienne*, a name that should be placed in *Capnodium*. This suggests that these type species are not congeneric and thus the generic names *Antennariella* and *Antennariella* are probably not synonyms.

Use *Cyclopeltella* Petr. 1953 (A)

The monotypic generic names *Cyclopetlis* and *Cyclopeltella* were described in the same article by Petrak (1953), however, *Cyclopeltis* is a later homonym of the fern genus *Cyclopeltis* J. Sm. 1846 (*Aspidiaceae*), and thus cannot be used. Only *Cyclopeltella* typified by *C. orbicularis* Petr. is available for use.

**References**


Rossman A (2014) Lessons learned from moving to one scientific name for fungi. IMA Fungus 5: 81–89.


Sivanesan A, Holliday P (1971) Elsinöe canavaliae. Descriptions of
Pathogenic Fungi and Bacteria 313: 1–2.


EDITORIAL BOARD

Editor-in-Chief
Pro. Dr. D.J. Hawksworth CBE, Departamento de Biologia Vegetal II, Facultad de Farmacia, Universidad Complutense de Madrid, Plaza Ramón y Cajal, 28040 Madrid, Spain; and Department of Life Sciences, The Natural History Museum, Cromwell Road, London SW7 5BD, UK; Comparative Plant and Fungal Biology, Royal Botanic Gardens, Kew, Surrey TW9 3DS, UK; E-mail: d.hawksworth@nhm.ac.uk

Managing Editor
Pro. dr M. van den Brink, CBS-KNAW Fungal Biodiversity Centre, P.O. Box 85167, 3508 AD Utrecht, The Netherlands; E-mail: p.crous@cbs.knaw.nl

Layout Editors
M.J. van den Hooven-Verweij & M. Vermaas, CBS-KNAW Fungal Biodiversity Centre, P.O. Box 85167, 3508 AD Utrecht, The Netherlands; E-mail: m.van_den_hooven-verweij@cbs.knaw.nl

Associate Editors
Dr. EV. Andriason, M.G. Khlopkov Institute of Botany, Terschekshevskova Street 2, Kizhi, M-3, 16041, Ukraine; E-mail: tandi@darwin.msk.com

Prof. dr D. Begerow, Lehrstuhl für Evolution und Biodiversität der Pflanzen, Ruhr-Universität Bochum, Universitätsstrasse 150, Gebäude ND 01-1, 44780, Bochum, Germany; E-mail: dominik.begerow@rub.de

Prof. dr M. Berbee, Department of Botany, University of British Columbia, 3528-6270 University Boulevard, Vancouver, BC V6T 1Z4, Canada; E-mail: maryberbee@gmail.com

Dr. S. Cantrell, Department of Plant Pathology and Crop Physiology, Louisiana State University, Agricultural Centre, 455 Life Sciences Building, Baton Rouge, LA 70803, USA; E-mail: scantrell@lsgsu.com

Dr. P.S. Dyér, School of Biology, Institute of Genetics, University of Nottingham, Nottingham NG7 2RD, UK; E-mail: paul.dyer@nottingham.ac.uk

Dr Ana Esperanza Franco Molina, Instituto de Biología, Universidad de Antioquia, A.A. 1226, Medellín, Colombia; E-mail: anesperfranco@gmail.com

Dr. K. Hansen, Kryptogambietika Naturhistoriska Riksmuseet, Box 50007, 104 05 Stockholm, Sweden; E-mail: karen. hansen@nrsm.nrm

Dr. Pro. David Hibben, Biology Department, Clark University, Luty Biological Science Center, 950 Main St., Worcester, MA 01610, USA; E-mail: dhibben@clarku.edu

Prof. Dr. Xingzhong Liu, School of Biology, Institute of Genetics, University of Nottingham, University Park, Nottingham NG7 2RD, UK; E-mail: x.liu@nottingham.ac.uk

Dr. Janet Jennifer Diviningasia Laosanga, and National Center for Genetic Engineering and Biotechnology (BIOTEC), 113 Thailand Science Park, Phashomathin Road, Khlong Nueang, Khlong Luang, Pathum Thani 12120, Thailand; E-mail: jjen@biotec.or.th

Dr. Pro. W. Meyer, Molecular Mycology Research Laboratory, CIDM, ICMP, Level 3, Room 3141A, Westmead Hospital, Darcy Road, Westmead, NSW, 2145, Australia; E-mail: w.meyer@weyer.edu.au

