IMA Fungus

THE GLOBAL MYCOLOGICAL JOURNAL

Volume 3 · No. 1 · June 2012

NEWS · REPORTS · AWARDS AND PERSONALIA · RESEARCH NEWS
BOOK NEWS · FORTHCOMING MEETINGS · ARTICLES

Colofon

IMA Fungus
Compiled by the International Mycological Association for the world's mycologists.

Scope: All aspects of pure and applied mycological research and news.

Aims: To be the flagship journal of the International Mycological Association. IMA FUNGUS is an international, peer-reviewed, open-access, full colour, fast-track journal.

Frequency: Published twice per year (June and December). Articles are published online with final pagination as soon as they have been accepted and edited.

ISSN 2210-6340 (print)
E-ISSN 2210-6359 (online)

Websites: www. imafungus.org
www.ima-mycology.org
E-mail: d.hawksworth@nhm.ac.uk

Volume 3 · No. 1 · June 2012

Cover: *Afrocantharellus symoensii* Photo taken in Tanzania by Donatha D. Tibuhwa. See also p. 35 of this issue.

EDITORIAL BOARD

HOLISTIC MYCOLOGY – BACK TO BIOLOGY!

The fungal world is enchanting for its biodiversity, complexity, and beauty! A long career has taught me that Mycology breaks easily into segments of specialization. But it makes sense and adds overall value to keep it connected and to keep a stable eye on biology as a whole.

My mycological career started early. I was inspired by my father, Morten Lange (1919–2003), to hunt for edible or rare fungi from kindergarden age. I was treading in the steps of my grandfather (Jakob E. Lange, 1864–1941), looking for plants and "sporeplants". Even before school age, I told my parents that the first thing I would rescue from our house if it caught fire was the cabinet with the original *Flora Agaricina Danica* drawings. *Mycological teaching and the training of youngsters to see, distinguish, and name are the basis of cognitive learning, and art is one of its powerful instruments.*

During my doctoral studies, I had the privilege of working with some of the grand personalities of mycology: I was an assistant to Frederick K. Sparrow (1903–1977), who taught me the wonders of chytrids and other zoosporic fungi in the inspiring surroundings of Ann Arbor and Douglas Lake in Michigan State. Observations not only of static morphological characters, but also dynamic ones such as motility patterns, are needed to determine the genus and species; even major groupings can be distinguished just by looking at the zoospores. This way of thinking, merged with Ralf Emerson´s heritage, brought to me through my wise colleague and close friend Lee Olson, provided *the conceptual precursor to bioimaging, experimental physiology, and new fungal genetics.*

In Thomas A. Shalla´s (1933–1983) laboratory at the University of California in Davis[1]. I learned immunological and ultrastructural techniques, helping to visualize and understand interactions between plant viruses and fungi. *Mycology can be developed by transferring technologies from other scientific areas and most importantly by studying the interactions between fungi and other parts of the biological world (viruses, archaea and bacteria, plants, and animals).*

My first boss was Paul de Neergaard (1907–1987), a specialist on *Alternaria* and head of the Seed Pathology Institute of the Danish Ministry of Foreign Affairs in Copenhagen. He dedicated his life to collaborations with the developing world, meticulously and strategically working to spread knowledge globally – thereby empowering all to control the spread of seed-borne plant pathogens through knowledge of their life-cycles and biology: "you must understand the strategy of the enemy to fight it"! Paul entitled one of his books on seed pathology *Seed: A Horse of Hunger or a Source of Hope* (1986), with the message that: *we by solid research efforts carefully disseminated, can avoid detrimental attacks of fungal pathogens*. But for this, global collaboration is a requisite!

Working in the Institute for eight years with scientists from Asia, Africa and South America, in the aftermath of the green revolution, I realized that we had the knowledge but not the products to use it at the field scale. K.M. Safeeula of Mysore University, India, was my main role model in how to combine laboratory and field studies. I saw a need to develop biocontrol measures, as all could not be achieved by agrochemicals alone. I also saw the need for state-of-the-art equipment for quality mycological research. Novo industries made that possible. They were already a world leader in the biological production of enzymes from fungi and bacteria. Further, they had large solution-focused and interdisciplinary R&D groups. I was part of a team being established to initiate work on biocontrol agents to control plant pathogens and insect pests. *Biological processes and products are complex, but when mastered, evidence-based, and with suitable equipment, can lead to more sustainable biological solutions worldwide – unlocking the magic of Nature!*

My 20 years with Novo, Novo Nordisk, and Novozymes A/S, provided a platform for productive international collaborations. Japanese mycologists and natural product chemists provided a new dimension to the study of antifungal compounds from fungi, later strengthened by inspiring collaboration with Danish, Dutch, and not least, Australian mycologists. The development of new biological production methods using filamentous fungi brought me into contact with talented research groups in South America, China,

the USA, and Europe. *Mycology united! And respect mycological expertise wherever you meet it, as it transcends geographical and cultural borders, and seamless collaboration is possible between academic and industrial researchers.*

Fashion and technological trends are also a factor in science. I have lived through periods in which different techniques have been seen as overarching. Electron microscopy and ultrastructure gave their names to entire institutes and funding schemes. Likewise for protein engineering; all proteins could be developed in the laboratory. Genome sequencing subsequently took the scene and the funding, threatening to submerge biology in data. Now synthetic biology is starting to attract funding and fame. *Let us help each other to see technologies as tools and new and inspiring ways of gaining even more and deeper insight. But do not forget that the main discipline is biology, and the main questions to answer are biological.*

Genomics is not just a tool for evolution, phylogenetic systematics, and identification. It is not just what fungi are, how they are related, and where they came from. Genomics can also be used to elucidate what fungi do and how they interact in nature. But here the focus must be not just genomics, but transcriptomics and secretomics, focusing on interactions both within populations and with other kinds of organisms. Now back in academia, the fungal secretome

[1]Undertaking a part of my PhD studies in Davis brought another fortunate inspiration to my onward mycological life, having John W. Taylor as a PhD colleague.

is a focus in my new research group – the filamentous fungi´s way of interacting with the rest of the world. *Contributing to new insights which may in turn contribute to new and more sustainable solutions to important global problems.*

Today, we are in the most fantastic era of biology. We have an entire new tool box filled with marvelous, powerful techniques, many of which can be used remotely through international collaboration, virtual thinking, and decentralized sharing – all leading to empowerment. *Let us use this fantastic time to develop mycology in an integrated, biological, social and environmental context.*

Mycologists all over the world have important work to do. One of the most important tasks is to contribute, with a true sense of urgency, to the feeding of the world, and to develop new biological processes, products, and also to facilitate the shift from being a fossil based world to a sustainable, biobased and globalized society. Fungi have a major role to play, and some aspects are unfolded in the article on pp. 87–92 in this issue. However, to realize this vision, we need to work together. Across all parts of the mycological specializations, and on a globally embracing scale of collaboration (see pp. (6)–(7) in this issue). Much of the new talent and investment in mycology is right now in South America, Asia, and southern Africa; a movement already making a very positive and encouraging impact on the development of world mycology – adding expertise and new thinking to that available in North America and Europe. I believe we can do even more to collaborate across continents, cultures and traditions. And also that we still have much to learn from what former generations used fungi for.

Lene Lange
IMA Executive Committee
(lla@adm.aau.dk)

STOP PRESS!

Registries of names and the new *Code*

The *International Code of Nomenclature for algae, fungi, and plants,* ratified by the 18th International Botanical Congress in Melbourne last July, dictated that, as of 1 January 2013, each new fungal name must be registered in a recognized registry prior to publication. The *Code* leaves to the permanent Nomenclature Committee for Fungi (NCF), established by the Congress, the task of which registries to approve.

With the year during which we must decide how to implement efficient nomenclatural registration almost half over, NCF deliberations are deceptively muted. 'Muted,' because formal committee discussion over the mechanics of name registration and registries is only beginning, but 'deceptive,' because behind the scenes it appears we are on the cusp of an impressive international accord.

The International Mycological Association (IMA) Executive Committee members who met in Utrecht after the 1F = ?N symposium in April agreed that registration of new fungal names must be handled as efficiently and accurately as possible. The majority agreed that MycoBank (MB), the fungal name registry initiated in 2004–2005 at the Centraalbureau voor Schimmelcultures (CBS) and transferred to IMA jurisdiction in 2010, was the logical choice to serve as either sole or central nomenclatural registry for fungi. Many felt that multiple registries would be less reliable, less synchronous, and possibly proliferate illegitimate homonyms. That MycoBank (MB) was the first registry to assign identifiers for fungal names with MB registration already required by most major mycological journals was also persuasive. Some, particularly those whose first language and alphabet is not English, spoke passionately for multiple registries. Recognizing that multi-language portals to a central registry that would track all identifiers simultaneously are also desirable, the Executive asked IMA President John W. Taylor to recommend that the NCF approve MycoBank as either a sole *or* central registry. After polling all IMA executive members, President Taylor sent the approved letter to the NCF Secretary on 20 May 2012.

Before the Amsterdam and Utrecht meetings, most were unaware that other fungal registries were already in operation. While virtually every mycological author has consulted the formidable resource that is Index Fungorum (IF), relatively few know that it has issued fungal name identifiers since 2009. Index Fungorum and MycoBank have coordinated their identifiers so that there is no numerical overlap and each number remains unique, even without the MB or IF prefix. Fewer are aware that a third fungal name registry — Fungal Name (FN)— has been established in China and has issued identifiers since mid-2011 that are also coordinated by IF to prevent numerical overlap with IF and MB. As noted in Taylor's letter: "At the time of 1F = ?N, neither of these new registries [IF, FN) was effectively synchronized with MycoBank, such that all three sites had to be searched to learn about novel taxa, thereby increasing the likelihood of simultaneous publication of different fungi with the same name."

The recognition of the existence of three independent registries in Amsterdam prompted a desire to see whether they could work together. Communication channels have been opened while their developers collaborate on how best to facilitate synchronization among the registries and present the best user-friendly multi-language interface(s). After two months, we now believe that coordination among the three main registries is not only possible, but probable if logistical hurtles between software can be overcome. At this time, it appears that MycoBank could serve as the central repository recognizing that it is more commonly used by mycologists worldwide, and that it, Index Fungorum, and Fungal Name will collaborate to launch mandatory registration on January 1 with relatively few problems. A vote on the registries by the NCF will take place by the end of August following meetings in the USA and China.

We ask all mycologists, including lichenologists, to log onto the three sites to see what each offers and urge all of you who are not already doing so to register all new names now, whether or not a journal editor requires it. The more who learn how to register their names now, the easier it will be for everyone when name registration is required in 2013.

MycoBank — www.mycobank.org
[now available in English, Chinese, German, Arabic, French]
Index Fungorum — http://www.indexfungorum.org/names/IndexFungorumRegister.htm
Fungal Name — http://www.fungalinfo.net/fungalname/fungalname.html

Lorelei L. Norvell (*NCF Secretary*) **and Scott A. Redhead** (*NCF Chair*)
(llnorvell@pnw-ms.com)

IMA Fungus full content available in PubMed (2010 onwards)

Since June this year, *IMA Fungus: the global mycological journal* is searchable on PubMed (http://www.ncbi.nlm.nih.gov/pmc/journals/1750/). We trust that this will further enhance the visibility and accessibility of the journal. PubMed comprises more than 21 million citations for biomedical literature from MEDLINE, life-science journals, and online books. Citations may include links to full-text content from PubMed Central and publisher web sites.

IMA Fungus continues to also be available on its own website (www.imafungus.org) and via Ingenta Connect (http://www.ingentaconnect.com/content/ima/imafung).

Fear of Fungi

The global significance of the threats to human well-being and the maintenance of ecosystems posed by fungi are rarely appreciated by policy makers, scientists in general, or the public at large. Now, Fisher *et al.* (2012) have spelled out the threats in a well-researched and extensively referenced review article which made the cover of the 12 April 2012 issue of *Nature*. Seven fungi are highlighted: *Batrachochytrium dendrobatidis* (amphibian decline), *Magnaporthe oryzae* (rice blast), *Geomyces destructans* (white-nose syndrome of bats; see also pp. **(3)**–**(4)** below), *Puccinia graminis* (wheat stem rust), *Aspergillus sydowii* (sea-fan aspergillosis of corals), *Nosema* species (colony collapse in bees), and *Fusarium solani* (hatch failure in loggerhead turtle nests). That list is necessarily eclectic, and designed to indicate a range of situations, and some would have included *Phytophthora ramorum* (sudden death of oak) – and a correspondent was quick to add fungal infections of fish to the slate (Gozlan 2012).

The review notes that reports of fungal Emerging Infectious Diseases (EIDs) are increasing worldwide as a proportion of all EID reports, considers dynamics that can lead to host extinctions, the evolution of virulence, and environmental change as a driver. Among other points it also draws attention to the role or trade and transport in the globalization of fungi, and stresses the risk fungi prose to both food security and ecosystem health.

In order to mitigate the threats, there needs to be much more attention paid to monitoring fungal inocula in wild populations, tighter control of trade, and understanding of the interactions between a host, its pathogens, and the environment. The authors conclude with a call for scientists in disparate research fields to be involved in global discussions to work towards strategies for the prevention and timely control of fungal diseases. It is a call-to-arms, and the issues raised must start to be addressed by appropriate agencies at the intergovernmental and governmental levels. At least no-one will now be able to claim that they was no alert as to the risks and global impacts of fungal diseases.

Anyone in doubt as to the importance of fungi in world affairs today, should be immediately directed to this important review.

Fisher MC, Henk DA, Briggs CL, Brownstein JS, Madoff LC, McCraw SL, Gurr SJ (2012) Emerging fungal threats to animal, plant and ecosystem health. *Nature* **484**: 186–194.

Gozlan R (2012) Monitoring fungal infections in fish. *Nature* **485**: 446.

White-nose fungus kills around six million bats

The US Fish and Wildlife Agency issued a press release on 17 January 2012[1] stating that there was a growing trend in the numbers of bats across the USA which were being killed by the white-nose syndrome fungus, *Geomyces destructans*. The mortality rates in colonies ranged from 70–90 %, and have been reported to be as much as 100 %

[1]http://us.vocuspr.com/Newsroom/Query.aspx?SiteName=FWS&Entity=PRAsset&SF_PRAsset_PRAssetID_EQ=129322&XSL=PressRelease&Cache=True

and between 5.5 and 6.7 million bats were estimated to have been killed since 2006, when the first cases were recognized in a cave in New York State. The disease has now spread to 16 states in the US and four Canadian provinces.

Conclusive experimental proof that *Geomyces destructans* was the causal agent was published on 15 December 2011 (Lorch *et al.* 2011). That study established that direct exposure of bats to the fungus caused the disease, and the fungus was recovered from diseased bats, so fulfilling Koch's Postulates. The authors also demonstrated that the disease could be transferred directly from infected to healthy bats. Previous uncertainty as to whether the fungus was the primary cause of the disease had arisen as the fungus occurs on the skin of European bats but is not associated with mortality in the region. It now seems probable that the fungus was transported into North America from Europe, where the bats are resistant to the disease, into North America where the native bats had no such resistance to a fungus they had never previously encountered.

For further information and to follow this developing situation, which poses a huge threat to the continuance of many bat species in North America, consult postings on the Bat Conservation International website (http://batcon.org/).

Lorch JM, Meteyer CU, Behr M, Boyles JG, Cryan PM, Hicks AC, Ballmann AE, Coleman JTH, Redell DN, Reder DM, Blehert DS (2011) Experimental infection of bats with *Geomyces destructans* causes white-nose syndrome. *Nature* **480**: 376–378.

The Top 10 fungal pathogens in molecular plant pathology

Ascomata of *Zymoseptoria tricti* (syn. *Mycosphaerella graminicola*) on infected wheat leaves.

The journal *Molecular Plant Pathology* conducted a poll amongst fungal pathologists associated with the journal to determine which species were of the most scientific or economic importance. The species selected, in rank order, on the basis of 495 votes, were:

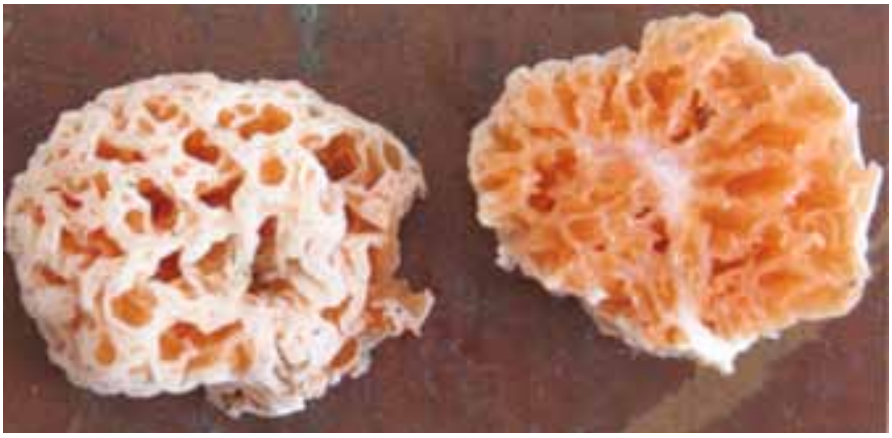
1 *Magnaporthe oryzae*
2 *Botrytis cinerea*
3 *Puccinia* spp.
4 *Fusarium graminearum*
5 *Fusarium oxysporum*
6 *Blumeria graminis*
7 *Mycosphaerella graminicola*
8 *Colletotrichum* spp.
9 *Ustilago maydis*
10 *Melampsora lini*

Phakopsora pachyrhizi and *Rhizoctonia solani* were the runners-up. The announcement of this result includes observations on each species and its significance (Dean *et al.* 2012) and os intended to stimulate debate amongst the plant mycology community.

Dean R, van Khan JAL, Pretorius ZA, Hammond-Kosack KE, Di Pietro A, Spanu PD, Judd JJ, Dickman M, Kahmann R, Ellis J, Foster GD (2012) The top ten pathogens in molecular pathology. *Molecular Plant Pathology* **13**: 414–430.

Botrytis cinerea on *Leucadendron* flower head.

Fungus makes the Top 10 species 2012



Spongiforma squarepantsii: Surface view and section of basidiome. Scale in mm. Photo courtesy Tom D. Bruns.

On 23 May 2012, the Spongebob Squarepants Mushroom, *Spongiforma squarepantsii,* a gasteroid bolete discovered in the Lambir Hills National Park in Sarawak, and described by Desjardin *et al.* (2011) was announced as one of the ten top species to be described in 2011. The selection is made by an international committee established by the International Institute for Endangered Species based at Arizona State University. The species are selected because they attract the attention of the committee for a variety of reasons, though perhaps the common and scientific names selected played a role in this case. The name recalls a resemblance to a North American Cartoon character, Spongebob Squarepants, who lives in a pineapple –

and the basidiome apparently has a fruity smell. Other organisms on the 2012 list include a monkey, jellyfish, nematode, orchid, wasp, poppy, millipede, cactus, and tarantula; all are figured on the Institute's website (http://species.ascu.edu/top10).

Two agarics made it into the 2011 list, as reported in *IMA Fungus* **2**: (2), 2011.

Perhaps *IMA Fungus* should run a parallel annual competition? If you would like to do that for the Association, please contact the Editor-in-Chief.

Desjardin DE, Peay KG, Bruns TD (2011) *Spongiforma squarepantsii,* a new species of gasteroid bolete from Borneo. *Mycologia* **103**: 1119–1123.

2013 CBS Spring Symposium – One Fungus : Which Gene(s) (1F = ?G)

The two important and successful CBS Spring Symposia, One Fungus = One Name (2011), and One Fungus = Which Name (2012) had great impacts on the mycological community. The CBS-KNAW Fungal Biodiversity Centre is now planning the 2013 Spring Symposium, One Fungus = Which Gene(s), now fixed for Wednesday-Thursday 10-11 April 2013. The main topic of the symposium will be to extend the concept of DNA barcoding to define how best to classify and identify fungi. Although a general consensus on the ITS barcode region has now been reached, it is important to clarify what additional gene(s) need to be targeted for specific fungal groups. Furthermore, best practices for obtaining and designating ex-type or ex-epitype isolates for whole genome analysis need to be addressed. The impact of fungal genome projects on fungal taxonomy and their utility for discovering new barcoding genes will be a focus, and the possible application of phylogenomic information to inform functional genomic annotation will also be discussed.

Contributed papers are welcome, and they will be selected for either oral or poster presentations.

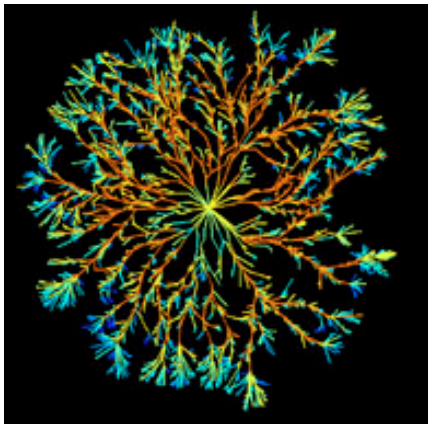
The venue, as for the previous two symposia, will be Trippenhuis, home of the Royal Netherlands Academy of Arts and Sciences, Amsterdam, and the registration fee is € 250 (which includes includes coffee/tea, lunches and a cocktail party). It is anticipated that on Friday 12 April meetings of the IMA Executive Committee, and of several ICTF and IUMS commissions and working groups will be held at the CBS in Utrecht, which will be concluded with a fungal barbeque.

Pedro Crous
(p.crous@cbs.knaw.nl)

Special issues of journals

Hyphal networks

The hyphal systems of fungi never cease to amaze in their complexity and adaptability. *Fungal Biology Reviews* **26**(1), April 2012, includes four review articles under the title "Hyphal networks: mechanisms, modeling and ecology". These address the self-fusion between conidial anastomosis tubes, analysis of fungal networks developed from block inocula, modeling of hyphal networks, and mycorrhizal networks. All contributions are by leading research groups in the field, and superbly illustrated. I also found the tabulation of the diverse mechanisms by which mycorrhizal networks may affect plant communities of value, and can see that being adapted for various taught courses. There is much to fascinate in the remarkable way these networks develop and function, and this read is a way of getting up-to-speed on this cutting edge research area in fungal biology,.



Phanerochaete velutina mycelium digitized and colour-coded to represent thicknesses of the major cords. From Boddy *et al.* (*Fungal Genetics and Biology* **47**: 522–530, 2010). Photo courtesy Mark Fricker.

Tropical fungi

The tropics are an immense store of unusual and undiscovered fungi, but our knowledge of them remains fragmentary. M. Catherine Aime and Francis Brearley have now put together a special issue of *Biodiversity and Conservation*, scheduled to appear as **21** (9) this August. It is anticipated that the issue will contain 12 original papers dealing with a range of groups of fungi, including aquatic fungi, lichen-fungi, mycorrhizal fungi, polypores, rusts, and trichomycetes, and also approaches to inventorying. Two previous special issues of the journal have been devoted to fungal diversity (**6**(5), 1997; and **16**(1), 2007) and these attracted considerable interest, and it is anticipated that this will also be the case with this number. Most papers are already available online-first *via* SpringerLink (www.

Favoleschia sp. nov. One of about 750 species of fungi new to science discovered in the Pakaraima Mountains of Guyana in 2010, and being featured on the cover of all 14 of the 2012 issues of *Biodiversity and Conservation*. Photo M. Catherine Aime.

springerlink.com/), and the possibility of making all open-access and free to download is under discussion at the time of going to press.

Endophytes

Two journals have recently issued special issues on endophytic fungi: *Fungal Diversity* **54**, May 2012, and *Fungal Ecology* **5** (3), June 2012. The *Fungal Diversity* issue has three review articles which concern the mediation of reactive oxygen species and antioxidants, the role of fungi in phytoremediation, and their value as a source of biocatalysts. Nine papers follow, on a wide range of aspects including cold adaptation, studies of particular species and endophytes of particular plants, and systematics. The *Fungal Ecology* issue, "The secret world of endophytes", has ten articles which range considerably in scope, but with an emphasis on the endophytic fungi of grasses in different regions, effects on the host plants, and also the production of alkaloids. The two issues are complementary rather than duplicatory, and are timely in view of the COST initiative recently launched on endophytes (see p. (7)).

Entomopathogenic fungi

Mycosystema **31** (3), May 2012 is devoted entirely to entomopathogenic fungi. It comprises 17 articles, almost all by Chinese authors, with the emphasis on species exploited for medicinal uses (particularly *Cordyceps* s. str. species) and of actual or of potential applications in biocontrol. The fungi considered in the latter category include species of *Beauveria, Metarhizium, Nomuraea, Paecilomyces,* and *Zoophthora*. Aspects covered include pathogenicity testing, marker genes for released strains,

gene cloning, screening, optimization of culture methods, and volatile products.

Mycophily, mycophilogy, and insect conservation

The grass *Festuca rubra* (red fescue) probably provides toxicity against predators to the butterfly *Melanargia galathea* (marbled white) by larval ingestion of pyrrolizidine alkaloids produced by the endophytic *Neotyphoidium* sp. Photos Roger Kemp.

The terms "mycophily" and "mycophilogy" have been proposed by Kemp (2010, 2011) for the association of fungi with living plants and animals, and the study of those associations, respectively. The author postulates that the chemicals produced by fungal endophytes in plants are required by or at least beneficial to the larvae of some insects, particularly butterflies and moths, for optimal growth. He then speculates that the declines in butterflies seen in the UK could be due to the loss of endophytic fungi in the host plants. There is clearly scope for the experimental testing of these novel cross-disciplinary ideas, and this could also encourage more entomologists and plant ecologists to take an active interest in the roles of fungi in the systems they investigate.

It should, however, be noted that in introducing the term "mycophily", Roger Kemp was not aware of the two previous uses of "mycophilic" for either a fondness for fungi (usually for food), or organisms growing on fungi.

Kemp RJ (2010) Mycophily – a new science for insect conservation. *Antenna* **34**: 13–15.

Kemp RJ (2011) Mycophily and its possible role in plant micro-distribution within habitats. *Botanical Society of the British Isles News* **118**: 23.

Global Mycology Initiatives

If you are interested in participating in the following project initiatives, please let us know at your earliest convenience. We do not have funding specifically for this, but want to get started anyhow. We have then two years to gather information before we hopefully can meet at IMC10 meeting in Bangkok!

Global Mycology Initiative I: Traditions, Technologies and Science

Topic: Fungal consortia used in food production (e.g. for production of soy sauce, fermented meat etc).

Objectives, Investigations and results: The basics: Description of production process (Starter culture? Enrichment culture? Process conditions? The experimental: characterization of microbial biodiversity, population dynamics, consortium signaling, secretome and transcriptome composition. Right now industrial biotechnology is basically one gene, expressed in one production host to produce one protein

which are sold as one product for one specific purpose. In future we will also be able to handle complex consortia to provide solutions for complex problems, e.g. conversion of biowaste materials. Here a comprehensive understanding of consortia would be very beneficial. Let us together move ahead of the business and share the interesting new knowledge, built on traditional cultural practices.

Join the initiative! Describe which system you are working on and what you can contribute with.

Global Mycology Initiative II: Enzymes from edible fungi

Topic: Enzymes, and enzyme expression and secretion, of cultivated, edible or medicinal fungi.

Objectives, Investigations and results: to obtain a more comprehensive understanding of the secreted enzymes from fungi, which can easily be grown on inexpensive substrates (e.g. old newspapers or straw). Edible and easily cultivated fungi with a rich enzyme profile and an efficient secretion systems may locally (in rural or other decentralized localities) be used for on-site production of enzymes for biomass conversion; allowing for low tech production of both feed, fuel, and fertilizer from biological waste or agricultural crop residues.

Join the initiative! Describe which fungal species and substrate you work on and which research technology you use and/or you are interested in using in the future.

Contact me or my science coordinator, Pia Haugaard Nord-Larsen (pnl@bio.aau.dk). with "Global Mycology Initiative" in the subject field of the message, to enabling us to search our mail box specifically for mails on this.

Lene Lange
(LLa@adm.aau.dk)

A network of European scientists investigating endophytic microorganisms: a new COST programme

Plants are associated with micro– and nano-organisms: endophytic bacteria and fungi, which live inter- and intra-cellularly in plants without inducing pathogenic symptoms, while interacting with the host biochemically and genetically. Endophytic microorganisms may function as plant growth and defence promoters by synthesising phytohormones, producing biosurfactants, enzymes or precursors for secondary plant metabolites, fixing atmospheric nitrogen and CO_2, or controlling plant diseases, as well as providing a source for new bioactive natural products with utility in pharmaceutical, agrochemical and other LifeScience applications. The use of these endophytic microorganisms to control plant-pathogenic bacteria and fungi is receiving increased attention as a sustainable alternative to synthetic pesticides and antibiotics. Furthermore, endophytes may be adapted to the presence and metabolism of complex organic molecules and therefore can show useful biodegradation properties. In order to reduce inputs of pesticides and fertilizers and add value to eco-friendly agriculture in Europe, it will be important to develop inocula of biofertilizers, stress protection and biocontrol agents. But there are currently bottlenecks limiting the development of endophytes for use in biotechnology and agriculture.

To increase understanding about these hidden associations between plants, bacteria and fungi, and to identify bottlenecks in the development and implementation of technologies using endophytes, a network of scientists was recently formed. This COST Action: "Endophytes in biotechnology and agriculture" will operate all over Europe during the next four years. COST (European Cooperation in Science and Technology) was founded in 1971 and is one of the longest-running European instruments supporting cooperation among scientists and researchers across Europe. The support of young researchers, scientific conferences and book publications are some of the activities which are organized by COST and paid for by the European Science Foundation. "My stay in Prague, Czech Republic, which was funded by COST, supported my trials very much. I was able to learn methods which I can now implement in my work at home", says Beate Ceipek, a young German researcher about her Short Term Scientific Mission at Czech Academy of Sciences.

This new COST Action will provide a forum for the identification of bottlenecks limiting the use of endophytes in biotechnology and agriculture and ultimately provide solutions for the economically and ecologically compatible exploitation of these organisms within Europe and beyond.

For more information on this Action and how you can become involved, visit the network's website (www.endophytes.eu).

Mark Stadler
(Marc.Stadler@t-online.de)

New funding for Australian medical mycology

The Molecular Mycology Research Laboratory at the Sydney Medical School, Westmead Hospital, University of Sydney, lead by Wieland Meyer has recently received two Australian National Health & Medical Research Council project grants to investigate two fundamental questions in modern mycology: (1) Which DNA region is the most appropriate one for DNA barcoding of human/animal pathogenic fungi, taking into account that there are serious limitations with the currently accepted barcode for fungi the ITS region; and the (2) What is the genetic basis of fungal virulence? The obtained funding, totalling 1 million dollars for three years, funds several postdoctoral and research assistant positions

The first grant is for collaboration between Wieland Meyer's laboratory, Vincent Robert (Bioinformatics Unit, CBS, Utrecht), and David Ellis (Mycology Unit, University of Adelaide). This project aims to identify the most appropriate loci for DNA barcoding by applying comparative bioinformatic genome analyses against all currently available fungal genomes. It will design loci-specific primers and test them against a broad range of fungi. The most informative loci will then be: selected and used to generate DNA barcodes; used to establish a reference barcode database; and applied as a tool in a diagnostic setting. The project's innovation lies in its use of comparative bioinformatics/genomics to determine novel universally applicable barcode regions and the build up of a barcode library, the first of its kind for human/animal pathogenic fungi, as a tool for fungal diagnosis. This has the promise of revolutionising fungal identification in medical diagnostic units, and reducing turn-around-time for species identification. This will allow earlier initiations of targeted antifungal therapy with improved patient outcomes. The barcodes will also be a key in providing border security with a novel tool to safeguard against fungal disease threats.

The second project is for collaboration between Wieland Meyer's laboratory, Gavin Huttley's laboratory working on genome analysis (Australian National University, Canberra), Helena Nevalainen's laboratory for fungal genetics (Macquarie University, Sydney), and June Kwon-Chung's laboratory for Medical Mycology (National Institutes of Health, Bethesda, USA). This project will compare the whole genomes of 16 high and low virulent cryptococcal strains, based on previously identified expression differences, to identify general virulence-associated genes. Knockout and animal virulence studies on a selection of the identified genetic candidate loci to establish the genetic basis of fungal virulence will be conducted, and used to generalise the cryptococcal findings by extending the whole genome comparison to other human/animal pathogenic fungi to establish fungal virulence-associated gene maps. The key knowledge generated in this project will provide the foundation of a greatly improved evidence base for the development of effective management guidelines for better patient outcomes, develop genetic markers to track the spread of fungal agents, and provide new targets for antifungal development.

Wieland Meyer
(w.meyer@usyd.edu.au)

China establishes State Key Laboratory of Mycology

October 2011 represented an important milestone in the history of research and development of Mycology in China. After a rigorous review by the Ministry of Science and Technology, the Institute of Microbiology, Chinese Academy of Sciences (IMCAS), has successfully obtained the final approval as the State Key Laboratory of Mycology (SKLM). The "state key laboratory" programme, which began in 1984, is an essential part of the national science and technology innovation system. It covers the major disciplines and has a high entry bar. Establishing this state key laboratory in China means a great deal for mycological studies because it brings with it sustainable funding from central government. This action will significantly increase the quality and quantity of the output of mycological research in China.

The history of the key laboratory can be traced back to the pioneer mycologists Fang-Lan Dai and Shu-Qun Tang, who established the first mycological research group in the Chinese Academy of Sciences (CAS) and trained generations of young scientists dedicated to fungal study. The CAS Key Laboratory of Systematic Mycology & Lichenology was established in 1985. Through 26 years' of development and extension in fields such as fungal systematics, ecology, genetics, metabolites, and other aspects, the CAS Key Laboratory has now been upgraded to the state level. The SKLM currently has 70 scientists including three CAS academicians and 18 principal investigators, and it will be further expanded to host 25 principal investigators in the next few years. The new Key Laboratory will highlight the following research areas: (1) fungal systematics and biodiversity; (2) fungal community and interaction; (3) fungal genetics and morphogenesis; and (4) the discovery, biosynthesis, and regulation of fungal secondary metabolites.

Lei Cai
(mrcailei@gmail.com)

FungalDC: a database on fungal diversity in genetic resource collections

FungalDC (Fungal Diversity in Culture Collections) assembles data on fungal diversity held in 264 collections of fungal cultures registered in the World Data Center of Microorganisms (WDCM) as well as ones held in individual laboratories around the world where catalogues are available. The database is open access through the All Russian Collection for Microorganisms (VKM) website (www.vkm.ru/fungalDC.htm), and provides current information on species by linking to the particular species/strain pages in sources such as: Index Fungorum (http://www.indexfungorum.org), MycoBank (http://www.mycobank.org), GenBank (http://www.straininfo.net), and StrainInfo (http://www.straininfo.net)

The integration of these resources mobilizes data from different databases, enabling their simultaneous use. FungalDC provides an opportunity to readily compare the diversity of fungal species available from collections and GenBank, to locate collections holding representatives of particular species and/or particular strains (including ex-type and authentic samples), and reveal species either omitted from the aforementioned information sources or not preserved in a collection as a living culture.

The ready availability of this information facilitates the location of living biomaterial for genetic studies. The data analysis shows that the fungal species diversity held in culture collections is represented only to a limited extent in GenBank (Table 1), thus indicating taxa where molecular studies could be rewarding

As the underlying data sources are constantly updated, the database also remains under constant change. A special format was developed to make it possible to perform real-time tracking to determine to what extent diverse fungal groups have been studied by molecular methods, and to identify type material of a particular species among the specimens studied. Each species name in the database is listed using the orthography of *Index Fungorum* and has the corresponding higher rank taxa indicated according to data from *Ainsworth & Bisby's Dictionary of the Fungi* (10th edn; Kirk PM *et al.*, 2008, Wallingford: CAB International). Further information is included in *Inoculum* **61** (3): 1–5 (2010).

We appreciated valuable comments from Bert Verslyppe and Peter Dawyndt (Ghent University, Belgium), P. Conrad Schoch (GenBank, National Institutes of Health/NLM/NCBI, Bethesda, MD, USA), Paul M. Kirk (CABI Bioscience, Egham, UK) and Vincent Robert (Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands). The work is supported by the Ministry of Education and Science of the Russian Federation (contract № 16.518.11.7035) and the programme "Molecular and cell biology" of the Russian Academy of Sciences.

S. M. Ozerskaya, N. P. Kirillova, and A. N. Vasilenko
(smo@dol.ru)

Table 1. Data in FungalDC

	Index Fungorum*	Collections of fungal cultures	GenBank
Number of genera	**19 705**	**3 728**	**4 087****
Number of species	**469 776**	**24 897**	**26 035**

*www.indexfungorum.org

**2 364 genera are common to the collections and GenBank

ONE FUNGUS = WHICH NAME ?

The special provisions that permitted asexual morphs of the same species of pleomorphic non-lichenized ascomycete and basidiomycete fungi to have separate names from that of the whole fungus, which was typified by a sexual morph, were ended at the International Botanical Congress in Melbourne in July 2011. These changes, that are embodied in the forthcoming *International Code of Nomenclature for algae, fungi, and plants*[1], followed after extensive debates and consideration by different committees, and in particular The Amsterdam Declaration[2]. The Declaration resulted from the "One Fungus = One Name" symposium organized by the CBS-KNAW Fungal Biodiversity Centre (CBS) under the auspices of the International Commission on the Taxonomy of Fungi (ICTF) and held in Amsterdam on 19–20 April 2011 (see *IMA Fungus* **2**: (7), 2011). Summaries of the changes which were introduced have been presented elsewhere[3, 4] and are not repeated here, but it is important that the published version of the *Code* is consulted for the final wordings.

Mycologists now have the tasks of implementing the changes in their own publications, and also contributing to the production of Accepted and Rejected Lists of names. Recognizing the uncertainties some mycologists expressed as how to proceed, and also the need to progress work on the Lists, CBS organized a follow-up symposium on "One Fungus = Which Name?" in the rooms of the Royal Netherlands Academy of Arts and Sciences in Amsterdam on Thursday and Friday 12–13 April 2012. The meeting was attended by 155 mycologists from 29 countries, almost all of whom were thrilled at the ending of the dual nomenclatural system and enthusiastic at the prospect of Accepted Lists which would place mycology at the cutting edge of biological nomenclature as a whole.

Each day of the symposium was organized in the form of a series of presentations in the morning, and discussion groups or debates in the afternoon. In a new venture aimed at making the presentations as widely available as possible, the talks were also videoed and made available *via* the Internet in real-time. Subsequently, a video-archive of the talks was compiled with a link to this through the CBS home-page to Youtube (http://www.youtube.com/playlist?list=PLF8BF8F71D5A3AEDC). It was gratifying that 220 mycologists watched the proceedings *via* the videolink while they were in progress, and that since the meeting there had been hundreds of downloads of presentations at the time this issue went to press. This means that hundreds of individual mycologists have so far been able to benefit from the full talks of the symposium and others still can do so.

There were 12 presentations in total, all of which are freely available in the video-archive:

One fungus which name: how do we proceed? (David L. Hawksworth, Spain/UK)[5].

Post-Melbourne fungal nomenclature: an overview (Lorelei Norvell,USA; Scott A. Redhead, Canada).

Why hyphomycete taxonomy is now more important than ever (Keith A. Seifert, Canada).

The nomenclature side of fungal databases, registration, etc (Joost A. Stalpers, The Netherlands; Paul M. Kirk, UK).

Single names in Hypocreales and Diaporthales (Amy Y. Rossman, USA).

Applications of old anamorph-typified names of genera and species (Uwe Braun)[6].

A strategy for fungal names with teleomorph-anamorph connections (Xing-Zhang Liu, China).

The future of fungal biodiversity research (Pedro W. Crous, The Netherlands).

Naming environmental nucleic acid species (ENAS) (John W. Taylor, USA).

The value of epitypification (Kevin D. Hyde, China/Thailand).

An official DNA barcode for fungi (Conrad Schoch, USA).

1000 fungal genomes and beyond (Joey Spatafora, USA).

A series of break-out group discussions, primarily focused on different fungal taxa, was held on the Thursday afternoon, and those groups were charged with reporting at the end of the next day. Prior to the presentation of these reports, which are reproduced below, an open discussion was held to clarify aspects of the new provisions or other matters that some present had found unclear, and further to ascertain the views of those present on various issues that needed to be addressed by those developing Lists and the Nomenclature Committee for Fungi (NCF) or ICTF; those discussions are also summarized below.

In addition to the formal parts of the symposium, two new books were formally launched at a cocktail party on the first evening. John W. Taylor (IMA President) was presented with copies of the *Taxonomic Manual of the Erysiphales (Powdery Mildews)* by Uwe Braun and Roger A. Cook, and the *Atlas of Soil Ascomycetes* by Josep Guarro, Josepa Gené, Alberto M. Stchigel, and M. José Figueras. Further information about these works is presented in the Book News section of this issue (pp. (35)–(36)).

[1]McNeill JM, Barrie FR. Buck WR, Demoulin V, Greuter W, Hawksworth DL, Herendeen PS, Knapp S, Marhold K, Prado J, Pru'homme van Reine WF, Smith GE, Wiersema JH, Turland NJ (eds) (2012a) *International Code of Nomenclature for algae, fungi, and plants (Melbourne Code) adopted by the Eighteenth International Botanical Congress Melbourne, Australia, July 2011.* [Regnum Vegetabile, in press.] Ruggell: A.R.G. Ganter Verlag.

[2]Hawksworth DL Crous PW, Redhead SA, Reynolds DR, Samson RA, Seifert KA, Taylor JW, Wingfield MJ [& 69 signatories] (2011) The Amsterdam Declaration on Fungal Nomenclature. *IMA Fungus* **2**: 105–112; *Mycotaxon* **116**: 91–500

[3]Hawksworth DL (2011) A new dawn for the naming of fungi: impacts of decisions made in Melbourne in July 2011 on the future publication and regulation of fungal names. *MycoKeys* **1**: 7–20; *IMA Fungus* **2**: 155–162.

[4]Norvell LL (2011) Fungal nomenclature. 1. Melbourne approves a new Code. *Mycotaxon* **116**: 481–490.

[5]Hawksworth DL (2012) Managing and coping with names of pleomorphic fungi in a period of transition. *Mycosphere* **3** (2): 52–64; *IMA Fungus* **3**: 15–24.

[6]Braun U (2012) The impacts of the discontinuation of dual nomenclature of pleomorphic fungi: the trivial facts, problems, and strategies. *IMA Fungus* **3**: 81–86.

The One Fungus = Which Name ? debate

Chair: David L Hawksworth
Rapporteur: John W. Taylor
Issues considered in this part of the meeting fell into two categories, a clarification of

concepts and possibilities, and view on topics where the ICTF and NCF would appreciate guidance.

Clarification of concepts and possibilities

(1) ***Names on an Accepted List are NOT conserved, BUT treated as if conserved***

Some speakers had used the term "conserved" for names that would be included on the Accepted Lists of names, but their status will not be identical to that of formally conserved names as, under the new *Code*, names included in the Lists of Conserved Names would have precedence over those on the Accepted Lists. Further, names that are formally conserved cannot be deleted, whereas there is no such restriction for names on the Accepted Lists. The meeting found this confusing, and felt that a different term should be found to replace "treated as if conserved." One possibility could be refer to names as "White-" or "Black-listed. It had also been suggested by Gams *et al.*[7], that the terms "prioritization" and "suppression" were preferable to help minimize possibilities of confusion, and that option should be referred to the NCF for consideration.

(2) ***What names can be included in the Accepted and Rejected Lists?***

There was uncertainty over the need to include names on Lists where there was no controversy or ambiguity. The Accepted Lists could include all names in use, including those where there was currently no dispute, as that would safeguard them from any earlier names that subsequently came to light. Alternatively, the Lists, could be restricted to cases where dual nomenclature had previously applied and which now had just one name.

It was felt that the Lists should be large enough to justify the time that would be spent on their preparation. The ideal would be a global checklist, though it was recognized that would not be realizable in the immediate future. However, there is no restriction on the ranks of names nor of taxonomic groupings. A List could be confined to all names in a particular rank, such as orders, families, genera or species, within a particular taxon. Alternatively, it could cover names at all ranks in use in a particular taxon. Thus, a List could deal with all accepted generic names of fungi, or just those in a particular order or family. It is really a matter for mycologists concerned with different groups of fungi to decide what protected Lists would be of most value to them and which should be prepared first. As there is evidently no obstacle to Lists being revised or replaced, unlike the situation with the already existing lists of conserved and rejected names, there could be some advantage in concentrating on generic names first, and adding species names at a later date.

There was almost unanimous and enthusiastic support for first producing a List covering all accepted generic names (including those of lichen-forming fungi, see below), whether or not they exhibited pleomorphism.

There was a strong feeling at the meeting that provisional Lists should be open for consideration by the community as a whole before submission, in order to iron out any controversy. It was suggested that draft Lists be put on the IMA website, with options for comment so as to work towards a consensus.

(3) ***Typification of names in Lists***

It is already possible to change the name-bearing type of a name by conservation, and there appears to be no obstacle to this in the new Lists. The new Lists can therefore include replacement types to deal with cases where well-known names have been misapplied, that bear both sexual and asexual morphs of the species when the previously designated type did not, or one has been sequenced and is widely available (for example as ex-type cultures).

(4) ***Terminology of specimens and cultures***

There had been some confusion over the terminology used for specimens and cultures other than name-bearing types by different workers. General usage is as follows:

Authentic: One named by the author of the name, generally after it was published, or, if the name is a combination, the author of the basionym.

Voucher: One used in a particular study, either for experimentation or to support an identification, enabling the same material to be used by or verified by later researchers.

Representative: One or more from a large set or specimens or cultures considered to serve as vouchers where it is impractical to preserve all those used or cited in a particular study.

(5) ***Continued use of binomials in synonymized genera***

There will be many cases in moving to one name per species in pleomorphic fungi, where it is uncertain whether all species currently under a particular name are congeneric with the type species of the generic name to be adopted. This situation is no different from that already occurring in non-pleomorphic genera where it has not been possible to ascertain the positions of all taxa previously referred to them. The *Code* does not rule on taxonomy, and, if there are no certain grounds to transfer a species from on genus to another, there is no nomenclatural obstacle to the continued use of the current name until the matter is resolved. This matter is discussed further elsewhere in this issue[8]. This situation is pragmatic not ideal, and one option used by some mycologists is to indicate in an informal way that a generic name is being retained in a wide sense, for example by the use of inverted commas, e.g. *'Mycosphaerella'* where it is unclear if the fungus is truly a *Cladosporium* (syn. *Davidiella*) in the new system. Wholesale uncritical transfer of names is to be discouraged.

(6) ***Who can prepare and submit Lists?***

There is no restriction on who can produce a draft List, and it could be an individual as well as formal or informal groups of mycologists. In view of the scale of the problem, the input of as many individuals as possible can only be welcomed. If you have information on particular families, genera, etc, prepare the first draft rather than wait and be angered by the content and quality of one someone else produces. However, be sure to inform the ICTF and NCF if you are willing to prepare a draft or contribute to a draft for a particular taxon so that duplication of effort can be avoided wherever possible. List preparation needs to be initiated quickly now to keep to the timetable necessary to achieve formal adoption at the 2017 congress[9].

[7]Gams W, Humber RA, Jaklitsch W, Kirschner R, Stadler M (2012) Minimizing the chaos following the loss of Article 59: suggestions for a discussion. *Mycotaxon* **119**: 495–507.

[8]Braun U (2012) The impacts of the discontinuation of dual nomenclature of pleomorphic fungi: the trivial facts, problems, and strategies. *IMA Fungus* **3**: 81–86.

(7) ***Operational dates***

There had been some confusion about when the one name for one fungus species system became effective, and in particular whether this was 30 July 2011 or 1 January 2013. The Preface to each edition of the *Code* now explains that all changes are immediately effective unless another date is indicated. This means that the special provisions ended on 30 July 2011, after which date all names of fungi compete on an equal footing, whether they are typified by material with the teleomorph or of the anamorph. The 1 January 2013 date in the new *Code* is there only to provide immunity to names published prior to that date that otherwise might be declared invalid or illegitimate. The use of a later date allows time for the change to be disseminated amongst researchers, and avoids works in press being contrary to the *Code,* i.e. introducing names that otherwise would be contrary to the *Code* and not available for use.

Issues requiring action or guidance

(1) ***Epitypes, teleotypes, and anatypes***

Epitypes are specimens selected to supplement a name-bearing type where that types does exist, but does not show the characters necessary to determine the species. An epitype is a formal category recognized in the *Code*, and once selected an epitype cannot readily be displaced. An increasingly common practice amongst mycologists is to designated as epitypes material that has been sequenced when no DNA could be recovered from the name-bearing type.

Redhead[10] had previously proposed the use of the term "teleotype" type as a special category of epitype selected to show the teleomorph when that was missing from the name-bearing type, but the proposal was withdrawn and not adopted at the Melbourne congress. Although Redhead did not propose it, logically the term "anatype" could also have been proposed for material selected to show the anamorph where that was not represented on the name-bearing type. As these two categories would not be epitypes, they could still be designated where there was already an epitype, and their existence would not preclude an epitype being selected subsequently where there was not.

The meeting rejected the idea of separate "teleotype" or "anatype" designations and considered that the type need not exhibit any particular morphology.

(2) ***The terms anamorph and teleomorph***

The issue of whether it was desirable or useful, when describing fungi, to continue to use the terms anamorph and teleomorph was also raised. These had been introduced into the *Code* at the Sydney congress in 1981 specifically for fungi that exhibited pleomorphism. The meeting felt that these terms were an unnecessary complexity, especially in teaching, and that they would be better dropped in favour of the familiar terms asexual and sexual, respectively.

(3) ***Defining widely used***

This issue was recognized as difficult, and the potential pitfalls in the use of the Google search engine in particular as an estimator of usage made it unreliable. Matches may not be exact for a variety of reasons. Google Scholar was considered probably better, if used critically. However, it was felt that experts in particular groups would have the best ideas of what was in the interests of mycologists as a whole. Those who disagreed, could make their own List for consideration, or comment on any posted. There was a strong view that applied usages and taxonomic usages were both important and neither should dictate.

(4) ***Evidence of holomorphy***

This was a matter considered too complex to debate in the session, but one on which guidance would be welcome. It was suggested that the ICTF should consider providing guidance on this matter.