Dr. Chihiro Nakashima, Graduate school of Bioresources, Mie University, Kurima-Machiya 1577, 514-8507, Tsu, Mie, Japan; E-mail: chihiro@bio.mie-u.ac.jp

Dr. Martin Riquelme, Department of Microbiology, Centro de Investigación Científica y de Educación Superior de Ensenada CICESE, Carrera Ensenada-Tijuana N. 3918, 22860 Ensenada Baja California, Mexico; E-mail: miquel@ci.cicese.mx

Pro. Dr. K.A. Sutti, Research Scientist / Biodiversity (Myco-logy, and Botany), Agricultural & Agri-Food Canada, K.W. Nearby Building, 960 Carling Avenue, Ottawa, ON. K1A 0C6, Canada; E-mail: sukti@agri.gc.ca

Dr. J.W. Taylor, Department of Plant and Microbial Biology, University of California, 111 Kohlhed Hall, Berkeley, CA 94720, USA; E-mail: jotayler@berkeley.edu

Dr. Pro. M.J. Wingfield, Forestry and Agricultural Research Institute (FABI), University of Pretoria, Pretoria 0002, South Africa; E-mail: m.j.wingfield@fabi.up.ac.za

Instructions to authors can be found on the IMA Fungus website http://www.imafungus.org/instruction.aspx
Editorial
Creative Commons, Open Access, and Living Cultures (37)

News
Major new research initiative to tackle life threatening fungal infections – Fungal Conservation and IUCN Red Listing (39)
- Volkswagen Foundation supports the African Mycological Association (AMA) to build mycological capacities in West Africa – FUNGEN – A national fungal genetic resource for India – Czech Culture Collection of Fungi (CCF)
- State Key Laboratory of Mycology (SKLM) in China celebrates 30 years – Fungi and Global Challenges – Advice to mycologists concerning Article 57.2 – Fungal Biodiversity Calendar 2017

Reports
Asian Mycological Congress (AMC) 2015 – 2nd Iranian Mycological Congress (IMyC2) – Essential Skills for Young Mycologists (45)

Awards and Personalia
Awards: The Gordon and Tina W asson Award (51)
- Birthday greetings: Katharina Bickerich-Stoll centenarian – Andreas Bresinsky’s 80th – Renowned lichenologist Irwin Brodo turns Eighty – Huub van der Aa at 80 years

Research News
Host-jumps drove rust evolution – Sensing host plant signals: a new role for pheromone-sensing machinery? – Fifty key events in fungal systematics (56)

Mycocenes
“Six simple guidelines for introducing new genera of fungi” by Else C. Vellinga, Thomas W. Kuyper, Joe Ammirati,

Correspondence
(69)

Book News
(70)

Erratum
(75)

Notices
(75)

Articles
“Phylogenetic placement of Itajahya: An unusual Jacaranda fungal associate” by Seonju Marincowitz, Martin P.A. Csertee, P. Markus Wilken, Brenda D. Wingfield, and Michael J. Wingfield 257
“Accepted Trichoderma names in the year 2015” by John Bissett, Walter Gams, Walter Jaklitsch, and Gary J. Samuels 263
“New sequestrate fungi from Guyana: Jimtrappea guayanensis gen. sp. nov., Castellanea pakaraimophila gen. sp. nov., and Costatisporus cyanescens gen. sp. nov. (Boletaceae, Boletales)” by Matthew E. Smith, Kevin R. Amses, Todd F. Elliott, Keiiske Obuse, M. Catherine Aime, and Terry W. Henkel 297
“Phytophthora boodjera sp. nov., a damping-off pathogen in production nurseries and from urban and natural landscapes, with an update on the status of P. alticola” by Agnes V. Simamora, Mike J. C. Stukely, Giles E. StJ. Hardy, and Treena L. Burgess 319
“Matsushimamyces, a new genus of keratinophilic fungi from soil in central India” by Rahul Sharma, Rohit Sharma, and Pedro W. Crous 337
“Phylogeny of Hirsutella species (Ophiocordycipitaceae) from the USA: remedying the paucity of Hirsutella sequence data” by D. Rabern Simmons, Ryan M. Kepler, Stephen A. Rehner, and Eleanor Groden 345
“New 1F1N Species Combinations in Ophiocordycipitaceae (Hypocreales)” by Joseph W. Spatafora, C. Alisha Quandt, Ryan M. Kepler, Gi-Ho Sung, Bhushan Shrestha, Nigel L. Hywel-Jones, and J. Jennifer Luangsa-ard 357

TABLE OF CONTENTS CONTINUED INSIDE BACK COVER