(5) ***Using the conserved/rejected mechanism while Lists are in preparation***

The existing mechanisms for the conservation and rejection of names in the ranks of family, genus, and species would continue to operate while Lists were in preparation, revision, and proceeding towards formal adoption. There was therefore the possibility that decisions made on conservation or rejection might not be in accord with the Lists themselves. The NCF made clear that it would nevertheless still entertain conservation proposals, but that it would prefer to see lists with lots of names rather than proposals dealing with a single taxon.

(6) ***Inclusion of lichen-forming fungi***

Under the proposals adopted at the Melbourne congress, lichen-forming and allied fungi were excluded from the Lists. However, many considered this illogical, and the meeting voted unanimously for the deletion of this anomaly. It is clear that a formal proposal should be made to rectify this in the near future so that it can be considered by the NCF and approved by the General Committee in a timely manner so that lichenized taxa can be included where appropriate in the Lists.

(7) ***Use of subgeneric names***

The issue of whether mycologists should use the rank of subgenus more frequently, especially in large monophyletic genera, proved very controversial. Some were completely against any subtaxa, whereas others saw good grounds for the use of subgenera in particular cases. The use of subgeneric names was a way of maintaining name stability as the generic and specific names would not be changed. On the other hand, some felt this meant that users might have to learn three names rather than two, were subgeneric names regularly to be inserted in parentheses between a generic name and a species epithet. No consensus emerged, and this may be a situation where the matter is best addressed on a case-by-case basis.

(8) ***Registration of typifications and First Revisers***

There was a unanimous view that details of types designated after the original introduction of a new taxon should be deposited in the registering database at the time of typification. At present it was very difficult to locate later epi-, lecto-, or neotypifications. It was considered that

[9]Hawksworth DL (2012) Managing and coping with names of pleomorphic fungi in a perioid of transition. *Mycosphere* **3** (2): 52–64; *IMA Fungus* **3**: 15–24.

[10]Redhead SA (2010b) Proposals to define the new term 'teleotype', to rename Chapter VI, and to modify Article 59 to limit dual nomenclature and to remove conflicting examples and recommendations. *Taxon* **59**: 1927–1929.

this was an issue that the NCF should consider, with a view to requiring accredited repositories to record such information.

The Amsterdam Declaration had included the proposal that the first authors to make a choice of names when uniting anamorph- and teleomorph-typified genera should be registered and accepted, unless that was subsequently challenged – in which case it would have to be considered by the appropriate mandated body, i.e. the NCF. This concept is similar to the principle of the first-reviser in zoological nomenclature, but has not been used outside zoology. This provision was not part of the package adopted at the Melbourne Congress, but some of those present at the meeting did consider the matter nevertheless merited careful consideration, and perhaps could be discussed during IMC10 in Thailand in 2014.

There was also a lengthy discussion and interchanges between representatives of MycoBank and Index Fungorum on the issue of accreditation of repositories of nomenclatural data, which is required for the valid publication of new fungal taxa from 1 January 2013. In particular, there was a debate as to whether more than one repository should be recognized by the NCF. The meeting saw MycoBank as the logical immediate choice, but it also recognized the value of several centres, especially ones operating systems in different languages, such as Chinese. It also recognized the depth of nomenclatural detail in Index Fungorum and the key role that had in underpinning all fungal nomenclatural databases. If a distributed system were eventually developed, the meeting felt it was absolutely essential that there was data-sharing in a timely manner, and ideally in real-time, but at least on a daily basis.

(9) ***Proposal by Walter Gams***

Gams and colleagues had recently published a proposal that when a binomial in a prioritized genus had a younger epithet than the corresponding name in the suppressed genus, priority should be granted to existing names in the prioritized genus[11]. This principle already applies in zoological nomenclature, and had been adopted by some botanists in the past where it became known as the "Kew Rule"[12] – but this practice has not been permitted under the various editions of the botanical *Code*. Some of those present saw some advantages in this suggestion as a further means of minimizing name changes, but it was recognized that a formal proposal on this matter would have to be prepared for consideration by the NCF and a future congress. Gams indicated that he was encouraged by the comments and would explore this possibility further.

(10) ***Desirability of a joint NCF/ICTF/IMA dedicated Lists committee***

The officers of the NCF, ICTF, and IMA present at the symposium did not see the need or value of establishing a dedicated Lists committee. There was a strong dialogue between the parties, and some mycologists were members of more than one of these bodies. It was recognized that the NCF was the body with mandatory responsibility for making recommendations on any Lists prepared, while the ICTF had a role in List preparation, through its various subcommissions.

(11) ***Environmental sequences***

The increasingly urgent need to address the issue of the naming of fungal taxa only known from environmental DNA sequences had been considered at the One Fungus = One Name symposium in 2011, and some suggestions were made in the report of that meeting[13]. After some discussion, the ICTF agreed to establish a working group on naming environmental strains.

Working group reports

Basidiomycota
Rapporteurs: Scott A. Redhead and Dominik Bergerow
Participants: 19
The group split into one dealing with heterobasidiomycetes, and the other with homobasidiomycetes (*Agaricomycetes* s.str.). For the heterobasidiomycetes, a web page in which it would be possible to comment on each name separately should be set up, if possible with a voting option. Most of the problems in these fungi were considered to be taxonomic rather than nomenclatural. The real need was for more people writing papers. For example, it is general knowledge that *Cryptococcus* is paraphyletic, but no one was resolving the problem, which in any case should be addressed together with the yeast commission and the group on medicinal fungi. In the rusts, the solution should be close to current practice. I.e. to maintain the use of *Uredo* for species only known from the uredinial stage and without any current possibility of assigning them to a monophyletic genus. If *Uredo* was to be restricted to it's type species, there was a possibility that some would propose names that prove superfluous in an intermediate time-frame; this was not ideal, but an *ad interim* alternative.

In the case of the homobasidiomycetes (agaricomycetes), a working list could be generated shortly. When that was available, invitations to assist in the evaluation should be sent worldwide to all who had expressed interest in helping and an invitation will be sent to them to participate in the decision making process. Initial tables had been provided for the Amsterdam meeting by CBS, but it was recognized these were not complete. Further it was evident that while there were issues, many would be easy to decide on. Taking the first four generic names: one required research (*Abortiporus vs. Fibrilklaria*), one had an obvious solution (*Abortiporus biennis vs. Sporotrichopsis terrestris*), one no obvious solution (*Aleurodiscus habgallae vs. Matula poroniforme*), and one conservation (*Armillaria* vs. *Rhizomorpha*). As such cases could be resolved during the meeting, the group opted to start an online working group as soon as the logistics could be worked out. In each case the types for each of the generic or species names would need to be confirmed, and the links between the names needed to be questioned or confirmed. It was planned to have a first List available for comment by the end of 2012.

Dothideomycetes
Rapporteur: Kevin D. Hyde
Participants: 18
It was agreed that a web page for *Dothideomycetes* should be set up within a few months, and all proposed committee members would be contacted by email or other social media (e.g. connect website). Of key importance was the type species of

[11]Gams W, Humber RA, Jaklitsch W, Kirschner R, Stadler M (2012) Minimizing the chaos following the loss of Article 59: suggestions for a discussion. *Mycotaxon* **119**: 495–507.
[12]Stevens PF (1991) George Bentham and the "Kew Rule". *Regnum Vegetabile* **123**: 157–168.
[13]Hawksworth DL Crous PW, Redhead SA, Reynolds DR, Samson RA, Seifert KA, Taylor JW, Wingfield MJ [& 69 signatories] (2011) The Amsterdam Declaration on Fungal Nomenclature. *IMA Fungus* **2**: 105–112; *Mycotaxon* **116**: 91–500.

generic names, and it is with those than links should be substantiated; if correlations were with species other than the type, this needed to be made clear in a note on any List or in a supporting paper. Linkages should be based on sexuality/phylogeny, and if not the case needed to be well- argued. In general, the group considered that the oldest names should be given priority, regardless of the nature of their types. In cases where a younger name was prepared, the logic in support of the retention needed to be provided. The group considered that initial Lists could be published by September 2012, with a view to submission by January 2013.

Eurotiomycetes
Rapporteur: Robert A. Samson
Participants: 32
The group recognized that many genera in the class were important for applied mycology, so the nomenclature should be simple, stable and not confusing. It was also noted that applied researchers are likely to ignore nomenclatorial changes. The phylogeny of *Trichocomaceae* was now well-established, and the IUMS International Commission on *Penicillium* and *Aspergillus* (ICPA) planned to tackle other genera in the family as well. However, in the case of *Onygenaceae* collaboration with medical mycologists would be sought. It was anticipated that ICPA would produce a list of accepted names in *Penicillium* within a short time, but it was recognized the case of *Aspergillus* would require more discussion with users. In *Aspergillus,* there were several options: retaining the name for all aspergillae, splitting the genus and re-naming the groupings according to their teleomorph names, changing the type of the genus to *A. niger* so that did not change in a splitting, or to use *Aspergillus* with an optional descriptor. It was also pointed out in open discussion that there was in addition the possibility of using subgeneric names, which could be those of the teleomorph-typified names if adopted in the Accepted List; it while names at the rank of subgenus or section could not be conserved under the *Code*, the Lists had no such rank restriction.sa noted that names in indicator. These matters would be discussed at a meeting of ICPA scheduled for the Saturday after the symposium, and open o all through the commission's website (www.aspergilluspenicillium.org).

Medical mycology
Rapporteurs: Sybren de Hoog and Vishnu Chaturvedi
Participants: 9
It was considered that the International Society for Human and Animal Mycology (ISHAM) should implement a democratic procedure to achieve a stable result, which would be adopted quickly by the entire community. There was a consensus for a practical approach, taking the needs of the user as the starting point. The community of medical mycologists must first decide which names we without doubt want to keep: for example, *Candida albicans* and *Aspergillus fumigatus* should be maintained, and *Trichophyton* used rather than *Arthroderma*. There could also be many other classical pathogens and opportunists that we wish to keep the current names for and which should be proposed for inclusion on an Accepted List. An important criterion over the choice of a name will be how frequently it has been used. However, "widely used" is an unclear criterion. How does one establish whether *Scedosporium* is more current than *Pseudallescheria*? For each name put forward, the reasons for the proposed retention should be specified. In cases where no single name was strongly favoured, the oldest name (whether anamorph- or teleomorph-typified) should have priority. For example: *Aspergillus* is older than *Neosartorya*, and therefore the *Neosartorya* species should be termed *Aspergillus* in the future.

Reclassifications can be phenotypic or molecular phylogenetic, but the key criterion of a group is the monophyly. The clade determines the group meriting a genus name, preferably the oldest available for that group is used, as for *Aspergillus*. Molecular taxonomy may reveal groups where all experts agree that they are clearly monophyletic, and also share essential characteristics such as pathogenicity or antifungal susceptibility, as in the yeasts. However, there are also groups where so many new data – often of environmental relatives – are being added, that the phylogeny is highly unstable, as in the rapidly developing black yeast taxonomy. The group felt it could be prudent to propose that for the time being we leave names as they are, even if some "genera" are polyphyletic. In the case of established but poorly differentiated genera, such as *Acremonium,* some may be highly polyphyletic and thus phylogenetically ambiguous. There was a proposal to abandon such generic names, but an alternative would be to redefine them in a modern sense on the basis of accessible type material.

The community of medical mycologists, including the ISHAM membership, is requested to propose Lists of preferred names on the basis of the above criteria. The names of many fungal pathogens have an ancient history and have become a source of confusion over the years. We therefore urge taxonomists, if necessary, to (re)define the groups of fungi they are working with by the deposition of (new) type material that can be protected in the Lists. As a first step, an *ad hoc* group has decided to provide a list of fungal names in current use based on the *Atlas of Clinical Fungi*[14] for the ISHAM membership to comment on (comments to be sent to: s.hoog@cbs.knaw.nl). The group hoped to have active involvement of as many medical mycologists as possible.

Sordariomycetes
Rapporteur: Joey Spatafora
Participants: *ca* 30
In discussing the criteria to be used to choose between two generic name options, considerations should include: taxonomic clarity (i.e. the genus name should be well circumscribed), the morphology most commonly encountered, names used in plant pathology and industry (etc), quarantine issues, stability, and relevance. The credentials of a particular taxonomist needed to be made clear when making a decision on a particular group. The strength of an argument should consider the number of name changes, monophyly, that names represented clades not morphologies, distinguish taxonomic and nomenclatural issues, consilience, and historical uses, and the possibility of retaining genera but with a different type species. Should there be a preference for names that commemorated the history of a taxon (e.g. *Cordyceps*) or ones that were history (e.g. *Tolpocladium*). It was felt that several subgroups would be needed: *Xylariales*; *Magnaporthales*/*Diaporthales*; *Fusarium*; *Hypocreales* I (*Bionectriaceae*, *Nectriaceae*, *Hypocreaceae*, and *Niessliaceae*; *Hypocreales* II (*Cordycipitaceae*, *Clavicipitaceae*, and *Ophiocordycipitaceae*); *Sordariales* and allies; and *Colletotrichum.*

[14]de Hoog G S, Guarro J, Gené J & Figueras M J (2000) *Atlas of Clinical Mycology*. 2nd edn. Utrecht: Centraalbureau voor Schimmelcultures.

Scenes from the One Fungus = Which Name symposium held in the Trippenhuis, headquarters of the Royal Netherlands Academy of Arts and Sciences, Amsterdam, on 12–13 April 2012.

Scenes from the One Fungus = Which Name symposium held in the Trippenhuis, headquarters of the Royal Netherlands Academy of Arts and Sciences, Amsterdam, on 12–13 April 2012; the launch and presentation of the *Atlas of Soil Ascomycetes* and *Taxonomic Manual of the Erysiphales (Powdery Mildews)* to John W. Taylor (IMA President); and the sun drenched Fungal BBQ at the CBS, following committee meetings on Saturday 14 April 2012.

IMA Executive Committee Meeting

In mid-April 2012, The Netherlands again turned into an international centre for mycology and the IMA Executive Committee met on 14 April 2012 in Utrecht parallel to meetings of the International Commission on the Taxonomy of Fungi (ICTF) and the International Commission on *Penicillium* and *Aspergillus* (ICPA). This was the largest Executive Committee meeting ever held between IMC congresses, and illustrated the amount and importance of activities organized by the Association, under the presidency of John Taylor. The meeting covered all aspects of advancing mycology on a global scale, and here I just wish to highlight a few of the points, which were discussed and decided.

First, the Executive Committee congratulates the winners of our young mycologist awards, which were finally completed with announcements on the two outstanding. The Elias Magnus Fries Medal was awarded to Cécile Gueidan (nominated by the European Regional Mycological Member Organization) and the Carlos Luis Spegazzini Medal to Luís Fernando Pascholati Gusmão (nominated by the Latin American Regional Mycological Member Organization; for further information see p. (25) in this issue. While the young mycologist awards are designated to the early years of a career and honours outstanding mycological research by young scientists from our regional member organizations, the Executive Committee also searches for ways of acknowledging substantial support of mycology by others. The introduction of a category of IMA Fellows as a midcareer award received great support from the Executive Committee, and guidelines will be available soon, so that a first round of mycologists can be recognized in this way during IMC10 in Bangkok.

Although the finances of the IMA are robust, we seek further external funding to increase our capabilities. While there is quite substantial support from external funding during our congresses, the IMA would like to attract companies and institutions to become patrons of the IMA for a yearly fee. The profits of several large international companies are based on fungi or fungal products, and the IMA supports the development of a closer link between research and economy. Mycology will become a big business in the future, and financial support to our work is highly appreciated.

To increase visibility and to provide better support for mycology worldwide, the Executive Committee agreed on the further development of our IMA Newsletter and the redesign of our webpage. IMA should reach all mycologists, worldwide, on a regular basis and information should be widely distributed in the age of the internet and free information exchange. Beside the issues of *IMA Fungus* volumes, the Executive wishes to enhance the Newsletter, and the option to subscribe will be highlighted much more often than has been the case before. In addition, we ask all members to contribute to the content of the IMA Newsletter and also to *IMA Fungus* to further increase the international visibility of global mycology.

Finally, the Executive Committee acknowledged the progress being made in the organization of IMC 10, which is to take place in Bangkok in 2014. Leka Manoch reported on progress made during the last year. Most exciting was the change of venue to the Queen Sirikrit National Convention Center, which will allows a great congress in Thai style. The Organizing Committee is already hard at work, with Leka Manoch and Morakot Tanticharoen as co-chairs. The call for symposia will be made soon, and the Executive Committee suggested that there should be seven concurrent sessions per day, two for fungal diversity, and one for each of the following themes: fungal cells, fungal genomes, fungal ecology, fungal pathogenesis and fungal biological technology. In addition there would be nomenclature sessions held on three days, as at IMC9. The congress aims to reflect the best of international mycology, and the needs of our communities.

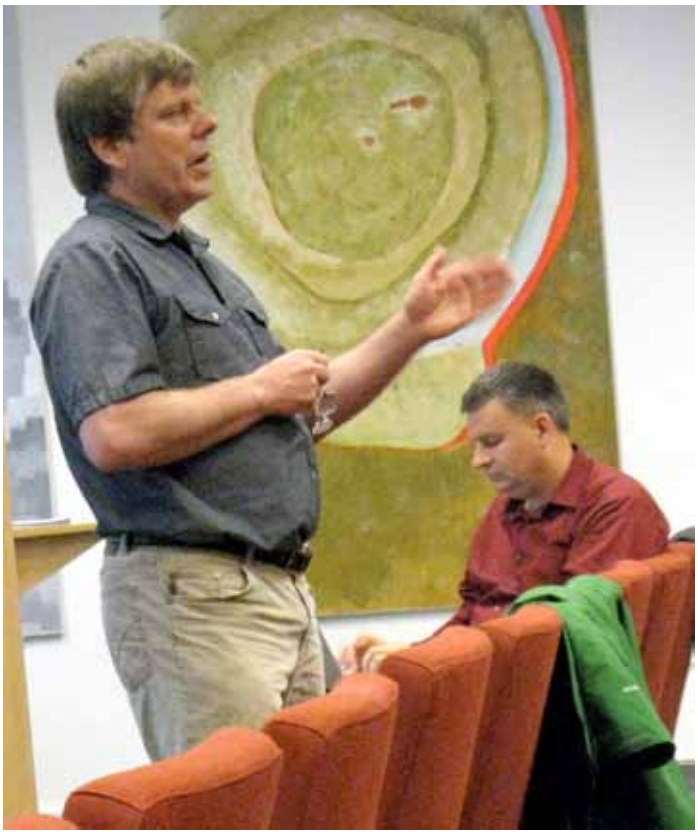
During the intensive discussions by the Executive Committee, a Skype conference was arranged to facilitate the participation of members who could not attend in person, so broadening the basis for discussions and decisions. Mycology is global, and the Executive Committee would like to get all of you who read this involved in discovering the future.

Dominik Begerow
(*Secretary-General, IMA*)

International Commission on the Taxonomy of Fungi (ICTF). 2012 General Meeting

(dominik.begerow@rub.de)

The International Commission on the Taxonomy of Fungi (ICTF) held a general meeting at the CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands on Saturday, 14 April



Keith Seifert (standing) and Andrew Miller (seated) at the ICTF meeting in Utrecht.

2012 following the "One Fungus : Which Name?" symposium held in the Royal Dutch Academy of Arts and Sciences in Amsterdam. Eleven members of the Commission attended the meeting, an unusually high turnout for this group at a meeting outside an IMC. Approximately 20 observers also attended, some of them taking an active role. With an ambitious programme of work already in front of it, the added expectation that the ICTF and its subcommissions will play active roles in the nomenclatural exercises currently developing made this meeting particularly relevant.

The ICTF website (www.fungaltaxonomy.org), has been hosted at the Technical University of Vienna by Irina Druzhinina and her colleagues since IMC7 in Oslo in 2002. Andrew Miller offered to host the website at the Illinois Natural History Survey (INHS) at the University of Illinois, and this transition is now complete. The ICTF plans to extend the contents of the website considerably, with a view to making it more attractive. The mandate of the ICTF is both to support fungal taxonomists and to provide information and tools that will be useful to those wishing to learn more about this subject. Until now, the website has primarily been a repository for the minutes of the Commission, links to the websites of subcommissions, and a small amount of other information. Plans are now being implemented to enhance the website with more visual information, to develop and make available information on good taxonomic practices (such as the article "How to describe a fungus", *IMA Fungus* **1**(2): 109–111, 2010), news items of general interest to fungal taxonomists, and other similar content.

The need to coordinate information on nomenclatural working groups addressing the changes in the International Code of Nomenclature (ICN) is discussed elsewhere in this issue of *IMA Fungus.* Some of these working groups will conduct their operations and post their draft lists of protected or rejected names on the ICTF website. Our intention is that links to all such working groups who develop their own websites, or those operating from the MycoBank website, will be listed on an ICTF webpage, allowing it to function as a starting point for taxonomists wishing to participate in these exercises.

The relationship between the ICTF, with its focus on promoting fungal taxonomy, and the Nomenclature Committee for Fungi (NCF), with its focus on nomenclature, was the topic of much discussion in Amsterdam and still seems to be a source of some confusion. For the nomenclatural exercises, the two bodies are cooperating as much as possible. While the ICTF envisions assisting in the coordination of the nomenclatural working groups in their preparation of lists, the NCF is the ultimate authority who will be making the final recommendations on the acceptance of these lists to the General Nomenclature Committee, a body appointed by the Melbourne International Botanical Congress in 2011. The existing subcommissions of the ICTF on *Penicillium* and *Aspergillus*, *Fusarium*, and *Trichoderma* and *Hypocrea*, are already actively leading the nomenclatural activities on these genera. We are particularly excited at the formation of new subcommissions on *Colletotrichum* (initiated by Cai Lei and Bevan Weir), and on rusts (initiated by Cathy Aime and José Dianese). Other nomenclatural working groups being formed will interact with the ICTF and the NCF as appropriate during their work.

One of the duties of the ICTF is to organize symposia and sessions at international meetings that will promote advances in fungal taxonomy to a broader scientific audience, as well as promote standards within the fungal community. For the 2014 IUMS congress in Montreal, Canada, we intend to organize a session addressing the changes to the names of economically important fungi resulting from the application of the new ICN. Further, we will propose a symposium on the interaction of genomics and taxonomy, which we hope will include presentations by bacteriologists, and virologists, as well as mycologists. The IMC10 in Bangkok, Thailand, will be held only a few days after the IUMS meeting, but will undoubtedly attract a larger but different crowd of mycologists. For IMC10, more detailed presentation and discussion of the nomenclatural lists will be organized by the ICTF, in collaboration with the NCF as appropriate. The ICTF will also offer a series of after lunch workshops on "Good Practice in Fungal Taxonomy", presenting information on microscopy, culturing, molecular methods, data analysis and other aspects of fungal taxonomy that would lead to a useful set of publications or exercises on the ICTF website.

Acknowledgement: We appreciate financial support from the IUMS Executive Board, which enabled Andrew Miller to attend the 2012 meetings in The Netherlands.

Seifert KA, Rossman AY (2010) How to describe a fungal species. *IMA Fungus* **1**(2): 109–116.

Keith A. Seifert (*Chair ICTF*),
Andrew N. Miller (*Secretary ICTF*)
(amiller@inhs.illinois.edu)

CBS Course Medical Mycology – Chinese edition

An international CBS Course on "Medical Mycology" was organized in Nanjing, China, on 19–27 November 2011. The course was a joint effort of the Chinese Society for Microbiology (CSM), the Chinese Society of Dermatology, and the CBS-KNAW Fungal Biodiversity Centre. The *Atlas of Clinical Fungi*[15] was used as the laboratory manual. This book is now also available in the Chinese language on a CD-ROM. A dedicated practical software was developed on fungal terminology, in order to assist Chinese participants in learning how to pronounce English and Latin names correctly. Eight specialist speakers from all over China were invited, while Sybren de Hoog gave presentations on biodiversity. The 70 participants that attended the course came from many parts of China, Taiwan, Hong Kong, and Indonesia. Their current positions were in hospital laboratories as clinicians, medical microbiologists, and medical technicians. The course was devoted to the identification of pathogenic and opportunistic moulds and yeasts. A large and representative set of organisms was offered for practical work and to introduce the participants to fungal diversity.

Sybren de Hoog
(s.hoog@cbs.knaw.nl)

Hidden Danger, Bright Promise: 4th Meeting of the ISHAM Working Group on Black Yeasts

A very successful meeting of the ISHAM-affiliated Working Group on Black Yeasts was held in Curitiba, Brazil, on 1–4 December 2011. Themes included new concepts on symbiotic interactions of black yeasts, bioremediation, extremophiles, and current overviews of diseases in humans and animals. There was much time for debate among scientists and clinicians, particularly on human diseases with significant impact such as chromoblastomycosis, a disease with impressive records in Brazil and China. Novel data on the lethargic crab disease in the *Uca* crab population at the northeastern Brazilian coast were also presented. A Brazilian Black Yeast Network was also introduced.

The presentations were organized in themes. The opening speech was by Sybren de Hoog with an overview of the latest achievements and future questions, followed by Flávio Queiroz-Telles who introduced the Brazilian Network. Sanjay Revankar reported on his recent experience on the MSG Phaeohyphomycoses Network, in cooperation with the ISHAM Working Group Fungiscope. Other themes, such as the biotechnological potential and biodiversity of melanized fungi, recent progress in melanin research, and the development of compounds with antifungal activity were debated. The workshop updated knowledge on treatment of diseases caused by black yeast infections.

A visit to the hospital of the Paraná State Federal University was part of the programme. Live patients with chromoblastomycosis and mycetoma were shown and discussed. On the last day a visit to a mangrove area was organized in order to draw the participants' attention to the natural habitat of edible crabs where currently a black yeast epizootic is taking place.

The meeting had 73 full participants from 11 countries, and comprised 43 speeches and 18 posters, with a broad diversity of topics showing recent results in taxonomy, molecular techniques, identification and diagnosis of clinical and environmental agents, besides genome analysis data. Elec-

[15]de Hoog G S, Guarro J, Gené J & Figueras M J (2000) *Atlas of Clinical Mycology*. 2nd edn. Utrecht: Centraalbureau voor Schimmelcultures.

tronic abstracts and lectures in PDF format are available on the website (http://www.blackyeast.org/Curitiba/report.html) and a film of the entire meeting can be viewed on YouTube.

From this event, new doors were opened for international and intercontinental cooperation involving both clinicians and scientists. At the end of the workshop a list was presented with all (about 20) full genomes that are currently being sequenced and annotated by different consortia, and plans were made for experimental reproduction and detection of agents of chromoblastomycosis in environmental sources.

The next meeting of the Black Yeast Working Group will be a symposium at the ISHAM Congress in Berlin on 12 June 2012, and a full meeting is planned in 2013 in Guangzhou, China.

Vania Vicente, Sybren de Hoog, Derlene Attili de Angelis, and Flávio Queiros Telles
(s.hoog@cbs.knaw.nl)

International cooperation in zygomycete research

Following the great success of the meeting of the ECMM-ISHAM Working Group Zygomycoses in Athens, Greece, in May 2010, a Special Interest Group meeting was organized in conjunction with IMC9 in Edinburgh in August 2010. Kerstin Voigt and Sybren de Hoog were privileged to organize this pre-conference meeting that was attended by 20 mycologists from seven countries (Egypt, Germany, Japan, Poland, Taiwan, The Netherlands, and the United Kingdom). The aim of the meeting was to bring together mycologists working in various areas of the zygomycetes, to share recent discoveries, to establish an international network for discussion, and to exchange materials and sequences. The plan is to build up a database allowing rapid and reliable identification of species, leading to understanding of ecology, routes of infection, and food safety.

Five presentations demonstrated divergent themes in research on morphology, systematics, phylogeny, physiology, and etiology of zygomycetes, and underlined their growing importance as agents of disease. An alarming rise in the incidence of zygomycosis was noted worldwide, especially in Asia and South America. Tropical climates seem to favour the manifestation of mucoralean infections. The percentage of cases of zygomycoses increased over the past seven decades from 0 % to above 70 % , as documented by cultures (Roden *et al.* 2005). However, during the same period mortality decreased from almost 100 % to below 40 % due to improved diagnostics (Roden *et al.* 2005). Therefore, correct identification and reliable diagnostics were major themes in the SIG meeting. It became evident that the taxonomy and phylogenetic reconstruction of the zygomycetes is changing fundamentally with the application of molecular methods, particularly ones involving the ITS and the D1/D2 domain of the large subunit (LSU) nuclear ribosomal DNA as barcoding and phylogenetic markers. The ITS domain is the preferred region for species distinction.

In the first presentation, Sybren de Hoog (CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands) pointed out that ITS and LSU sequences do not fully determine the species level, because significant intraspecific variability is observed. Generic circumscription is also difficult, with ITS dissimilarities of up to 30 % observed between species of the same genus. *Mucorales*, the most prominent order of zygomycetes, was recently raised to the rank of subphylum, underlining the molecular divergence of these organisms. De Hoog advocated a multigene approach which also utilizes protein-coding genes and their diagnostic power hidden in the introns, supplemented with classical mating experiments. Members of *Mucorales* are ubiquitous in homes, bath- and bedrooms, as well as in refrigerators and pantries. They are known to have an ecological "hit-and-run-strategy", which means that they arrive on virgin food sources prior to most other microbes, eat fast, grow fast, and get away before competing microorganisms arrive. This strategy leads to rapid spoilage of unattended food batches by abundant production of extracellular enzymes. Within hours, they form a disgusting hairy felt on fruits, vegetables, and cereal products alike. Humans have learnt to manipulate the decomposition process. Especially in Asia a wide variety of mucoralean fungi is applied for pre-digestion of fermented foods, such as soy sauce or Indonesian tempeh. However, de Hoog drew the attention to a possible downside to the use of *Mucorales* in food preparation, since the order also harbours confirmed causative agents of mycoses. Human infections tend to produce severely disfiguring and often fatal symptomatologies. These infections have been encountered particularly in patients with severe underlying disease, such as ketoacidotic diabetes or leukemia, but recently a species was found consistently causing chronic skin infections in otherwise healthy patients in East Asia. Inappropriate therapy of such lesions due to poor diagnostics of the causative agent of the infection may lead to fulminant growth and severe mutilation. Agents of these destructive infections in part belong to the same species that are used for food preparation. An example is *Rhizopus microsporus*, where the varieties classically maintained for food preparation and those responsible as agents of severe disease appear to be identical. Further research is needed to establish whether pathogens are consistently being used to prepare food.

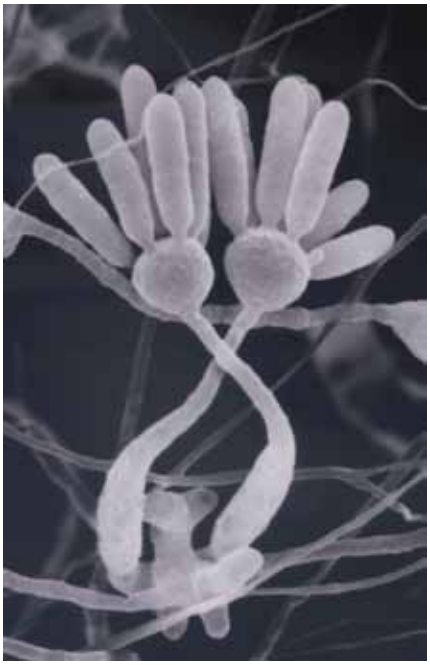


Fig. 1. *Syncephalis parvula* (*Piptocephalidaceae*, *Zoopagales*), SEM micrograph. Photo courtesy Hsiao-Man Ho.

The status of zygomycete research in Taiwan outlined from historical and contemporary points of view was presented by Hsiao-Man Ho (National Taipei University of Education, Taiwan). Special emphasis was placed on thermotolerant species in *Mucorales* with a potential to

cause human infections. The study of zygomycetous fungi in Taiwan started in the 1920s, and since that period a number of local mycologists recorded 38 genera with 123 species. The fungi comprise the following nine families, with their most prominent genera between parentheses: *Chaetocladiaceae* (*Chaetocladium*), *Dimargaritaceae* (*Dispira*), *Kickxellaceae* (*Coemansia*, *Linderina, Ramicandelaber*), *Lichtheimiaceae* (*Lichtheimia*), *Mortierellaceae* (*Mortierella*), *Mucoraceae* (*Absidia*, *Gongronella*, *Cunninghamella*), *Pilobolaceae* (*Pilobolus*, *Utharomyces*), *Piptocephalidaceae* (*Piptocephalis*, *Syncephalis*), and *Thamnidiaceae* (*Thamnidium*, *Thamnostylum*). The morphological beauty of the zygomycetes is demonstrated exemplarily for *Syncephalis parvula* (Fig. 1) and *Zygorhynchus moelleri* (Fig. 2). At present, most of the zygomycete research is carried out in the mycology laboratory of Hsiao-Man at the National Taipei University of Education. Species identification is based on morphological characters combined with ITS, LSU-D1/D2, SSU data for most of the taxa.

Kerstin Hoffmann (Jena Microbial Resource Collection, Department of Microbiology and Molecular Biology, Institute of Microbiology, Jena, Germany) gave an overview of the zygomycetes as emerging pathogens in recent years. Traditionally, the phylum *Zygomycota* has been divided into two classes, *Zygomycetes* and the *Trichomycetes* (Alexopolous *et al.* 1996). However, since the *Zygomycota* appeared to be polyphyletic, multi-gene based phylogenies suggested the elimination of the classical *Zygomycota* as a separate phylum and its subdivision into five distinct subphyla: *Mucoromycotina*, *Entomophthoromycotina*, *Kickxellomycotina*, *Zoopagomycotina* (Hibbett *et al.* 2007) and the newly described *Mortierellomycotina* (Hoffmann *et al*. 2010). Members of *Entomophthoromycotina* produce indolent subcutaneous and mucocutaneous infections in immunocompetent hosts, whereas the *Mucoromycotina* mostly cause rapidly progressing, fatal and often systemic infections in immunocompromised or severely debilitated hosts (Voigt *et al.* 1999, Ribes *et al.* 2000). Members of *Mucorales* are very significant in hospital settings. Of a total of 205 known species in the order, 25 species, belonging to the genera *Apophysomyces*, *Cunninghamella*, *Lichtheimia*, *Mucor*, *Rhizomucor*, *Rhizopu,s* and *Saksenaea* have been reported to be pathogenic, whereas only 4 four out of a total of 277 species described in *Entomophthorales* are reported as causing infection. Within *Mortierellales,* only a single species was found to be clinically relevant, *Mortierella wolfii,* causing abortion in cattle. Infection routes are variable, including inhalation, ingestion or direct inoculation into pre-damaged tissue. Ketoacidotic diabetes, burns, major surgery, severe trauma and immune disorders trigger the establishment of mucoralomycoses. Roden *et al*. (2005) listed malignancy, organ transplantation, desferoxamine therapy, injection drug use, bone marrow transplantation, renal failure, and malnutrition as additional risk factors, in order of decreasing significance. A relationship between predisposing factors and type of infection was reported, demonstrating that diabetes, malignancy, and desferoxamine therapy predispose for rhinocerebral, pulmonary, and disseminated infections, respectively. Differences between entomophthoromycoses and mucormycoses can be shown in virulence tests using a hen egg model (Fig. 3). While the mucoralean fungus *Rhizopus oryzae* produces a 40 % mortality at day six in hen egg embryos, infection with the entomophthoralean fungus *Conidiobolus coronatus* resulted in 60 % mortality of the embryos within one day, using comparable spore concentrations.

The hen egg model for testing virulence appears to be particularly suitable for large scale assessments of the pathogenic potential of zygomycetes. Ilse D. Jacobsen (Department of Microbial Pathogenicity Mechanisms, Leibniz Institute for Natural Product Research and Infection Biology - Hans-Knöll-Institute, Jena, Germany) gave a summary of embryonated eggs as an alternative infection model to study virulence. She emphasized that zygomycetes are increasingly recognized as pathogens in both humans and animals. However, relatively little is known of their pathogenesis and virulence. Infection models for zygomycetes have only been described in a very few species. Based on her experience with embryonated eggs as alternative infection model for *Candida albicans* and *Aspergillus fumigatus* (Jacobsen *et al.* 2010, Olias *et al.* 2010), Jacobsen elucidated the suitability of this model for species of *Lichtheimia* (formerly *Absidia*; Hoffmann *et al.* 2009, Alastruey-Izquierdo *et al.* 2010), using *L. corymbifera* as the reference species. Eggs were infected on developmental day 10 on the chorioallantoic membrane (CAM) with 10^6 to 10^2 spores (n = 20 per dose and experiment). Survival was determined daily by candling, a standard method which allows visualization of embryonic structures and movement by applying a strong light source to the surface of eggs. Mortality upon infection with the reference strain was dose-dependent, with infectious doses of 10^6 to 10^4 spores per egg resulting in 95–100 % mortality within two days. 10^3 spores per egg killed 70–80 % of infected eggs, and the LD_{50} was found

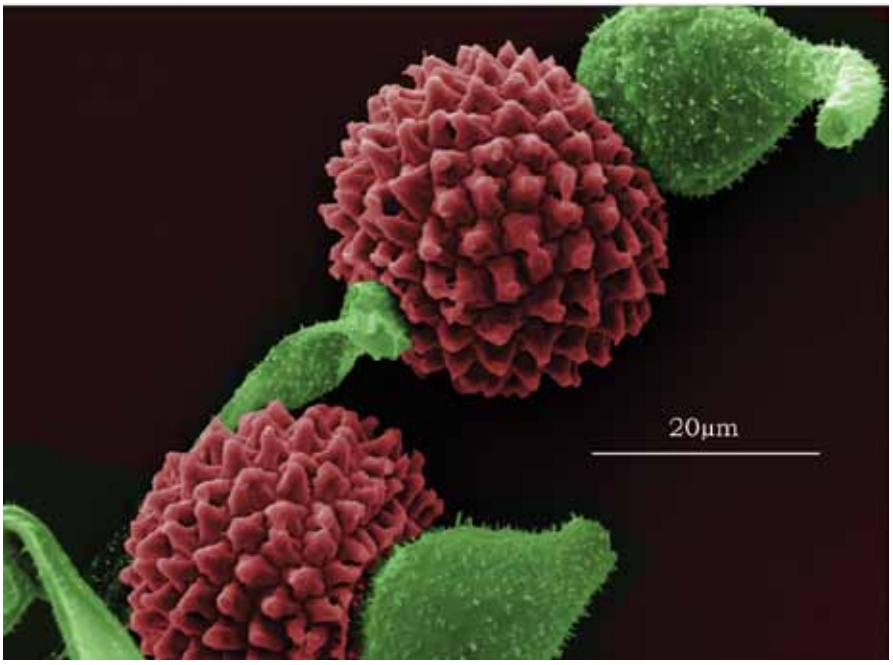


Fig 2. *Zygorhynchus moelleri* (*Mucoraceae*, *Mucorales*), SEM micrograph. Photo Martin Eckart and Kerstin Hoffmann.

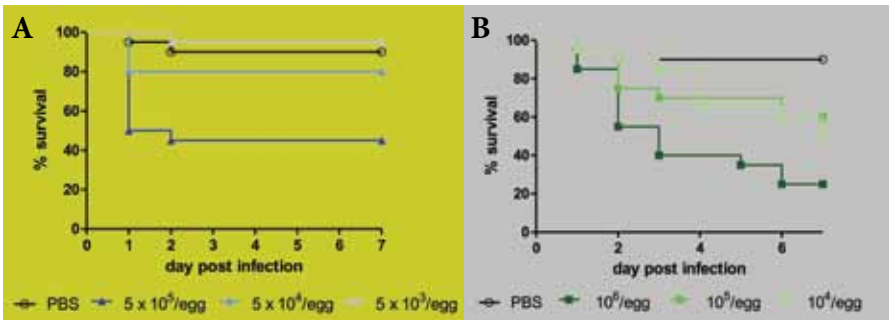


Fig. 3. Virulence tests in the hen egg model. Survival of embryos by application of *Conidiobolus coronatus* (A) and *Rhizopus arrhizus* (syn. *R. oryzae*) (B). Courtesy Ilse Jacobsen and Volker Schwartze.

to be 10^2 spores per egg. These results were highly reproducible (2–4 experiments per infectious dose). *Lichtheimia corymbifera* could readily be re-isolated from the CAM of infected eggs, while the CAM of PBS-mock infected controls remained sterile. The three clinically relevant *Lichtheimia* species complexes, *L. ramosa*, *L. corymbifera,* and *L. ornate,* displayed a comparable virulence potential in embryonated eggs. In contrast, the *L. sphaerocystis* and *L. hyalospora* complexes were significantly attenuated in comparison to *L. corymbifera*. The embryonated egg model is reproducible, inexpensive, easy to handle and does not require specialized facilities. It could serve as alternative model to analyse the virulence potential of different zygomycetes and to directly compare the virulence potential between species, strains and isolates. As the model allows determination of fungal burden, histological analyses and measurement of the host's cytokine response, it can also be used to assess potential pathogenicity mechanisms.

Guido Fischer (Arbeitsmedizin, Umweltbezogener Gesundheitsschutz, Landesgesundheitsamt Baden-Württemberg, Stuttgart, Germany) introduced "Fungiscope - a Global Rare Fungal Infection Registry" and its services for the scientific community. The registry is supported by the pharmaceutical industry as well as by scientific communities (as an ISHAM working group) and is hosted at the University of Cologne (www.fungiscope.net). While the registry focuses on the detailed documentation of cases of rare infectious fungi from different taxa, a number of zygomycete infections have been included. Of 41 recently published cases of zygomycote infections (Rüping *et al*. 2009), 63.4 % occurred in patients with malignancies, 17.1 % in patients with diabetes mellitus, and 9.8 % in patients having undergone transplantation. Diagnosis of zygomycete infection was made by culture in 68.3 % and/or histology in 63.4 % of the cases. The sites of infection were: lung (58.5 %), soft tissue (19.5 %), rhino-sinuorbital region (19.5 %), and brain (14.6 %). In 82.9 %, a targeted treatment against zygomycetes was applied and the overall survival rate of patients was 51.2 % (Rüping *et al*. 2009). All strains collected within Fungiscope are stored in the collection of the mycology laboratory of the State Health Office Baden-Württemberg (LGA-BW, Germany) and were re-identified by morphology-based methods to cross-check the initial identification in the hospital. In addition, all strains were sequenced at CBS. 29 % (4 of 14) of the identifications carried out in the respective centers were incorrect at the genus level; 50 % of the strains had only been identified to that level. *Lichtheimia corymbifera* was the most frequent infectious agent (6 of 14) with a preference for lung infection, followed by *Rhizopus microsporus* and *R. oryzae* (each 3 of 14), and two single isolates of *Mucor racemosus* and *M. circinelloides*. From these findings, two questions could be raised: (1) how reliable is the statistics on clinical cases reported in the literature for different fungal taxa?; and (2) does the correct identification have any implication for therapy? For the cases reported here, application of liposomal amphothercin B was associated with a higher survival rate (*cfr* Rüping *et al.* 2009). For *Rhizopus microsporus/oryzae* infections, the ratio of fatal outcomes tended to be higher than that of *Lichtheimia corymbifera* infections. In general, antimycotic therapy of zygomycetes is difficult because: (a) clinical and microbiological diagnosis of zygomycete infections is difficult in practice, while species may have different susceptibility profiles; (b) zygomycetes grow very quickly causing fulminant infections; and (c) zygomycetes are resistant to some azoles, except posaconazole, and may show reduced susceptibility to amphotericin-B. Exposure prophylaxis may be relevant to high-risk patients, as infectious zygomycetes occur ubiquitously in the environment. Effective risk assessment is based on knowledge of fungal concentrations in the environment and of possible sources of infection. Quantitative data were presented at the SIG meeting from Fischer's preliminary studies. The concentration of *Rhizopus* species lies below 1 cfu m^{-3} air in natural environments, and is thus one order of magnitude lower compared to *Aspergillus fumigatus*. Concentrations can be higher due to human activities, such as waste-handling. *Lichtheimia* species are associated with composting facilities (up to 4×10^2 cfu m^{-3}), and are rarely encountered in air in natural habitats. A study in a suburban area showed that *R. pusillus* was the most frequently encountered species, followed by *R. oryzae* and *R. microsporus*; *L. corymbifera* was encountered infrequently. It was concluded that knowledge on distribution and habitats of potentially infectious zygomycetes may help to improve risk assessment and infection prophylaxis for immuno-compromised patients.

All participants came to the conclusion that networking of scientists with research interests in zygomycetes on a global basis is necessary to exchange and calibrate materials and data. A platform for future collaboration was created with an expansion of the clinically oriented ECMM-ISHAM Working Group of Zygomycetes by a section on biodiversity and ecology. A follow-up meeting, on "The dynamics of zygomycete research in a changing world", was held at the CBS-KNAW Fungal Biodiversity Centre in Utrecht, The Netherlands, on 3–5 March, 2011. That workshop was organized by Kerstin Voigt (Jena, Germany), Anna Skiada (Athens, Greece), and Sybren de Hoog (Utrecht, The Netherlands). The keynote speakers were Mary Berbee (University of British Columbia, Canada), Hsiao-man Ho (National University of Taipei, Taiwan), Ashraf Ibrahim (Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center, Torrance and David Geffen School of Medicine at UCLA, Los Angeles, USA), Ilse D. Jacobsen (HKI, Jena, Germany), and Paul M. Kirk (CAB International, Egham, UK).Topics covered all areas of zygomycete biodiversity, including genomic, phylogenetic, morphological, physiological and ecological aspects. Participants were able to present

their latest research data on the many beautiful and bizarre members of these fungi. The meeting will culminate soon in a special issue on zygomycete phylogeny in the journal *Persoonia*, scheduled for publication in December 2012.

Mycologists, food and nutrition scientists, medical microbiologists, infection and immune biologists, molecular biologists, and bioinformaticians, are welcome to join the Working Group in any of its upcoming initiatives. For more information please consult the Group's web page (www.zygomycota.eu).

Alastruey-Izquierdo A, Hoffmann K, Hoog GS de, Rodriguez-Tudela J-L *et al.* (2010) Species recognition and clinical relevance of the zygomycetous genus *Lichtheimia* (syn. *Absidia* p.p., *Mycocladus*). *Journal of Clinical Microbiology* **48:** 2154–2170.

Alexopoulous CJ, Mims CW, Blackwell M (1996) *Introductory Mycology*. 4th edn. New York: John Wiley & Sons.

Hibbett DS, Binder M, Bischoff JF, Blackwell M *et al.* (2007) A higher-level phylogenetic classification of the *Fungi*. *Mycological Research* **111**: 509–447.

Hoffmann K, Walther G, Voigt K (2009) *Mycocladus* vs. *Lichtheimia*, a correction (*Lichtheimiaceae* fam. nov., *Mucorales*, *Mucoromycotina*). *Mycological Research* **113**: 277–278.

Ribes JA, Vanover-Sams CL, Baker DJ (2000) Zygomycetes in human disease. *Clinical Microbiological Reviews* **13:** 236–301.

Roden MM, Zaoutis TE, Buchanan WL, Knudsen TA *et al.* (2005) Epidemiology and outcome of zygomycosis: a review of 929 reported cases. *Clinical Infectious Diseases* **41:** 634–653.

Rüping MJGT, Heinz WJ, Kindo AJ, Rickerts V *et al.* (2010) Forty-one recent cases of invasive zygomycosis from a global clinical registry. *Journal of Antimicrobrobial Chemotherapy*. **65:** 296–302.

Voigt K, Cigelnik E & O´Donnell K (1999) Phylogeny and PCR identification of clinically important zygomycetes based on nuclear ribosomal-DNA sequence data. *Journal of Clinical Microbiology* **37**: 3957–3964.

Kerstin Voigt, Sybren de Hoog, Hsiao-Man Ho, Kerstin Hoffmann, Ilse D. Jacobsen, and Guido Fischer
(kerstin.voigt@uni-jena.de)

AWARDS

CBS-KNAW Fungal Biodiversity Centre Awards

The CBS-KNAW Fungal Biodiversity Centre presented its two prestigious awards at the start of the second day of the "One Fungus = Which Name" symposium in Amsterdam on Friday 13 April 2012. The awards are made at irregular intervals by the institute following discussions by its senior staff. This is the third time these awards have been made, and the citations were read, and the presentation of certificates made, by the Centre's Director, Pedro W. Crous.

Johanna Westerdijk Award: Michael J. Wingfield

Awarded on special occasions to an individual who has made an outstanding contribution to the culture collection of the CBS Fungal Biodiversity Centre, marking a distinguished career in mycology. Nominees for the award will be evaluated on the basis of quality, originality, and quantity of their contributions to the collection, and on the basis of associated mycological research in general.

"Mike" Wingfield is Professor and Director of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa. The nomination clearly outlines the extraordinary high level of achievement he has attained. His scientific output is truly remarkable, as evidenced by nine books and 585 scientific publications that have attracted more than 7000 citations in the scientific literature; this makes Mike one of the highest cited scientists in his field. He has received numerous awards from societies worldwide, including The Hendrik Christiaan Persoon gold medal, from the Southern African Society for Plant Pathology, Honorary Membership from the Mycological Society of America, Fellowship of the American Phytopathological Society, an A-rated scientist in the National Foundation for Research Development in South Africa, and later this year he is to be awarded an Honorary Doctor of Science degree by the University of British Colombia (Vancouver, Canada).

Mike studied at the University of Natal in South Africa, where he majored in botany and plant pathology, did an MSc on tree diseases at the University of Stellenbosch in South Africa, and then a PhD on the pine wood nematode at the University of Minnesota in the USA. He is a remarkable mentor, and has supervised over 100 MSc and PhD students. His counsel is not only continuously sought by students but by academics, foresters, and leading forestry companies worldwide. He has the exceptional ability to motivate others and to bring out the best in everyone. Mike has provided a home for a generation of biologists to study and work in Africa at the cutting edge of science. One of the biggest gifts he ever gave his students, was to teach them how to culture fungi. The remarkable aspect of Mike Wingfield's cv, is that his papers are backed up by cultures and DNA evidence to test and retest his hypotheses. A further remarkable aspect is that the majority of his designated ex-type strains have over the years been deposited in the CBS collection. So one day, when we have moved on, the students of the future will still be able to retest his hypotheses with the latest techniques available.

Mike's passion for collecting and culturing fungal biodiversity make him an excellent recipient for the Johanna Westerdijk award. Westerdijk had 56 PhD students in her career, and one of them, Susara Truter, returned to South Africa, and became a professor in plant pathology, and the first female dean in Agriculture. She also taught classes to a young Mike Wingfield. By handing Mike the prestigious Westerdijk award today, the circle is complete.

Josef Adolf von Arx Award: John W. Taylor

Awarded on special occasions to an individual who has made an outstanding contribution to taxonomic research of fungal biodiversity, marking a distinguished career in mycology. Nominees for the award will be evaluated on the basis of quality, originality, and quantity of their contributions in the field of fungal taxonomy.

It is no exaggeration to state that John's name is universally known within our field. His research focuses in two main areas: one concerns barriers to reproduction that are essential to the persistence of species, and the other is comparative genomics that takes into account variation within species. Furthermore, he is also working to make *Neurospora* a model evolutionary organism to study the timing of deep divergences in fungal evolution and the application of molecular evolution to socially important problems involving fungi. Arguably, some of his biggest contributions include the papers on genealogical concordance species recognition, and the use of the ITS (Internal Transcribed Spacer) region as a gene for species recognition.

Of the peer-reviewed works that John has produced, many have appeared in *Proceedings of the National Academy of Sciences* (USA), *Science,* and *Nature*, giving him an

H-index above 50. One paper in particular has been outstandingly influential, namely that introducing the ITS primers which became widely used in fungi, and which has received more than 4000 citations.

John has received many awards, including the Rhoda Behnam Medal for Research from the Medical Mycological Association of the Americas, the Lucille Georg Medal for Research from the International Society for Human and Animal Mycology, and the Alexopoulos Award for Research from the Mycological Society of America. He is a fellow of the Mycological Society of America, the American Academy of Microbiolog, and the California Academy of Sciences. John is also the current President of the International Mycological Association, and a former President of the Mycological Society of America.

We are extremely proud today to be able to honour John with the Josef von Arx award. I think that, similar to von Arx, John is also seen as a trailblazer in fungal taxonomic research.

IMA Young Mycologist Awards 2011

The recipients of the IMA Young Mycologist Awards for 2011 for Africa, Asia. Australasia, and North America were announced in *IMA Fungus* **2** (2): (52)–(53), 2011. At that time, the IMA regional mycological member organizations for Europe and Latin America had not finalized their selections, so they are announced here. The recipients will receive their awards, which include a cheque for 500 €, at IMC10 in Thailand in 2014.

Elias Magnus Fries Medal

Cecile Gueidan is unusual in that her broad mycological interests started in fieldwork with one of Europe's most experienced lichenologists, Claude Roux. She went on to learn molecular phylogenetic methods at Duke University (NC, USA) and tackled some of the most difficult pyrenocarpous lichens that grow on rocks in terrestrial as well as marine and freshwater habitats. Her studies demonstrated enormous polyphyly and convergence in some thallus characters in the verrucarioid lichens (especially those with simple spores), and by combining her molecular work with careful observations of ascomatal features, she laid the foundations for a modern taxonomy of this huge group of lichenized fungi.

During a post-doctoral period at CBS, Cecile applied her talents to other groups of non-lichenized fungi, including the fascinating rock-inhabiting taxa, some lichenicolous species, and also certain moulds. This led her to become interested in and to make contributions to the discussion of the overall system for ascomycete classification, and the origins of rock-inhabiting fungi.

It is also of note that Cecile co-operates with a wide range of lichenologists and other mycologists, and as Elias Magnus Fries worked and published on lichen-fungi as well as other fungi, this makes her a particularly fitting recipient of this award.

Cecile is now employed as a research scientist in the Department of Life Sciences of the Natural History Museum in London, where she continues her research on the molecular systematics of verrucarioid lichen-forming and also other ascomycetes.

Carlos Luis Spegazzini Medal

Luis Fernando Gusmao has been a very productive mycologist since he obtained his PhD at the Universidade de São Paulo, Brazil, in 2004. This concerned the microfungi on decaying leaves of native plants. He has already published 50 papers, most in peer reviewed journals. His main research has continued to be on the taxonomy of mitosporic fungi from Brazil, contributing to the knowledge of this important and little-known group in South America and also worldwide. He has coordinated the research activities of several laboratories, and has conducted several research projects himself.

Luis is now at the Universidade Estadual de Feira de Santana (Feira de Santa,Bahia State, Brazil) where, in addition to his own research, he has become very committed to the training of both undergraduate and graduate students interested in mycology. Indeed, to date he has been the advisor of 14 undergraduate and graduate Brazilian students.

Luis has also participated in national and international congresses, workshops, and other activities, at which he has given talks related to his research activities.

The Committee considered that he has all the attributes to making him a deserving recipient of the Spegazzini Medal: dedication, enthusiasm, national and international visibility, and a strong commitment to the study of mycology in Latin America.

Anton de Bary Medaille: Walter Gams

Walter Gams is to be honoured with the Anton de Bary Medaille of the Deutschen Phytomedizinischen Gesellschaft (DPG) at a ceremony in Braunschweig on 11 September 2012. The award is named after Anton Heinrich de Bary (1831–1888) who had enormous influence on mycology in the mid- to late nineteenth century. The medal was initiated in 1989, and is now generally awarded in alternate years to persons who have made outstanding contributions to mycology and phytopathology. Walter, for many years a mycologist at the KNAW-CBS Fungal Biodiversity Centre in Baarn and later Utrecht, developed an international reputation for his thorough systematic revisionary work on critical and difficult groups of hyphomycetes, especially in the genera *Acremonium*, *Fusarium*, *Trichoderma* and *Verticillium*, but further on soil fungi and hyphomycetes in general. His books include *Cephalosporium-Artige Schimmelpilze* (1971), *CBS Course of Mycology* (1975, 1980, 1983, 1998; with various other CBS staff), *Compendium of Soil Fungi* (1980, 2007; with K. H. Domsch and T.-H. Anderson), and most recently the stupendous *Genera of Hyphomycetes* (2011; with K.Seifert, G. Morgan-Jones and W. B. Kendrick). He collaborates with and assists mycologists world-wide, and played a major role in training courses and supervising students and visiting researchers at CBS, and also at the University of Aachen. For many years he served as Secretary of what is now the Nomenclature Committee for Fungi (NCF), and willingly shares his deep knowledge of the intricacies of fungal nomenclature.

Queen's Award for Forestry: Jolanda Roux meets the Queen of England

Jolanda Roux of the University of Pretoria's Forestry and Agricultural Biotechnology Institute (FABI), as the recipient of the Queens Award for Forestry of the Commonwealth Forestry Association (CFA), was invited to Buckingham Palace and able to spend ten minutes in private conversation with Her Royal Highness Queen Elizabeth II on 13 December 2011. The Queen has been a patron of the CFA since 1987, and this award aims to recognise outstanding international contributions to forestry and recognizes the achievements of outstanding mid-career foresters, based on a combination of exceptional contributions to forestry and an innovative approach to his or her work. Since its inception, the award has been made only nine times and Jolanda is the first woman to receive this honour. She was accompanied to the Palace by Jim Ball, the current Chair of the CFA.

Jolanda is a forest pathologist and mycologist and one of the team of academics that lead FABI and also has an appointment in the Departments of Microbiology and Plant Pathology at the University of Pretoria. Her research focuses on tree diseases, and she is particularly passionate about tree health in general and fungi that cause diseases of trees on the African continent. She collaborates with researchers on many other parts of the world and has travelled widely to undertake her research. She has already published close to 100 papers in international respected journals and has supervised numerous post-graduate students at the University of Pretoria. In addition to the Queen's Award, she has received many other forms of recognition for her work, notably in 2011, the "Distinguished Young Women in Science" award of the South African Department of Science and Technology.

IN MEMORIAM

Vernon Ahmadjian (1930–2012)

Vernon Ahmadjian, pioneer in the culture of the fungal and algal partners of lichens, and explorer of the lichen symbiosis, died on 13 March 2012. The son of Armenian immigrants, Vernon graduated from Clark University, Worcester (MA, USA) in 1952, and received a PhD from Harvard University in 1960 where he worked with the lichenologist Ivan Mackenzie Lamb (1911–1990). For almost all his life he was based at Clark University where he experimented with the culture of the isolated components from lichens, and strove to resynthesize lichen thalli and understand the nature of the lichen symbiosis. His early work is drawn together in *The Lichen Symbiosis* (1967). His scanning electron micrograph of an algal cell being clasped by a fungal hypha featured in *Nature* in 1981 captivated numerous biologists. He took a particular interest in the taxonomy of the green trebouxioid algae, and in collaboration with Chicita F. and William L. Culberson showed that "lichen products" much used in chemotaxonomy were manufactured by the fungal partner alone. He extended his interests to symbiotic systems in general, producing an influential textbook on the subject with Surindar Paracer (*Symbiosis: an introduction to biological associations*, 1986). He was the first editor of the International Association for Lichenology's Newsletter, and was awarded the Association's Acharius Medal in 1986. He presented his personal lichen collections with supporting literature and documents to the Farlow Herbarium in Cambridge (MA) in 2007. Lichen biology has lost one of its foremost and much respected pioneers.

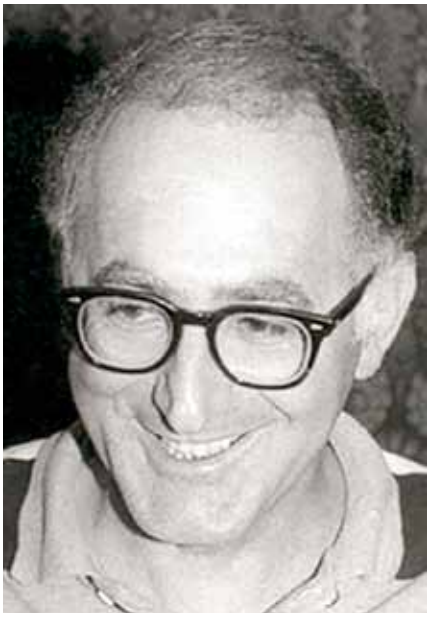
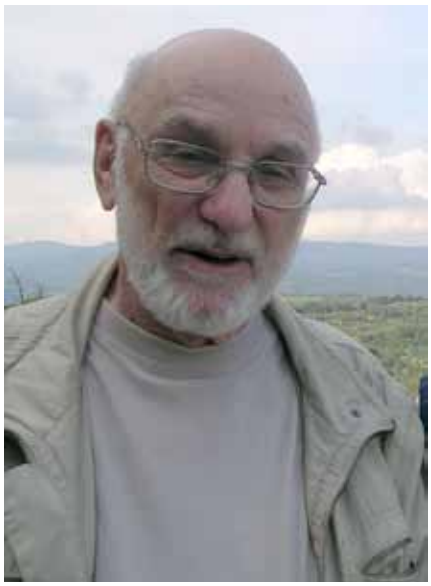


Photo Hannes Hertel

Ovidiu Constantinescu (1933–2012)



On 23 January 2012 our dear friend Ovidiu Constantinescu passed away at the age of 79. He was a passionate mycologist already in his Romanian years, specializing in fungi growing on plant leaves. In spite of the difficult situation under the communist regime in Romania, he managed to acquire the relevant literature in exchange for herbarium specimens, and his papers were always perfectly documented. He was always keen to improve standards in mycology, and to that end published a book *Metode si Technici în Micólogie* (1974), which sadly was not translated into English. In a second effort he succeeded to leave his country and came to The Netherlands, moving from there to Sweden in 1982. Using his technical skills, he built up a culture collection, Mycoteket, in Uppsala. His most prominent expertise was the taxonomy of biotrophic *Peronosporaceae*, about which he published several relevant papers, including a compilation of names in *Peronospora* (1991). More recently he collaborated with Jamshid Fatehi and others, in order to unertake molecular work with his favourite group of fungi.

Developed from a draft provided by Walter Gams.

Walter Friederich Otto Marasas (1941–2012)

It is with great sadness that we must share the news of the passing of our dear friend and colleague Walter ("Wally") Marasas on 6 June 2012. Wally, famous for his ground-breaking research on mycotoxins, especially those associated with *Fusarium* species, had friends and admirers in many parts of the world. He was a larger than life character who inspired people around him, and he will be deeply missed by his many friends and colleagues. Wally's passion for biology, and mycology in particular, was infectious and he shared his experience and skills with great numbers of people. He mentored students (ourselves included) and encouraged many to pursue their mycological dreams. Those who knew Wally only as a mycologist/mycotoxicologist probably did not know that he was an accomplished botanist with a deep love for the flora of South Africa, spending long hours with his wife Rika identifying and photographing flowering plants. He was also an avid philatelist, and post his formal retirement

five years ago, he worked furiously to complete a book illustrating most of the world's fungal stamps – which he classified taxonomically. During an illustrious career, Wally published in excess of 300 scientific papers, numerous books, and was amongst the worlds' most highly cited mycologists. He was a founder member (fellow and honorary member) of the Southern African Society for Plant Pathology, Fellow of the American Phytopathological Society, Fellow of the South African Veterinary Association, and Foreign Associate member of the US National Academy of Science. He was the recipient of many international awards, and held honorary doctorates from the University of the Free State and the University of Pretoria. Wally's death leaves a great void, perhaps most so in the tremendous support, guidance and mentorship that he provided to friends and colleagues, both young and old. He had a special knack of being able to focus on the real issues and to provide wise council. Many mycologists will know that he held very strong views regarding mycological issues and principles and he was not shy to share these openly. This firm commitment to what he believed to be "good practice" and the courage to express his feelings is what many of us relied on most. His loss will be felt for many years to come. Wally is survived by his wife Henrika (Rika) Marasas and two children Carissa and Walter jr and two grandsons. He was not only a wonderful friend to many, but also a loving family man, and a great biologist that will be fondly remembered by all who knew him.

Michael J. Wingfield and Pedro W. Crous
(mike.wingfield@fabi.up.ac.za)

Erast Parmasto (1928–2012)

Erast Parmasto at IMCV in 1994. Photo Karen Nakasone.

A leading and much respected expert in the taxonomy, nomenclature, and phylogeny of fungi, Erast died on 24 April 2012 at the age of 83. He graduated from Tartu State University in 1952, and studied at the Institute of Biology of the Estonian Academy of Sciences in Tartu, supervised by the renowned polypore specialist Apollinari Bondarzew, gaining a PhD in 1955 and a DSc in 1969. He was based from 1950 until his death at the Institute of Biology of the Estonian Academy of Sciences (now part of the Institute of Agricultural and Environmental Sciences of the Estonian University of Life Sciences) with roles from senior gardener to director – and after retirement as a senior researcher.

From 1951–1977 he also taught part-time at the Department of Botany, University of Tartu, on mycology, methodology of science, cladistics, cladistic biogeography, computer applications, and principles of biosystematics. He was awarded the title of Emeritus Professor of Mycology in 1980, and later worked as a part-time professor in the department from 1987–1995.

His main reserch interest was in the corticioid fungi, especially *Hymenochaetales*, on which he published extensively with particular care being paid to nomenclature. In total, he published more than 400 scientific or popular scientific studies, and described more than 200 new taxa. He was an early devotee of e-mail, and initiated and for many years ran Mycologists Online, which compiled the electronic addresses of mycologists world-wide. He was always passionate about new approaches, and he was one of the first mycologists to embrace cladistics. He jumped also at the potential of databases, initiating Cortbase, a continuing nomenclator of the corticiod fungi which swelled to over 8000 species names. But he was also philosphical on issues such as generic and species concepts, on which he edited a book (*Problems of Species and Genus in Fungi*, 1986).

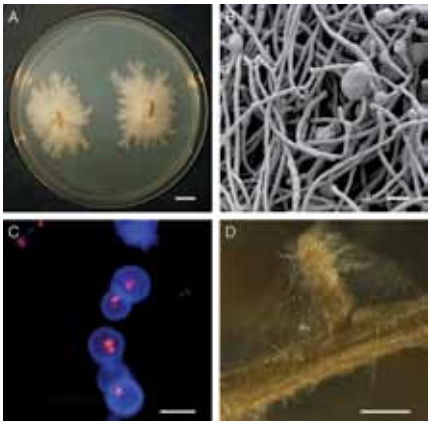
In addition to his scientific work, Erast was one of the academician-secretaries of the Estonian Academy of Sciences in 1973–1981, established the series *Scripta Mycologica* in 1970, and served as editor of *Eesti Loodus* (Estonian Nature) in 1957–1960. He organized the Tenth Congress of European Mycologists (CEMX) in Tallin in 1989, and was a major contributor to nomenclatural discussions at congresses, and also for many years an astute and much-valued member of what is now the Nomenclature Committee for Fungi (NCF). His bright eyes, wry smile, and sharp intellect will be missed by all mycologists who came to know him, not least his many close colleagues in Estonia.

Urmas Kõljalg kindly provided key biographical information on Erast's career.

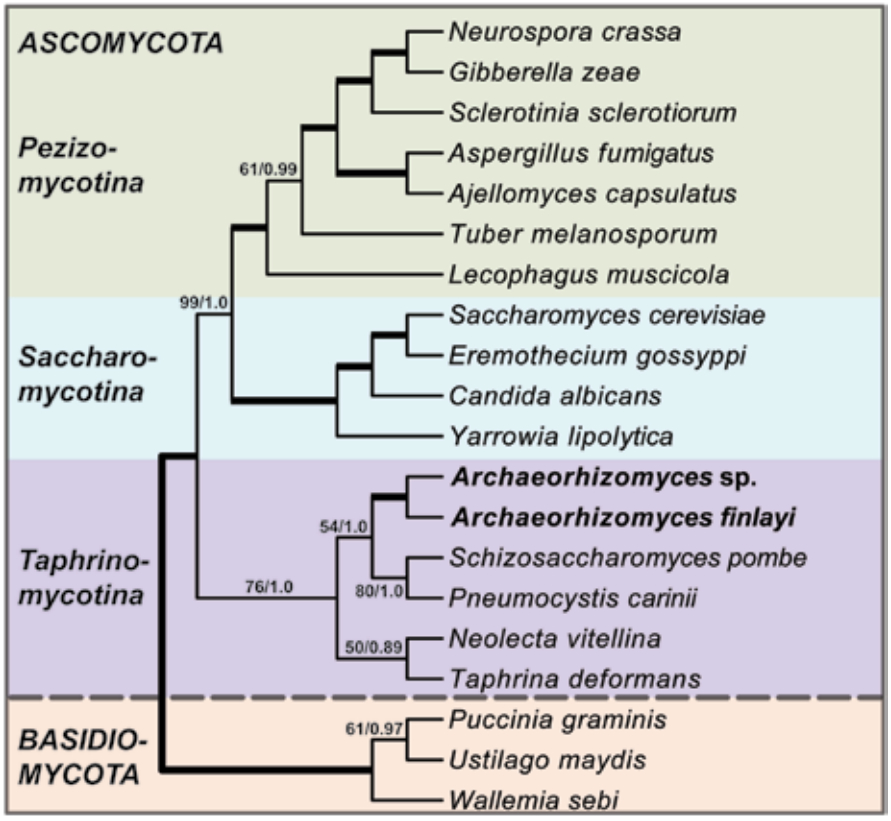
Archaeorhizomycetes: a new class for a major clade of soil fungi

The pioneering molecular study on Alaskan soils by Schadt *et al.* (2003) was a huge surprise to mycologists in claiming that there were even major fungal lineages in soils that did not correspond to known fungal groups. Subsequent work by many researches has shown just how right their seemingly brash claims were. Two major clades of unnamed soil ascomycetes repeatedly emerge, that have come to be termed Soil Clone Groups 1 and 2. Group 1 is the most ubiquitous, especially in boreal and tundra soils, and data from 52 studies with 162 environmental sequences have been analyzed by Rosling *et al.* (2011). In addition, the authors obtained cultures of one species from soil in Sweden, and studied its behaviour on *Pinus* roots in the laboratory. It forms pale colonies and occurs on root surfaces, often mixed with other fungi, but is not mycorrhizal. No sexual spores or undeniable conidia were found, but chlamydospore-like structures were noted. The fungus seems to have seasonal tendencies which the authors suggest may be indicative of a saprobic habit in which it depends on carbon compounds released by roots in the summer.

The new genus *Archaeorhizomyces* is introduced for *A. finlayi* and another unnamed species. The new class *Archaeorhizomycetes,* order *Archaeorhizomycetales*, and family *Archaeorhizomycetaeae* are introduced to accommodate these. The class is diagnosible by rRNA sequences, and is clearly extremely ancient as it belongs to the subphylum *Taphrinomycotina*, which includes *Neolecta*, *Pneumocystis* and *Schizosaccharomyces*. It is notable that the new genus is filamentous as previously *Neolecta* seemed somewhat anomalous in the subphylum.



Archaeorhizomyces finlayi. Culture (A), SEM micrograph (B), chlamydospore-like structures (C), and growth on a *Pinus* root (D). Photos courtesy Anna Rosling.



Consensus phylogeny showing the position of *Archaeorhizomyces* species in the phylum *Ascomycota*. Modified from Rosling *et al.* (2011).

The choice of class name is perhaps somewhat unfortunate, however, in view of the class name *Archiascomycetes* Nishida & Sugiyama 1994. Although that name was not validly published, it has also been used by other authors and is essentially a synonym of *Taphrinomycotina* in circumscription.

It is to be anticipated that the numerous other taxa that appear in the class will eventually be named as they are obtained in culture or when procedures for naming taxa only known environmental sequences have been agreed. And Soil Clone Group 2 yet has to receive formal recognition.

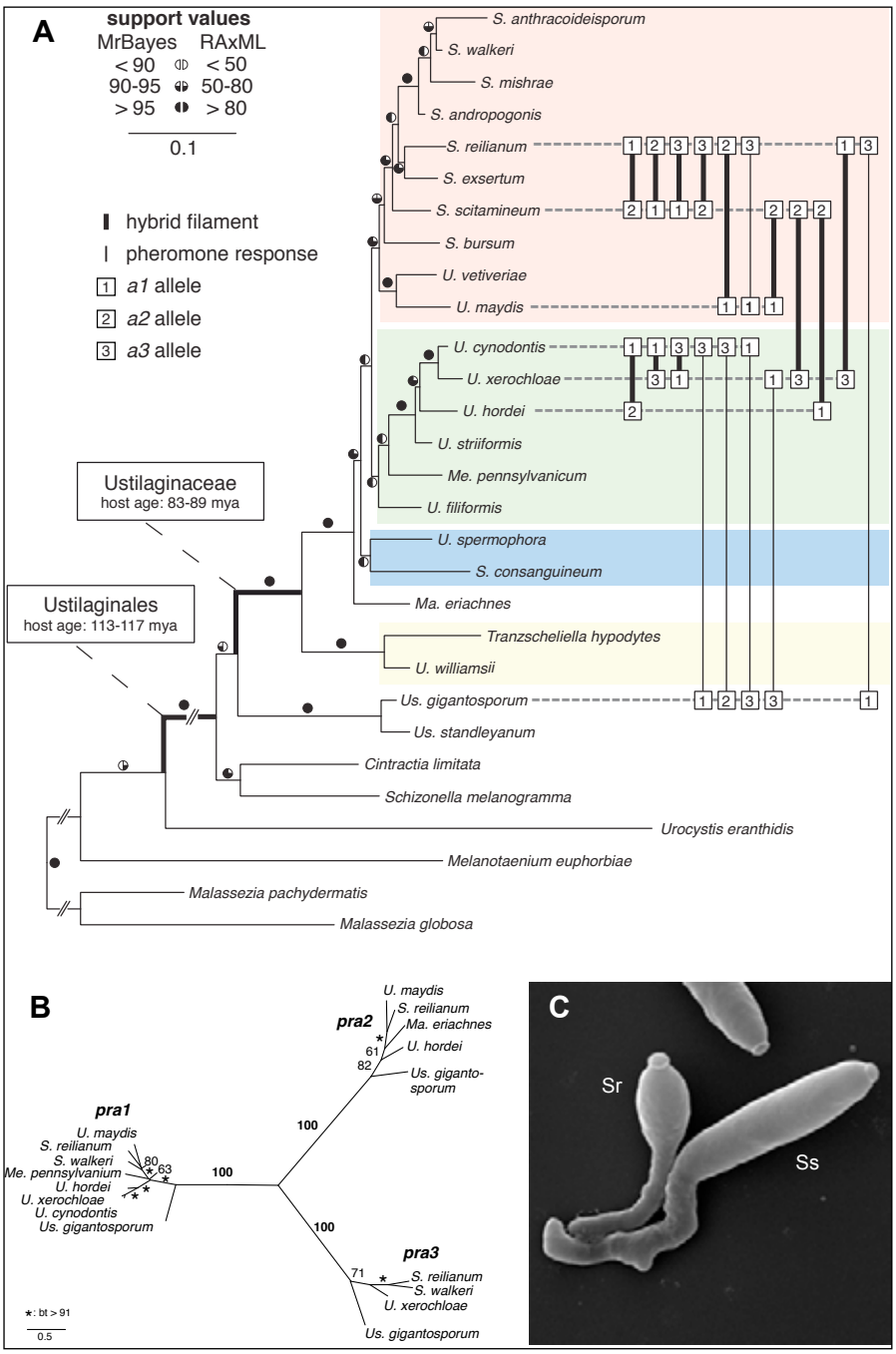
Rosling A, Cox F, Cruz-Martinez K, Ihrmark K, Grelet G-A, Lindahl BD, Menkis A, James TY (2011) *Archaeorhizomycetes*: unearthing an ancient class of ubiquitous soil fungi. *Science* **333**: 876–879.

Schadt CW, Martin AP, Lipson DA, Schmidt SK (2003) Seasonal dynamics of previously unknown fungal lineages in tundra soils. *Science* **301**: 1359–1361.

Inter-specific sex in grass smuts

Mating is of key importance to grass smut fungi (*Ustilaginaceae*) as it initiates parasitism by switching from a yeast phase to a filamentous one with hyphae able to invade the plant tissues. The sexual identity is due to particular genomic alleles that code for variants of a pheromone-receptor (PR) system and the transcription factors, operating in a bisphasic mating process;

this involves recognition, directed hyphal growth leading to conjugation, and then plasmogamy of compatible mating partners. In order to investigate this complex and fascinating system, last December Kellner *et al.* (2011) reported on elegant investigations designed to illuminate our understanding of the evolution of the PR system. Ten species spanning 100 Myr of evolution of the system were selected for genomic and biological study. In addition to detailed comparative information on the alleles, they performed interspecific sex tests which revealed a high potential for hybridization between species linked to pheromone signalling. While the system is optimized for within-species sex, it reveals that there are possibilities for hybrid generation which could lead to smuts with new host specificities. This possibility was confirmed by the demonstration of actual fusions between not only species of the same genus, but ones in different genera, as illustrated in the accompanying figure. The authors comment that the system now revealed may serve as a valuable model for the study of the hybrid-based genesis of novel genotypes.



Interspecific sex in grass smuts (modified from Kellner *et al.* 2011). **A**, Multi-gene phylogeny and interspecific sexual compatibility of *Ustilaginales*. Concatenated Maximum Likelihood (ML) analysis of 2571 bp of ssu, ITS, lsu rDNA, ef1-a and rpb1. Circles next to branches indicate bootstrap support values and *a posteriori* probabilities of Bayesian and ML analyses, respectively. Branch lengths correspond to substitutions per site and abbreviated branches indicate longer branches. Connected squares illustrate hybrid filament formation (bold lines) or pheromone response (thin lines). Numbers in squares represent respective a mating types. Coloured boxes depict different phylogenetic clades (see text). Host ages refer to Prasad *et al.* (*Science* **310**:1177–1180, 2005. **B**, Phylogeny of mating type-specific pheromone receptors. Maximum Likelihood analysis of complete pheromone receptor-coding sequences. Numbers and asterisks next to branches indicate bootstrap (bt) support values and branch lengths correspond to substitutions per site. **C**, Interspecific mating of haploid sporidia of *Sporisorium reilianum* (Sr) and *S. scitamineum* (Ss); SEM micrograph.

Kellner R, Vollmeister E, Feldbrügge M, Begerow D (2011) Interspecific sex in grass smuts and the genetic diversity of their pheromone-receptor system. *PLoS Genetics* **7**: e1002436.

Prions and phenotypic inheritance in wild yeasts

It has previously been recognized that self-perpetuating changes in protein structure can be heritable elements in yeasts separate from and preceding genetic change (True & Lindquist 2000); such self-perpetuating epigenetic structures are termed prions. Halfmann *et al.* (2012) investigated the yeast translation-termination factor prion Sup35 that is not essential to the function of the protein and can adopt an amyloid configuration which self-perpetuates and leads to increased stops in codon read-through; that leads to a variety of new traits. The prions had been considered an artefact of strains kept in culture, but these authors examined occurrences and screened for new prions in around 700 wild *Saccharomyces* strains. Prions proved to occur in about one third of the wild strains examined. Modifications of the Sip35 prion were demonstrated to confer characters likely to be beneficial to the yeasts under selective pressures, that is to develop beneficial phenotypes. Indeed, 40 % of the prions in

the wild yeasts were beneficial to growth under 12 sets of conditions tested. In yeasts, it has consequently now been established that prions are a naturally present supplementary source of inheritable material of adaptive value. The extent of prions in filamentous fungi as a whole has yet to be assessed, but they clearly have the potential to contribute to adaptability and fitness.

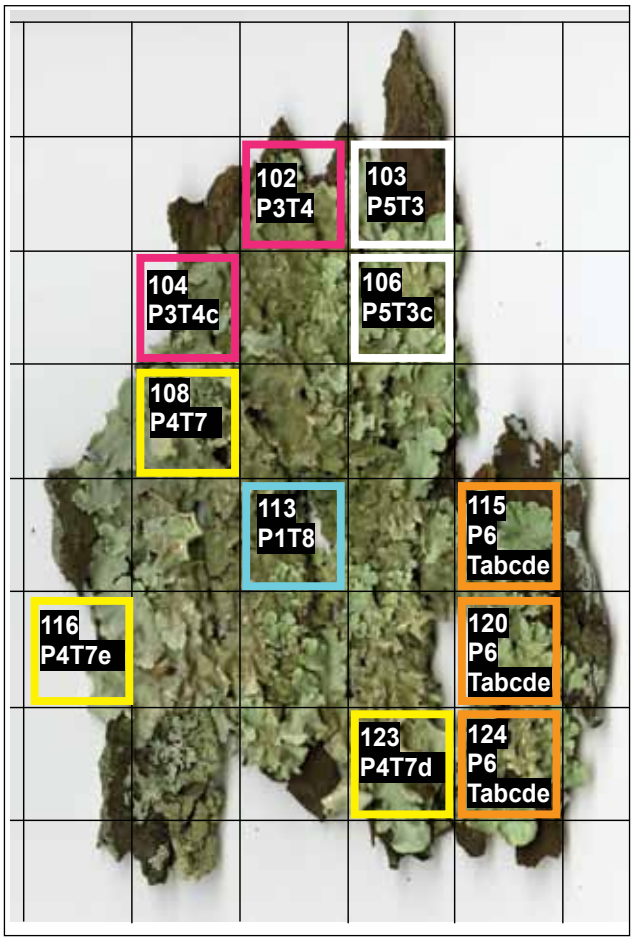
Halfmann R, Jarosz DF, Jones SK, Change A, Lancaster AK, Lindquist S (2012) Prions are a common mehcnisms for phenotypic inheritance in wild yeasts. *Nature* **282**: 363–368.

True HL, Lindquist SL (2000) A yeast prion provides a mechanism for genetic variation and phenotypic diversity. *Nature* **407**: 477–478.

Yeast colonies, light and tramsmission electron micrograph photos of *Saccharomyces cerevisiae*.

Different fungal and algal genotypes demonstrated within one lichen specimen

Observations on the development of lichens in the field reveal that multiple propagules of a species developing on a surface often coalesce to form a single structure. This is frequently observed where the propagules are asexual soredia or isidia, which may or may not have come from the same parent, and is well-documented. However, whether all had to be of a single genotype for this to occur was uncertain. The first study to suggest that a single lichen specimen might not just have a single fungal partner experimentally was the study of Larson & Carey (1986) who found that single specimens of two *Umbilicaria* species showed variations in physiological parameters and isoenzyme profiles. With the advent of DNA PCR technology, and especially the use of microsatellite (SSR) markers, it has become possible to explore the issue of the degree of individuality of single lichen specimens with respect to both the fungal and the algal populations that comprise them.



A single specimen of *Parmotrema tinctorum* showing the different fungal and algal genotypes determined with PCR of SSR markers. Codes prefixed by P are of the fungal partner, and those by T are of the algal partner; five fungal genotypes and one algal genotype were detected within this particular specimen. Adapted from Mansournia *et al.* (2012).

Parmotrema tinctorum is a rather common tropical lichen that reproduces mainly by asexual isidia. Mansornia *et al.* (2012) studied populations growing on *Pinus thunbergii* in Japan, and used microsatellite markers to characterize the partners at different levels: within single specimens, on single trees, and within 10 x 10 cm quadrats. Of particular interest were the results from single specimens in which they studied numerous small pieces of tissue. They found that a single specimen could be formed from a single fungal partner with or without changes in the algal partner, or fusion of several independent partners. In total 12 fungal genotypes and 37 algal genotypes were recognized. An example in which there were five fungal genotypes and a single algal genotype is illustrated here. Further, specimens from individual trees or which were close together tended to have similar genotypes, suggesting limited dispersal in the site.

This study provides evidence to support what has long been suspected, that one cannot presume that what looks like a single individual lichen specimen represents a single fungal genotype.

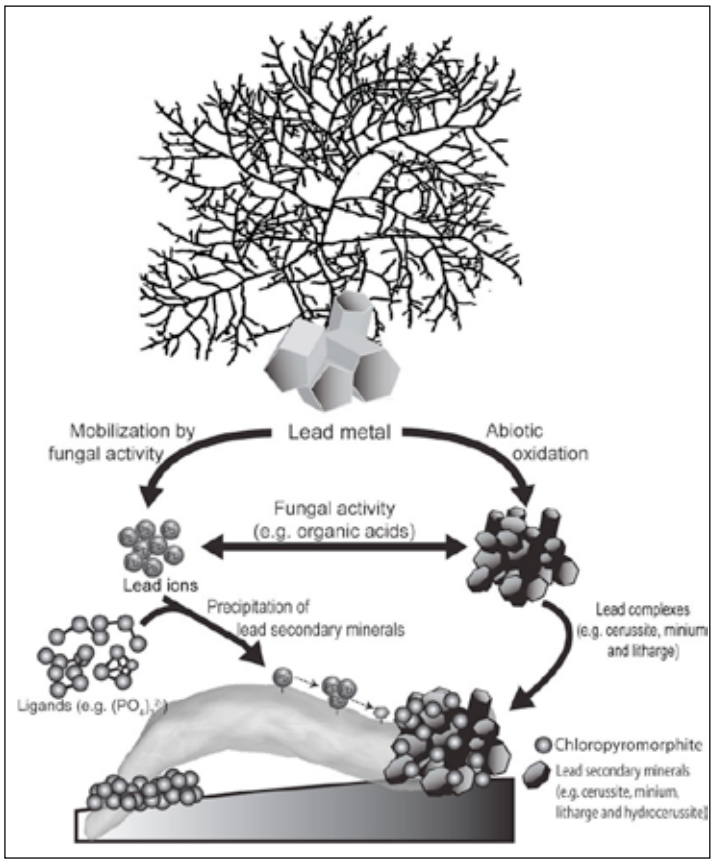
Larson DW, Carey CK (1986) Phenotypic variation within "individual" lichen thalli. *American Journal of Botany* 73: 214–223.

Mansournia MR, Wu B, Matsushita N, Hogetsu T (2012) Genotypic analysis of the foliose lichen *Parmotrema tinctorum* using microsatellite markers: association of mycobiont and photobiont, and their reproductive modes. *Lichenologist* **44**: 419–440.

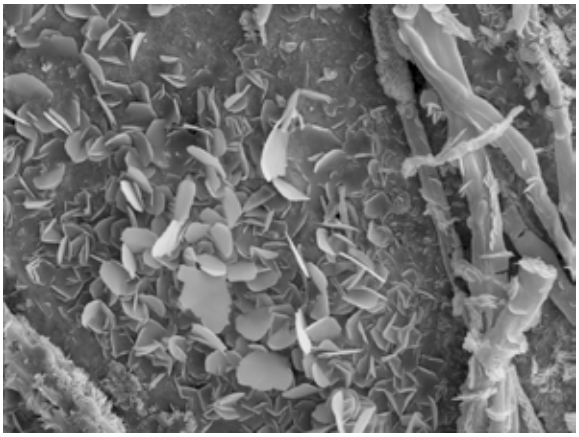
Fungi that can transform lead

Soil and rock-inhabiting fungi, especially lichen-forming fungi, are well known to be able to convert different minerals to oxalates through the extracellular secretion of oxalic acid. Now, Rhee *et al.* 92012) have found that two fungi, *Metarhizium anisopliae* and *Paecilomyces javanicus*, are able to act directly on lead metal to form chloropyromorphite, the most stable lead mineral known. The strains were isolated from a former lead-mining area in Scotland, and their activity was demonstrated using incubated lead shot, and examination by two methods of X-ray analysis; it should be noted that in controls without the fungi, different compounds were formed. The lead shot was visibly corroded after one month, and minor amounts of some other lead compounds were also noted. The paper includes superb environmental scanning electron (ESEM) micrographs, and amazingly shows that the pyromorphite develops as minute spherules even inside the fungal hyphae. This finding is not only of interest in demonstrating a previously unknown biogenic step in the corrosion of lead metal and as a contribution to lead biogeochemistry, but could have applications. Soils can become lead-contaminated through, for example, the deposition of industrial wastes, battery casings, pipes, paints, inks, and shot, and lead has dangerous toxic effects on humans. The potential of using the tested strains, and other isolates of those and additional species of fungi, in the bioremediation of actual lead-contaminated soils clearly merits further exploration and assessment.

Rhee YJ, Hillier S, Gadd GM (2012) Lead transformation to pyromorphite by fungi. *Current Biology* **22**: 1–5.



Schematic representation of the processes involved in lead transformation by fungi. Courtesy Geoffrey M. Gadd.



Secondary mineral formation on the surface of metallic lead resulting from the activities of *Metarhizium anisopliae*. Photo courtesy Geoffrey M. Gadd.

Nutritional value of fungi in animal diets

Humans along with many other animals, including a wide range of terrestrial mammals, eat fungi as components of their diets to various degrees. The actual nutritional value of fungi has, however, been unclear and much-debated. This is as while chemical analyses can give very positive indications, the extent to which they are digestible is unclear. In order to ascertain the extent of digestibility, Wallis *et al.* (2012) analyzed the fibre, amino acid composition, and both total and available nitrogen in a about 60 samples of sporocarps of diverse epigeous and hypogeous macrofungi from Australia and the USA; they then examined the digestibility *in vitro*. Amongst the genera of fungi studied, were species of *Agaricus, Boletus, Cantharellus, Gauteria, Hysterangium, Morchella, Rhizopogon*, and *Tricholoma*. The results showed that while in general the mushrooms and truffles tested

A northern flying squirrel (*Glaucomys sabrinus*) holding a truffle in its paws, evidently devouring the white flesh. Photo Jim Grace.

were a reasonable source of amino acids and digestible nitrogen, there were large differences between species, and the protein had a poor balance of digestible amino acids. The authors consider that this explains why mammals that are primarily mycophagous tend to eat a wide range of sporocarps, and in some cases have developed foregut-fermentation to maximise the available nutritional value. In addition, they note that many mycophagous mammals supplement their diets with insects which are a source of high-quality protein. In Australia, the combination of mycophagy, foregut fermentation, and coevolution may explain the potororine marsupials which are obligate or preferential mycophagists. It is suggested that their use of hypogeous fungi enables them to survive the destructive effects of devastating fires as the hypogeous fungi tend to remain in the aftermath. The authors, perhaps tactfully, largely avoid the issue of the dietary value of fungal sporocarps in the human diet . . . . A single experience can hardly be taken as representative but, after repeatedly consuming meals with different mushrooms as the major component over several weeks about 15 years ago, I found I had shed quite a few pounds.

Wallis IR, Claridge AW, Trappe JM (2012) Nitrogen content, amino acid composition and digestibility of fungi from a nutritional perspective in animal mycophagy. *Fungal Biology* **116**: 590–602.

Archaeolichenology: a novel use of lichens

A novel application of lichens has just been developed by lichenologists at the Royal Botanic Garden Edinburgh. These are being used to reconstruct species' regional distributions, and so indicate habitat types, for the historic period prior to the industrial revolution that started in the mid-18th century. The rationale is that epiphytic lichens grow on the outer-bark surface of trees, and trees harvested and used as the frame for pre-industrial buildings were not likely to have been transported far from where they were used. Consequently, where bark occurs on the timber structures of pre-industrial buildings, it might be possible to find preserved lichens which may suggest something of both past distributions and local ecologies.

This proved to be the case, and Yahr *et al*. (2011) discovered 87 epiphytic lichen species in a survey of 78 buildings dating from the period 1300-1750 across southern England. The best-preserved material tended to be found in the roof-spaces of low-status homes with continuous occupancy, where conditions are not so dissimilar to those of many herbaria today. Many of the pre-industrial records are from outside the species' current range, and estimates suggest an 80 % loss of epiphyte diversity from areas such as south-east England (Ellis *et al*. 2011). This study has demonstrated an intriguing new tool for environmental reconstruction, with the potential of re-evaluating environmental and conservation base-lines. This is of particular interest as current knowledge from the literature and preserved specimens is necessarily biased towards the mid-18th century onwards, a period where industrialization was already starting to become widespread in much of lowland Britain.

Information related to changes over time can be accrued, but to ensure accuracy, it was necessary to consider the accurate dating of the timbers (based on styles of carpentry), possible timber re-use within buildings, and the local transport networks. The group plans to focus on increasing the resolution of the data, using data available from wattles, and also dendrochronology. The work is being undertaken in collaboration with archaeologists at University College London.

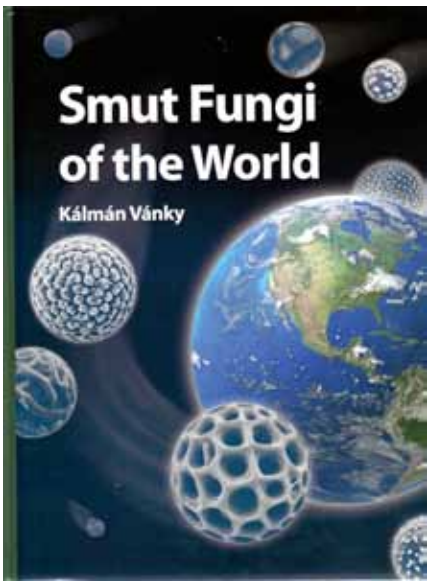
Based on material kindly supplied by Christopher J. Ellis.

Ellis CJ, Yahr R, Coppins BJ (2011) Archaeobotanical evidence for a massive loss of epiphyte species richness during industrialisation in southern England. *Proceedings of the Royal Societyof London, Biological Science, B*, **278**: 3482–3489.

Yahr R, Coppins BJ, Ellis CJ (2011) Preserved epiphytes as an archaeological resource in post-medieval vernacular buildings. *Journal of Archaeological Science* **38**: 1191–1198.

A specimen of a *Physconia* species preserved on the bark of a 400 year-old timber. Photo courtesy Christopher J. Ellis.

Smut Fungi of the World. By Kálmán Vánky. 2012. ISBN 978-0-89054-398-6. Pp. xvii +1458, figs 650, micrographs 2800. St Paul, MN: APS Press. Price US$ 499.00.



The doyen of smut fungi, Kálmán Vánky, building on a succession of substantial and well-illustrated regional monographs, has now brought together his immense knowledge accumulated over almost half a century into this major crowning world treatment. There has been no similar attempt to draw all the data on the world's smut fungi into a single volume since that of Zundel (1953). However, unlike Zundel, whose work was largely a compilation of previously published descriptions, Vánky's monograph is based almost entirely on his personal examination of material; his own herbarium is said to contain some 21 500 specimens and 6 500 slide preparations.

The number of species accepted in the main body of the work is 1650, and these are dispersed through 93 genera. The genera are pragmatically treated alphabetically, which greatly facilitates use of the work, but an outline classification placing these in higher taxa is provided at the start (pp. ix–x), although without a simplified phylogenetic tree. Each species entry has full bibliographic details of both accepted names and synonyms, along with information on the name-bearing types and a "!" indicating collections he studied. Detailed descriptions of symptoms, anatomy, and morphology are supplemented by line drawings of infected plants, and in almost all cases by light by photomicrographs and SEM micrographs of the spores – showing the details of surface ornamentation and sculpturing so critical in the identification of these fungi. Host plants are listed by family and genus, followed by a perhaps too brief note on distribution, surprisingly in most cases only indicating the continents in which a species is known or using words such as "cosmopolitan". As the author surely has so much more information on rarity and distributions at his fingertips, it was unfortunate that the opportunity to cite the actual countries for all but the commonest species was passed by; elimination of the superfluous author citations after the names of the host plants (this is not a taxonomic work on plants!) could have helped the generate extra space required. The entries on both genera and species sometimes include comments, but in general these are terse notes on separations from other taxa, especially ones on the same or allied plants.

Many species are evidently rare, or at least rarely collected, 25 % of those treated having been found only once. Further, Vánky estimates the true number of smut fungi on Earth as 4500, implying that only about one third have so far been recognized. That there are many new taxa to be discovered is substantiated by 37 additions made after the work was completed; these taxa are treated more briefly in an Addendum, which also includes one new combination – the only nomenclatural novelty in the whole work to catch my eye. I was pleased to see that there was a substantial section on doubtful, excluded or invalidly published smut taxa which included full explanations on the reasons for non-acceptance. There is also a most helpful alphabetical list of plant genera with the smuts known from them, and an epithet-based index to fungal names. There is a 44-page "Selected smut fungi literature", but no glossary nor any introductory material describing either life-cycles or spore-germination types which would have been an asset for non-specialists. It will be necessary to use this in conjunction with his superbly presented earlier account of smut genera which includes extensive introductory material and illustrations of germinating spores and a glossary (Vánky 2002). For mycologists and plant pathologists not used to working with smut fungi, the information on methods of examination he used (*cfr* Vánky 1994: 8–9) could also have proved helpful. However, weighing in at 4.21 kg, topping *The Genera of Hyphomycetes* (Seifert *et al*. 2011) at a "mere" 3.3 kg, it is unlikely to be used far from a library where his complementary texts may also be kept to hand.

Identifications are facilitated first by a key which takes each family alphabetically and has a key to the genera represented on it based on the characters of the fungi. Then, under each generic entry, there is a key to all known species of that genus, based on a combination of host plant names and morphological features of the fungi. While this may be pragmatic for the identification of known species, I would have also expected that to be complemented by a key to genera with no mention of the hosts, perhaps developed from that he previously published for European smuts (Vánky 1994) or his later world keys (Vánky 2002, 2008) with the host-based dichotomies in that also eliminated. If one of the expected 3000 or so yet undiscovered smuts is found, it will be difficult to place it in a genus in the absence of such a fungus-character-based key.

That Vánky has been able to generate such an extraordinarily full monograph, published in his 82nd year, is both a major service to mycologists and plant pathologists worldwide, and at the same time a tremendous and extraordinary personal achievement. This is especially so as he trained and practiced as a physician, developing an interest in smuts as an amateur in his home country of Romania, obtaining a PhD in Uppsala in 1985 (Vánky 1985) while still working as a physician, but then devoting himself fully to them on moving to Germany in 1986. This is a landmark publication, destined to be the major reference work on smut fungi for decades to come, and a must-have for all key mycological and plant pathological libraries – despite the unavoidably high price.

Seifert KA, Morgan-Jones G, Gams W, Kendrick B (2011) *The Genera of Hyphomycetes.* Utrecht: CBS-KNAW Fungal Biodiversity Centre.

Vánky K (1985) Carpathian *Ustilaginales*. *Symbolae Botanicae Upsalienses* **24** (2): 1–309.

Vánky K (1994) *European Smut Fungi*. Stuttgart: Gustav Fischer Verlag.

Vánky K (2002) *Ilustrated Genera of Smut Fungi.* 2nd edn. St Paul, MN: APS Press.

Vánky K (2008) Taxonomic studies on *Ustilaginomycetes* – 28. *Mycotaxon* **106**: 133–178.

Zundel GL (1953) *Ustilaginales of the World.* [Contribution no. 176.] University Park, PA: Department of Botany, Pennsylvania State University.

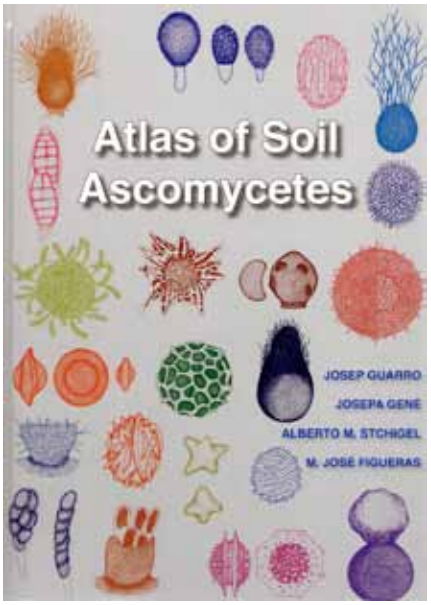
Atlas of Soil Ascomycetes. By Josep Guarro, Josepa Gené, Alberto M. Stchigel, and M. José Figueras. 2012. ISBN 978-90-70351-88-5. Pp. iv + 486, numerous figs. Utrecht: CBS-KNAW Fungal Biodiversity Centre. [CBS Biodiversity Series no. 10.]. Price: 70 €.

The scarcity of authoritative well-illustrated and comprehensive texts poses a major problem for anyone wishing to identify fungi that are isolated from soil. Previous works dealing with soil fungi have either been selective and concentrated on the most commonly isolated species (Domsch *et al.* 2007), or regional in scope (Moubasher 1993, Subramanian & Wasser 2001), or concerned those found by one group of workers (Watanabe 2010; see *IMA Fungus* **2**: (33), 2011). This new work stands apart in worldwide scope and comprehensiveness, though it has to be remembered that it embraces only fungi in which ascomata are known and form in pure culture, and neither yeasts, nor truffles, nor ascomycetes only known as conidial fungi. However, where anamorphs are known in ascoma-forming species, these are embraced. In total, 146 genera and 698 species are treated in detail, and helpfully, notes are added on other species known in the genera so far not known from soil.

Following a key to the treated genera, the generic accounts are alphabetically arranged. Key bibliographic information is provided on both accepted names and synonyms, followed by descriptions of colonies and microscopic features, notes on the known distribution, and pertinent references to sources of further information. The authors are well-known for the high quality of their line-drawings, and the volume does not disappoint, but rather excels, in that regard. There are also numerous half-tones, often including scanning electron micrographs which are so helpful in visualizing the nature of ascospore ornamentation. The line-drawings including spores of different species of a genus are of especial value in making comparisons.

The taxonomic treatment is generally up-to-date, but in some cases follows that adopted in previous papers by members of the group that are not all accepted by mycologists, such as the inclusion of *Gelasinospora* in *Neurospora*. It is also somewhat unfortunate that the changes in the nomenclature of pleomorphic fungi made in 2011 were not accommodated. In consequence, ascoma-forming species of *Aspergillus* and *Penicillium*, for example, are treated under the names of the teleomorph-typified genera, such as *Neosartorya* and *Eupenicillium*, rather than the anamorph-typified generic names.

The authors, all at the Universitat Rovira I Virgili in Reus, Spain, are to be congratulated on producing a work which will be of lasting value and also a major impetus to those struggling to identify ascomycetes not only from soil, but also from other substrates as well, such as decaying plant materials and dung. The realization of this work was facilitated by grants from the Ministerio de Educacion y Ciencia in Spain; a model not uncommon in Spain, but which is too rarely emulated elsewhere. All mycological centres should purchase a copy!



Domsch KH, Gams W, Anderson T-H (2007) *Compendium of Soil Fungi.* 2nd edn. Eching: IHW Verlag.

Moubasher AH (1993) *Soil Fungi in Qatar and other Arab Countries.* Doha: University of Qatar.

Subramanian CV, Wasser SP (2001) *Soil Microfungi of Israel.* Ruggell: A. R.A. Gantner Verlag.

Watanabe T (2010) *Pictorial Atlas of Soil and Seed Fungi: morphologies of cultured fungi and key to species.* Boca Raton: CRC Press.

Taxonomic Manual of the Erysiphales (Powdery Mildews). By Uwe Braun and Roger T. A. Cook. 2012. ISBN 978-90-70351-89-2. Pp. vi + 707, figs 860 (7 col.). Utrecht: CBS-KNAW Fungal Biodiversity Centre. [CBS Biodiversity Series no. 11.] ISBN 978-90-70351-89-2. Price: 80 €.

It is 25 years since Uwe Braun's world monograph of *Erysiphales* appeared (Braun 1987). It was immediately sought after by mycologists ranging from plant pathologists to what are now termed citizen scientists. That work accepted 516 species, and clearly stimulated fresh interest in these fungi as this new book, prepared with plant pathologist Roger Cook, has 873 species, no less than 55 of which are described as new to science here. The addition of 357 species represents an increase in the number of known species of the order of 69 %, indicating just how much remains unknown even within a relatively well-studied order of ascomycetes. I anticipate that the number will swell further now the field has this new very moderately priced monograph as a stimulus.

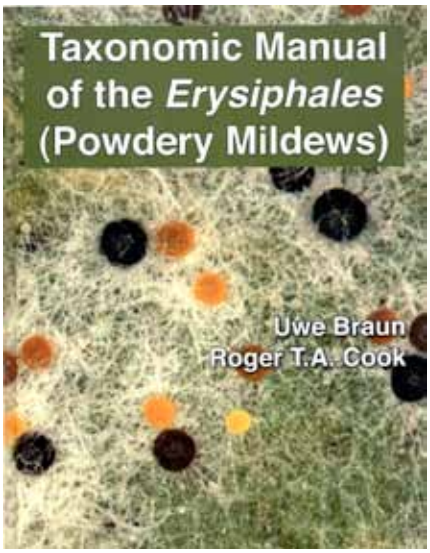
The monograph starts with an overview of the powdery mildews and the characters used in their taxonomy and identification; all well-illustaretd by line-drawings or photographs, and embracing haustorium and conidium germination types. A most helpful table (pp. 31–32) summarizes the conidium germination types and ornamentation of the conidia as seen in scanning electron micrographs. Amongst other aspects covered are accounts of ascoma development, fungicolous fungi, and fossil representatives.

The phylogenetic systematics of the order is now much clearer than it was in the pre-molecular age in which the 1987 monograph was produced. A series of papers giving an overview of the current systematics and evolution of these fungi was published separately in *Mycoscience* **52** (3) last year (see *IMA Fungus* **2**: (60)–(61), 2011).

Four tribes are now accepted within *Erysiphales*. Thirteen genera are recognized, which can be distinguished morphologically by the teleomorphs, but not always so readily by the anamorphs. A fourteenth "genus", *Microidium* comb. stat. nov., is pragmatically used for three species only known from the anamorph which appear to form a distinct taxon, while 27 of uncertain affinity are left in "*Oidium*". Keys to the genera and sections are presented based on teleomorph and anamorph features, with a separate key to species based on the host families. Within each genus or section account, however, I was pleased to see that the characters used in making separations in the couplets were almost exclusively of the fungi rather than the hosts.

The species accounts are meticulously prepared, with full synonymies, information not only on types, but exsiccatae, illustrations, and literature reports; details not generally seen in many modern monographs. Comprehensive descriptions and information on host species and distribution are followed by often full and informative notes about the species, including doubtful or erroneous reports. Careful line drawings illustrate both teleomorph and anamorph where known. In such cases, the names of named anamorphs are presented with separately grouped synonyms under that of the teleomorph. The changes in the *Code,* effective from 30 July 2011, under which one fungus species can have only one correct name came too late to enable the new provisions to be accommodated in the nomenclatural presentations. Very few names, a mere 2 %, need any change as a result, and Braun (2012) has helpfully provided details of 18 cases where there is an earlier anamorph-typified name for inclusion in a future approved *List* of accepted names. In reality, the full slate of 873 accepted names could be included in such as *List* to stabilize the nomenclature of this order for posterity.

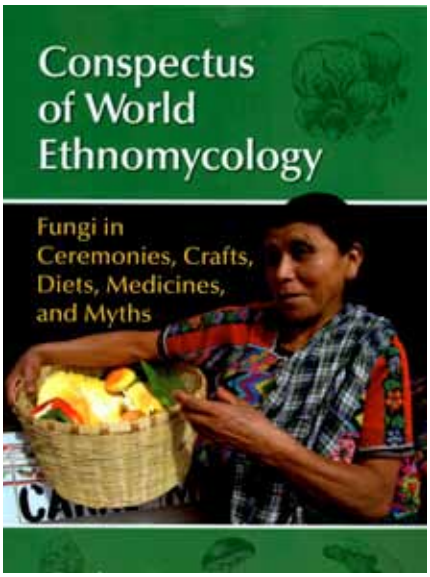
The use of "Taxonomic" in the title should not put off the non-specialist, and might have been expanded to "Taxonomic and Identification" as there is so much information here that it can be utilized by the neoerysiphalean with little or no previous knowledge of these fungi. This work is the pinnacle in the careers of two exceptional and dedicated mycologists, and is destined to be of everlasting value. Individual mycologists and plant pathologists, as well as institutions, are urged to secure a copy of this superb work while they can.



Braun U (1987) A monograph of the *Erysiphales* (powdery mildews). *Beihefte zur Nova Hedwigia* **89**: 1–700.

Braun U (2012) The impacts of the discontinuation of dual nomenclature of pleomorphic fungi: the trivial facts, problems, and strategies. *IMA Fungus* **3**: 81–86.

Conspectus of World Ethnomycology: fungi in ceremonies, crafts, diets, medicines, and myths. By Frank M. Dugan. 2011. ISBN 978-0-89054-395-5. Pp. viii + 151, figs 27 (18 col.), tables 6, Appendices 2. St Paul, MN: APS Press (American Phytopathological Society Press). Price US$ 69.95.



Frank Dugan has already addressed the role of fungi in ancient civilizations (Dugan 2008), but now goes global. APS Press), but is much broader in scope and has a wealth of vignettes that have the potential to liven-up mycology classes.

There is perhaps almost no end to what might be encompassed in such a title, especially as Dugan adopts a particularly broad definition of "ethnomycology", as the study of the multifarious uses of fungi by humans since pre-historic times. The first chapter of the book provides a well-referenced global overview, while the second has an interesting take on the role of women, specially market women, as sources of information for herbalists since at least the sixteenth century – and accompanied by several pertinent early illustrations. The bulk of the volume, however, is organized by regional chapters: Europe and the Mediterranean; Asia and the Pacific; Sub-Saharan Africa; Latin America and the Caribbean; and North America. He aims to list the exploited fungi in each of these regions, which conclude with a table of those "commonly documented as ethnomycologically important". This is an almost impossible task, and the author makes a laudable effort, but mycologists who have visited rural markets and traditional medicine shops in Africa and China in particular will note numerous species they encountered remain uncited. A final chapter looks at the nature of "folklore", and touches on the issue of the exploitation and conservation of wild mushrooms. The literature cited covers over 25 pages, and this compilation is of considerable value in its own right as some sources are little-known, but again is inevitably incomplete, and I missed Findlay's (1982) book in particular. The whole concludes with lists of utilized fungi available from selected fungal genetic resource collections, and some 20 recipes compiled

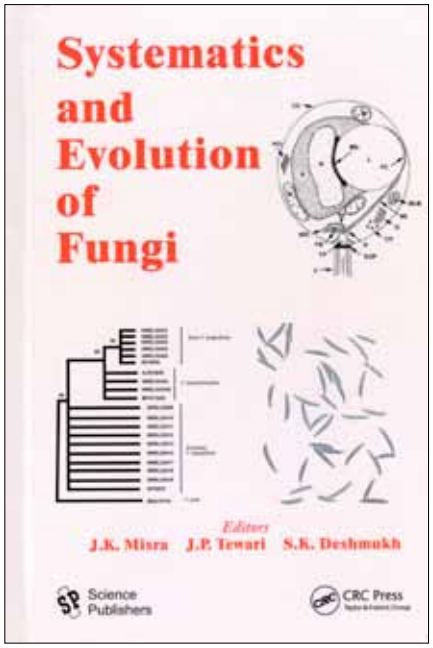
from various sources around the world.

Overall, I found the book, while necessarily eclectic, rather absorbing and with many often fascinating footnotes and asides, and I especially enjoyed some of illustrations. I am sure both professional and other mycologists would similarly enjoy it, but fear that the price might be seen as prohibitive – especially for a small-format slim paperback as compared to, for example, the *Atlas of Soil Ascomycetes* reviewed above in this column.

Dugan FM (2008) *Fungi of the Ancient World: how mushrooms, mildews, molds, and yeast shaped the early civilizations of Europe, the Mediterranean, and the Near East*. St Paul, MN: APS Press.

Findlay WP (1982) *Fungi, Folklore, Fiction & Fact*. Richmond, Surrey: Richmond Publishing.

Systematics and Evolution of Fungi. Edited by J. K. Misra, J. P. Tewari, and S. K. Deshmukh. 2012. ISBN 978-1-57808-723-5. Pp. xii + 412, illustr. (2 col. plates). Enfield, NH: Science Publishers. Price: £ 76.99.



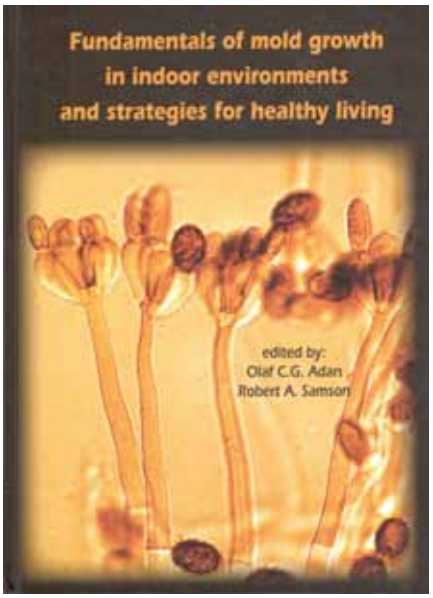
This book, according to the Preface, is "intended to present the progress and shifts that have taken place towards the understanding of systematics and evolution of fungi in recent years". Indeed, the impact of molecular phylogenetics on fungal systematics at all levels, from kingdom to population, can only be viewed as traumatic. The capturing of the excitement as new relationships emerge and long-cherished hypotheses fall was always going to be difficult one to capture in a single volume. The editors of this work approached this challenge by inviting 13 papers concerned with different aspects and levels of fungal systematics today. Three of those papers are broad in scope, dealing with the integration of morphological and molecular data (Hawksworth), perspectives from the fossil record (Tripathi), and an overview of comparative methods (Nagy *et al.*). The remainder concern particular groups of fungi: *Chytridiomycota* (Powell & Letcher); *Zygomycota* (Benny, including a detailed synopsis down to and including all generic names proposed); *Trichomycetes* (Lichtwardt); *Stachylina* and *Smittium* (Misra); *Morchella* and *Macrolepiota* (Barseghyan *et al.*); mushroom-formers (Zmitrovich & Wasser); *Phellinus* and *Inonotus* (Tura *et al.*); toxigenic *Fusarium* species (Yli-Mattila); alternarioid hyphomycetes (Gannibal); and rapid diagnostic methods for candidiasis (Nagy *et al.*). These studies give a flavour of the current situation, rather than a comprehensive overview, which would have been a much larger task, but mycologists working on the selected topics will wish to access a copy. The title is also available in a kindle version.

Fundamentals of Mold Growth in Indoor Environments and Strategies for Healthy Living. Edited by Olaf C. G. Adan and Robert A. Samson. 2011. ISBN 978-90-8686-135-4. Pp. 523, illustr. Wageningen: Wageningen Academic Publishers. Price 97.00 €.

Indoor fungi continue to be a matter of considerable concern, to the extent that the World Health Organization (WHO) issued "Guidelines on Air Quality: dampness and mould" in 2009. There are already numerous publications on the matter, including several recent books, for example on identification (e.g. Samson *et al.* 2010) and sampling and analysis methods (e.g. Yang & Heinsohn 2007). However, this new work, which is also available as an e-book, has a somewhat different aim, to describe the fundamentals of indoor mould growth as a perquisite to tackling the problem in buildings that exist and ones yet to be designed and built. In order to do this, it brings together 23 specialists from diverse pertinent disciplines, including materials science, physics, and public health as well as mycologists.

The result is a book which has three main threads that the editors consider set it apart: (1) the response of moulds to indoor climate dynamics; (2) the crucial role of materials in control strategies for indoor mould; and (3) the newest insights into adverse health effects.

As many who consult the work will not have a mycological or microbiological background, it starts with five chapters which together present fundamental information on water relations, growth and humidity fluctuations, the fungal cell, ecology and general characteristics of indoor fungi, and the characteristics and identification of indoor wood-decaying basidiomycetes. This is followed by a section on health implications, including epidemiological studies, aerosolization of fungal fragments,



mycotoxins in building materials, and a detailed discussion of the WHO Guidelines mentioned above. Strategies for measuring moisture content, the fungal resistance of interior finishing materials, and for the detection of indoor fungal aerosols follow. Strategies for remediation discuss the situation from experience in North America and western European buildings, the protection of wood, and coating and surface treatments of wood.

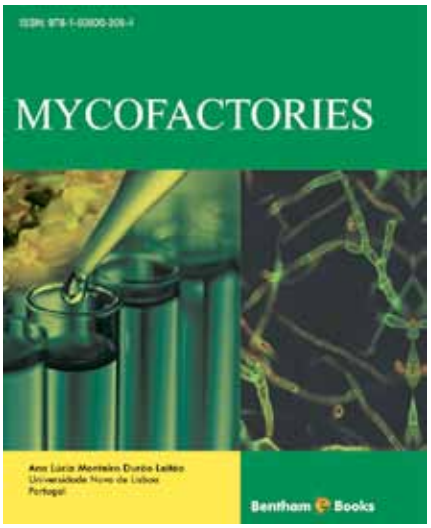
Of particular interest to practicioners is a series of recommendations prepared by the editors (pp. 491–498) and based on those of the Second International Workshop on Fungi in Indoor Environments" held in Utrecht in March 2005. In addition to aspects of inspection and detection, three "pillars" are recognized as important for building and construction: thermal performance, ventilation, and finishing materials. Five statements and recommendations on matters of policy conclude the chapter.

Many of the contributions inevitably have a "western" focus, as that is the region where most research on indoor fungi has been conducted. Nevertheless, this well-edited and thoughtfully constructed book, together with its recommendations merits wide dissemination in the public health sectors of all countries, including those of the tropics. Only in that way will the risks to human health posed by indoor fungi come to be more widely recognized and appropriate prophylactic actions taken.

Samson RA, Houbraken J, Thrane U, Frisvad JC, Anderson B (2010) *Food and Indoor Fungi.* Utrecht: CBS-KNAW Fungal Biodiversity Centre.

Yang CS, Heinsohn P (eds) (2007) *Sampling and Analysis of Indoor Microorganisms*. Hoboken, NJ: Wiley-Interscience.

Mycofactories. Edited by Ana Lúcia Monteiro Durão Leitão. 2011. eISBN 978-1-60805-223-3. Pp. v + 147, illustr. Bentham e-Books. Price: US $ 59.00.

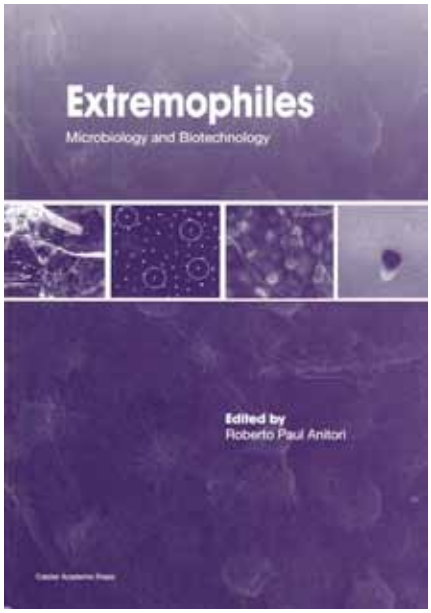


The term "mycofactory" was new to me, and is used here in the sense of the use of fungi, and particularly fungal enzymes, in industrial processes, especially those conducted within a factory. This e-book focuses on recent developments, future trends, and realizable potentials in the exploitation of fungi as a main source for the production of enzymes and for the manufacturing of food derivatives, applications in bioremediation, and the production of pigments and other food additives. This is such an enormous field that a selection of topics had to be made, and seven are chosen. These concern: (1) Hydrolases, especially thermotolerant amylases in starch utilization for the baking, sugar, sweetener, textile, brewing and paper manufacturing industries; pectinases in fruit juice extraction and coffee and tea fermentation; and phytases in animal and fish feed. (2) Lignocellulose biodegradation, and applications of lignocellulolytic fungi or their enzymes in the biotransformation and biodegradation of wastes, and the conversion of biomass into useable products. (3) The emerging potential of the fungal secrotome in biomass degradation as revealed from genomic and proteomic analyses. (4) Multicopper oxidases, especially laccases and tyrosinases, and their potential applications in the oxidation of aromatic compounds. (5) The use of *Penicillium* species as ripening agents in cheese and meat products, including rarely compiled information on *P. nalgiovense* on the surface of certain meat products. (6) *Monascus* pigments used in food colouring and flavouring, and further dietary supplements to ameliorate hyperlipidemia, hypercholesterolemia, and hypertension. And (7) the development of biofilters to purify or deodorize waste gases by passing them though fungal.

I found this an exciting and stimulating book, with numerous fine colour diagrams explaining the processes, and with extensive reference lists for each chapter. In addition to clear and full explanations of current applications, it gives a topical overview of cutting-edge research and glimpses as to the potential fungi have to play an increasingly important role in industry – and at a time where it endeavours to develop novel strategies to meet current and emerging human needs and challenges. The editor, from the Universidade Nova de Lisboa in Portugal, is to be congratulated on marshalling her authors to prepare such a fine prospectus for, and glimpses of, future directions in industrial mycology.

Extremophiles: microbiology and biotechnology. Edited by Roberto Paul Anitori. 2012. ISBN 978-1-9904455-98-1. Pp. xii + 299. Caister, Norfolk, UK: Caister Academic Publishing. Price US$ 319.00, £ 159.

"Microbiology" is used here in what is increasingly the usual sense of being almost synonymous with prokaryotology. Nevertheless, it does have some content of interest to mycologists. Thomas D. Brock poignantly notes in a Foreword (p. ix) that the term "extremophile" is essentially anthropocentric and that it could be more aptly defined taxonomically on the basis of environments where particular organisms can grow but others cannot. There is a helpful table (p. 4) with definitions of eight categories of extremophiles with commercial applications, ranging from hyperthermophiles (optimal growth > 70 °C) to piezophiles (growth at > 38 MPa). The 11 individual chapters are either reviews of a particular extremophile niche (e.g. acidophiles, deep sea environments, ionizing radiation resistant, psychrophiles, or a particular exploited thermophile (e.g. a cold-loving archaeon). Fungi are almost entirely ignored except for a chapter by Helena Nevalainen and co-workers (pp. 89–108) devoted to psychrophilic microfungi; this provides a valuable overview (though omitting to mention lichens) and also detailed information on a cold-active li-



pase from an Antarctic strain of *Penicillium expansum* -- which has potential application in the degradation of crude oil and has been tested on a range of islands. Nevertheless, this book will be of some value to mycologists wishing to categorize extremophile fungi, or wishing to learn of possible novel commercial applications for particular enzymes. Unfortunately, the opportunity was missed to treat or critically review what is known of the numerous fungi of extreme environments alongside the archaea and bacteria; that would have enhanced its value to mycologists considerably.

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

2012

2nd Annual International Symposium on Mycology (ISM-2012)
30 July–1 August 2012
Guangzhou, China
Contact: Maya Chen, East Area F11 Building 1, Dalian Ascendas IT Park, 1 Hui Xian Yuan, Dalian Hi-Tech Industrial Zone, LN 116025, China; maya@bitconferences.com
<bitlifesciences.com/wcm2012/>

New Era in Fungal Nomenclature. State Key Laboratory for Lichenology and Mycology (SKLM) and Mycological Society of China (MSC)
8–10 August 2012
Institute of Microbiology, Chinese Academy of Science, Beijing, China
Contact: Lei Cai; mrcailei@gmail.com
<www.mycolab.org.cn>

13th International Congress on Yeasts (ICY): Yeasts for a Sustainable Future
26–30 August 2012
Monona Terrace Community and Convention Center Madison, WI, USA
Contact: Thomas Jeffries, Forest Products Laboratory, One Gifford Pinchot Drive, Madison, WI 53705, USA; twjeffri@wisc.edu
<conferencing.uwex.edu/conferences/icy2012/>

Fungal Interactions [British Mycological Society Annual Meeting]
3–6 September 2012
Universidad de Alicante, Alicante University, Spain
Contact: imem@ua.es
<britmycolsoc.org.uk/science/scientific-meetings/2012-alicante/>

2nd Annual World Congress on Marine Biotechnology (WCMB-2012)
20–23 September 2012
World Expo Center, Dalian, China
Contact: Doris Han, East Area F11 Building 1, Dalian Ascendas IT Park, 1 Hui Xian Yuan, Dalian Hi-Tech Industrial Zone, LN 116025, China; doris@bit-ibio.com
<bitconferences.com/wcmb2012/>

Australasian Mycological Society Conference
26–28 September 2012
Flecker Botanical Gardens Vistior Centre, Cairns, QLD, Australia
Contact: Sandra Abell; sandra.abell@jcu.edu.au
<australasianmycology.com>

International Mycological Conference 2012
[German Society of Mycology (DGfM) and the Mycology and Lichenology section of the German Botanical Society (GML)]
1–3 October 2012
Drübeck, Germany
<dgfm-ev.de>

Integrated Soil Fertility Management in Africa: from microbes to markets.
Tropical Soil Biology and Fertility Institute of CIAT (CIAT-TSBF) and the Faculty of Agriculture of the University of Nairobi
22–28 October 2012
Safari Park Hotel, Nairobi, Kenya
Contact: Ken Dashiell; k.dashiell@cgiar.org
<isfmafrica2012.org/>

2013

One Fungus = Which Gene(s)?
10–11 April 2013

Royal Dutch Academy of Arts and Sciences, Amsterdam, The Netherlands.
Contact: Pedro Crous; p.crous@cbs.knaw.nl
<cbs.knaw.nl/>

23rd European Congress of Clinical Microbiology and Infectious Diseases (ECCMID)
27–30 April 2013
Berlin, Germany
Contact: ECCMID 2013, c/o Congrex Switzerland Ltd, Peter Merian-Strasse 80, 4002 Basel, Switzerland; basel@congrex.com
<congrex.com>

Asian Mycological Congress and 13th International Marine and Freshwater Mycology Symposium
14–19 August 2013
Beijing International Convention Center, Beijing, China
Contact: Na Jiang; AMC2013@163.com
<amc2013.com>

Bio-security, Food Safety and Plant Pathology: The Role of Plant Pathology in a Globalized Economy 10th International Congress of Plant Pathology
25–31 August 2013
Beijing International Convention Center, Beijing, China
<bicc.com.cn> and <icppb2013.org/file/>

6th Trends in Medical Mycology (TIMM)
11–14 October 2013
Tivoli Hotel and Conference Center, Copenhagen, Denmark
Contact: Congress Care, P.O. Box 440, 5201 AK 's-Hertogenbosch, The Netherlands; info@congresscare.com
<congresscare.com>

2014

IUMS XIV International Congress of Mycology
[with the Congresses of Bacteriology and Applied Microbiology, and also Virology]
27 July–1 August 2014
Montreal, Canada
Contact: e-mail: iums3014@nrc-cnrc.gc.ca
<montrealiums2014.org/>

10th International Mycological Congress (IMC10)
3–8 August 2014
Queen Sirikit National Convention Center, Bangkok, Thailand
Contact: Lekha Manoch; agrlkm@ku.ac.th

NOTICE

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.hawksworth@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.

doi:10.5598/imafungus.2012.03.01.01

IMA FUNGUS · VOLUME 3 · NO 1: 1–7

ARTICLE

Pilidiella tibouchinae sp. nov. associated with foliage blight of *Tibouchina granulosa* (quaresmeira) in Brazil

Bruno E.C. Miranda[1], Robert W. Barreto[1], Pedro W. Crous[2], and Johannes Z. Groenewald[2]

[1]Universidade Federal de Viçosa, Departamento de Fitopatologia, 36570-000, Viçosa, MG, Brazil; corresponding author e-mail: Robert W. Barreto, rbarreto@ufv.br

[2]CBS-KNAW Fungal Biodiversity Centre, P.O. Box 85167, 3508 AD, Utrecht, The Netherlands

Abstract: *Tibouchina granulosa* (*Melastomataceae*), Brazilian glorytree (Brazilian common name – quaresmeira), a common tree of the Atlantic Forest of Brazil, is widely used as an ornamental for its violet or pink blossoms. Little is known about fungal diseases affecting this species, although these represent a known limitation for its cultivation in nurseries. Among these there is a foliage blight that occurs in combination with distortion of branch apices and die-back. A consistent association of a species of *Pilidiella* with the diseased tissues was observed. The fungus was isolated in pure culture and based on its morphology and DNA phylogeny, we conclude that it represents a new species, for which the name *Pilidiella tibouchinae* is introduced.

Key words:
Coniella
Diaporthales
ITS
LSU
Sordariomycetes
systematics

Article info: Submitted: 9 January 2012; Accepted: 29 February 2012; Published: 5 April 2012.

INTRODUCTION

Tibouchina granulosa (*Melastomataceae*), the Brazilian glorytree, is a fast growing tree native that occurs in the Atlantic Forest of Brazil. It is a common and important component of the native flora, particularly in secondary forests. It is also a highly prized ornamental, which is widely used in gardens and parks because of its spectacular violet or pink blossoms that each year appear prior and during Easter. The period of lent (quaresma, in Portuguese) is an important period in the catholic tradition, from which the Brazilian common name for this tree, "quaresmeira" is derived (Lorenzi 2002). Usually there is a high demand for young *T. granulosa* plants in the garden nursery market in Brazil, particularly for the variety that produces pink flowers. Nevertheless, diseases are known to be a limiting factor in nursery production of *T. granulosa.* However, very little is known about the diseases affecting this tree. The record of *Chrysoporthe cubensis* (syn.: *Cryphonectria cubensis*) on *T. granulosa* in Brazil by Seixas *et al*. (2004) is the sole record of a fungal pathogen on this host in the Brazilian database (http://pragawall.cenargen.embrapa.br/aiqweb/michtml/fichahp.asp?id=1912)–and there are only three records of fungi on this host in the USDA fungal database (Farr & Rossman 2010). This is somewhat surprising for such a common plant in the neotropics, and possibly reflects the limited existing knowledge about plant pathogenic fungi occurring on wild plants in this region. Here we clarify the identity of the fungus associated with a severe foliage blight (often evolving into a form of die-back) of quaresmeira. This is one of the most widespread and damaging diseases affecting *T. granulosa* in the field, in gardens, and also in nurseries.

MATERIAL AND METHODS

Isolates

Samples of young abnormal branches of *Tibouchina granulosa* bearing diseased leaves were collected at two localities in Brazil (states of Rio de Janeiro and Minas Gerais), dried in a plant press and brought to the laboratory for further examination. Representative specimens of the fungus were deposited in the herbarium at the Universidade Federal de Viçosa (VIC). Pure cultures were obtained by transfer of conidia, using a sterile fine-pointed needle, from lesions onto plates containing VBA (vegetable broth-agar) as described in Pereira *et al.* (2003). Pure cultures are deposited in the fungal culture collection at the Universidade Federal de Viçosa and also at the CBS-KNAW Fungal Biodiversity Centre (CBS) in Utrecht, The Netherlands. Representative voucher specimens are deposited in VIC and at CBS.

DNA isolation, amplification and analyses

Genomic DNA was isolated from fungal mycelium grown on MEA, using the UltraCleanTM Microbial DNA Isolation Kit (MoBio Laboratories, Inc., Solana Beach, CA, USA) according to the manufacturer's protocols. The primers V9G (de Hoog & Gerrits van den Ende 1998) and LR5 (Vilgalys & Hester 1990) were used to amplify part of the nuclear rDNA operon spanning the 3' end of the 18S rRNA

gene (SSU), the internal transcribed spacer 1, the 5.8S rRNA gene, the internal transcribed spacer 2 (ITS) and the first 900 bases at the 5' end of the 28S rRNA gene (LSU). The primers ITS4 (White *et al*. 1990) and LSU1Fd (Crous *et al*. 2009a) were used as internal sequence primers to ensure good quality sequences over the entire length of the amplicon. The PCR conditions, sequence alignment, and subsequent phylogenetic analysis followed the methods of Crous *et al*. (2006, 2009b). Additionally, partial translation elongation factor 1-alpha (TEF) sequences were determined as described by Bensch *et al*. (2010). Sequences were compared with the sequences available in NCBI's GenBank nucleotide (nr) database using a megablast search and alignments were constructed based on these results for ITS. For LSU, the novel sequence were added to an alignment modified from Lamprecht *et al*. 2011 (TreeBASE study S11805). Sequences derived in this study were lodged at GenBank, the alignment in TreeBASE (www.treebase.org/treebase/index.html), and taxonomic novelties in MycoBank (www.MycoBank.org; Crous *et al*. 2004).

Morphology

Slides containing fungal structures were mounted in lactophenol or lactofuchsin, with 30 measurements determined per structure. Sections were prepared with the help of a freezing microtome (Microm HM 520). Observations of fungal structures and measurements, as well as preparation of photographs, were performed with an Olympus BX 51 light microscope fitted with an Olympus E330 camera. Colony characters and pigment production were noted after 6 d of growth on 2 % malt extract agar (MEA) and potato carrot agar (PCA) (Crous *et al*. 2009c) plates incubated at 25 ºC. Colony colours (surface and reverse) were rated according to the colour charts of Rayner (1970).

RESULTS

Phylogeny

Approximately 1700 bases, spanning the ITS and LSU regions, were obtained from the sequenced culture.

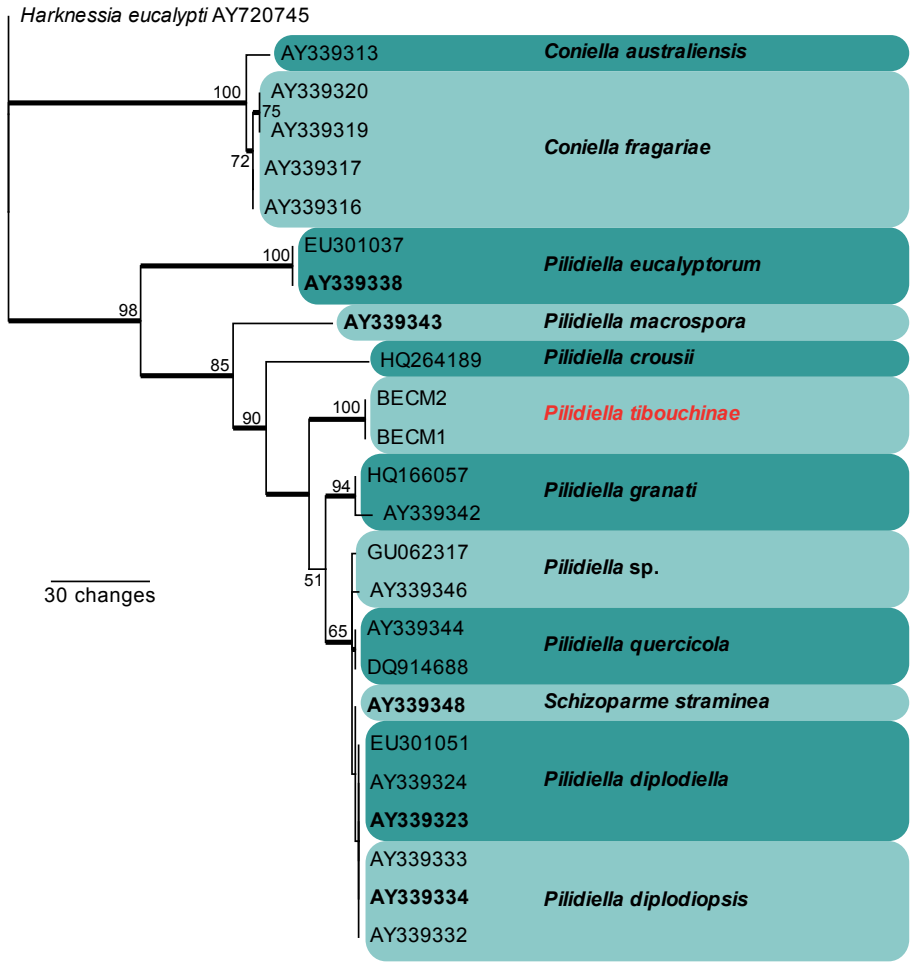


Fig. 1. The first of 24 equally most parsimonious trees obtained from a heuristic search with 50 random taxon additions of the ITS sequence alignment (Tree length = 229, CI = 0.834, RI = 0.904, RC = 0.754). The scale bar shows 30 changes, and bootstrap support values from 1000 replicates are shown at the nodes. Accession numbers of ex-type strains are shown in **bold** and the novel species in this study in red. Branches present in the strict consensus tree are thickened and the tree was rooted to a sequence of *Harknessia eucalypti* (GenBank accession no. AY720745).

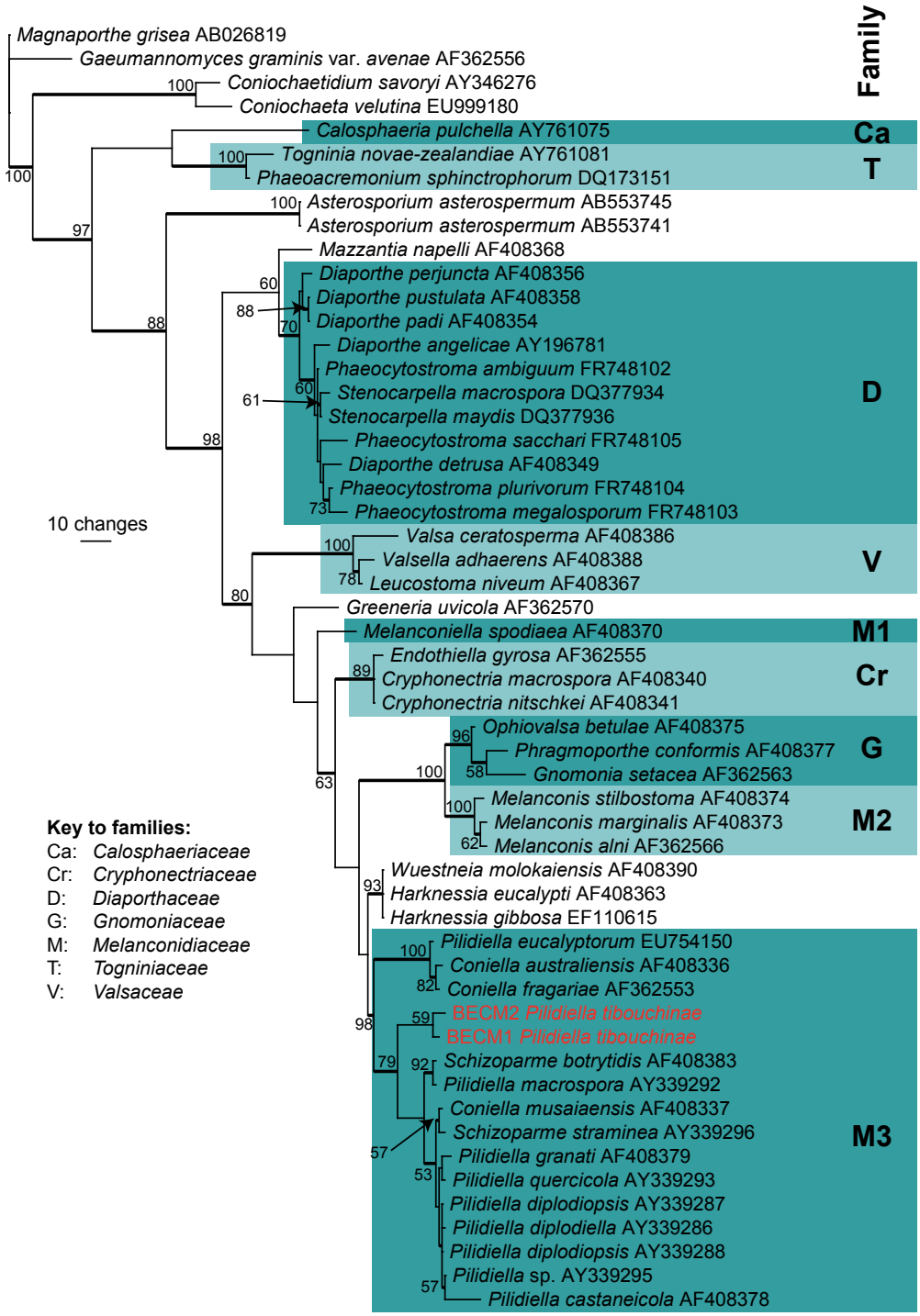


Fig. 2. The first of 180 equally most parsimonious trees obtained from a heuristic search with 100 random taxon additions of the LSU sequence alignment (Tree length = 594, CI = 0.515, RI = 0.817, RC = 0.421). The scale bar shows 10 changes, and bootstrap support values from 1000 replicates are shown at the nodes. The novel species in this study is indicated in red and families are indicated to the right of the tree according to the key on the figure. Branches present in the strict consensus tree are thickened and the tree was rooted to sequences of *Magnaporthe grisea* and *Gaeumannomyces graminis* var. *avenae* (GenBank accession nos AB026819 and AF362556, respectively).

The ITS region was used in the phylogenetic analysis to determine species-rank relationships (Fig. 1) and the LSU region for the generic placement (Fig. 2). The manually adjusted ITS alignment contained 25 taxa including the outgroup sequence and, of the 501 characters used in the phylogenetic analysis, 86 were parsimony-informative, 49 were variable and parsimony-uninformative, and 366 were constant. Twenty-four equally most parsimonious trees were retained from the heuristic search, the first of which is shown in Fig. 1. The phylogenetic tree of the ITS region (Fig. 1) shows that the obtained sequences cluster between *Pilidiella crousii* and *Pilidiella granati*. The manually adjusted LSU alignment contained 54 taxa including the two outgroup sequences and, of the 840 characters used in

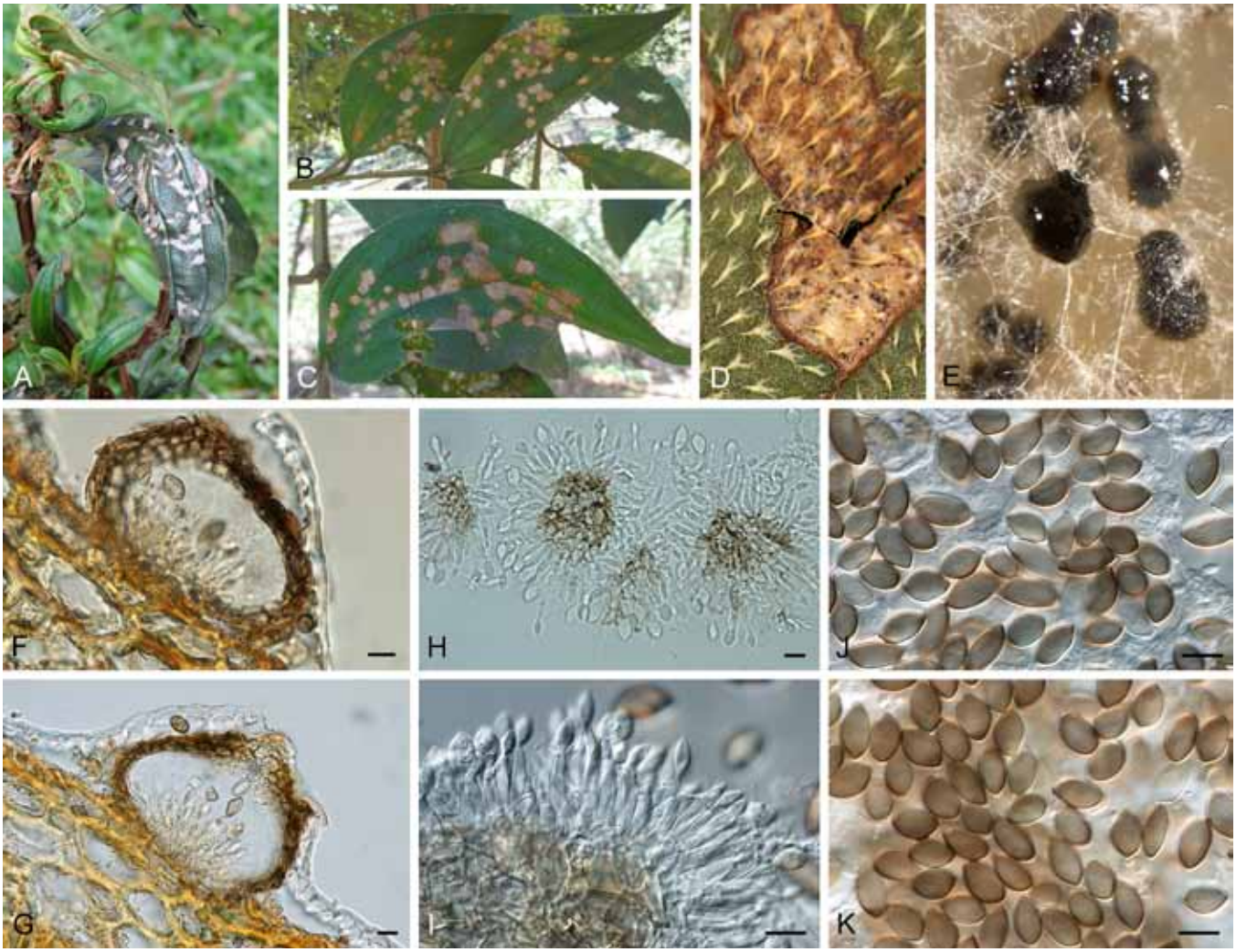


Fig. 3. *Pilidiella tibouchinae*. **A–D.** Leaf spots and curling on *Tibouchina granulosa*. **E.** Colony on oatmeal agar. **F, G.** Vertical section through pycnidia. **H, I.** Conidiogenous cells. **J, K.** Conidia. Bars = 10 µm.

the phylogenetic analysis, 190 were parsimony-informative, 54 were variable and parsimony-uninformative, and 596 were constant. From this heuristic search, 180 equally most parsimonious trees were retained, the first of which is shown in Fig. 2. Phylogenetic analysis of the LSU region (Fig. 1) confirms the placement of the novel sequences in *Pilidiella*. The partial TEF sequences did not have any high identity to those sequences available in GenBank (data not shown).

Taxonomy

A pycnidial coelomycete was regularly associated with diseased tissues on the samples collected at the two separate localities. Its morphology conformed to that of species in the genus *Pilidiella* (Nag Raj 1993, van Niekerk *et al.* 2004), although it appeared to represent a distinct taxon. Accordingly, a new species name is introduced below to accommodate the fungus occurring on *T. granulosa.*

Pilidiella tibouchinae B.E.C. Miranda, R.W. Barreto & Crous, **sp. nov.**
MycoBank MB563992
(Fig. 3)

Etymology: Named after the host genus on which it occurs, *Tibouchina*.

Diagnosis: Similar to *Pilidiella eucalyptorum* but lacking conidial germ slits, and similar to *P. petrakioidea* but lacking mucoid appendages on conidia.

Type: **Brazil**: *Minas Gerais*: Viçosa, campus of the Universidade Federal de Viçosa, on leaves of *Tibouchina granulosa,* 8 March 2010, *B. C. Miranda* (VIC 31443 – holotype; CBS H-20827 – isotype; cultures ex-holotype CPC 18511, CPC 18512 = CBS 131595).

(GenBank accession numbers for VIC 31443 and VIC 31444: ITS = JQ281774, JQ281775; LSU = JQ281776, JQ281777; TEF = JQ281778, JQ281779)

Other specimen examined: **Brazil**: *Minas Gerais*: Viçosa, campus of the Universidade Federal de Viçosa, on leaves of *T. granulosa,* 17 May 2010, *B. C. Miranda* (VIC 31444).

Description: Lesions on living leaves and young stems, firstly as adaxial straw-coloured necrotic spots, mostly appearing near the leaf veins, becoming yellowish to greyish with a dark brown to dark purple border, irregularly shaped, coalescing and leading to necrosis and distortion of large parts of the leaf lamina; loss of necrotic leaf parts usually creating the impression of insect damage. In conjunction to these symptoms, a shortening of branch internodes, leaf distortion, bud death, necrosis and die-back of young stems are also observed. Stunting and decline of severely affected plants are observed even for adult plants. *Conidiomata* pycnidial, adaxial, subcuticular, solitary, globose to depressed globose, 42.5–75 × 75–112.5 µm, wall composed of dark greyish brown *textura angularis* of 1–3 cell layers, 7–12 µm thick, dark brown; dehiscence ostiolate, central; *conidiophores* formed on a dense, basal, cushion-like aggregation of hyaline cells, mostly reduced to conidiogenous cells, subcylindrical, branched below, 8–15 × 3–4 µm, smooth, hyaline, 1–2-septate. *Conidiogenous cells* enteroblastic, phialidic with apical periclinal thickening, 5–10 × 2–3 µm, smooth, hyaline, with minute collarette, and covered in mucilage. *Conidia* mostly broadly ellipsoidal, often somewhat flattened on one side, oblong, subreniform, ovoid to subovoid, 10–13 × 6–8 µm (l:b = 1.7), apex rounded, subtruncate at base, hilum sometimes slightly protuberant, aseptate, hyaline when immature, becoming smoky-brown at maturity, smooth, guttulate (usually with one large guttule but sometimes biguttulate or eguttulate).

Culture characteristics: (MEA or PCA either under a 12 h light regime or in the dark): Colonies fast-growing (up to 86 mm diam after 6 d); flat, occasionally slightly raised centrally; mostly composed of immersed mycelium, aerial mycelium mostly sparse (but very dense cottony to woolly aerial mycelium on MEA in the dark); cottony to woolly to spider web-like white to grey olivaceous, sometimes with some small cinnamon areas centrally, occasionally becoming powdery towards the periphery, abundant olivaceous black fruit bodies crowded in zone rings on MEA/light. On PCA greyish black to greenish black centrally, with saffron margin in reverse; black fruit bodies less abundant, and in more distinct rings on PCA/light.

DISCUSSION

The fungus on *Tibouchina granulosa* clearly belongs to the *Coniella/Pilidiella*-complex that has *Schizoparme* teleomorphs (*Schizoparmaceae*, *Diaporthales*; Rossman *et al*. 2007). Fungi in *Schizoparmaceae* include several species associated with foliar diseases, sometimes occurring as secondary invaders of plant tissues infected by other organisms or injured by other causes (Ferreira *et al.* 1997). There is no record of any teleomorphic species of *Schizoparmaceae* in association with members of the genus *Tibouchina,* and a single doubtful record of a *Coniella* on another member of the *Melastomataceae, Miconia serrulata* (Farr & Rossman 2010). Several members of *Myrtales*, which according to Bremer *et al.* (2003) includes up to 14 families, are known hosts of *Schizoparmaceae* (Farr & Rossman 2010). For instance, several species are known from *Myrtaceae* (*Acca*, *Blepharocalyx*, *Eucalyptus*, *Eugenia, Heteropyxis, Myrcia*, *Syzygium)*, *Lythraceae* (*Lythrum, Punica*), and *Combretaceae* (*Anogeissus, Anogeissus, Terminalia*) (van Niekerk *et al*. 2004, Farr & Rossman 2010)

Sutton (1980) and Nag Raj (1993) treated *Pilidiella* as a synonym of *Coniella*. However, based on analyses of large subunit (LSU) nuclear ribosomal DNA (nrDNA) sequences, Castlebury *et al.* (2002) concluded that *Pilidiella* is distinct from *Coniella*. *Pilidiella* has two main morphological criteria separating it from *Coniella*: the presence of conidia that are hyaline when young becoming pale brown with age (consistently brown in *Coniella*) (Castlebury *et al.* 2002) and having a length to breadth ratio larger than 1.5 (equal to or smaller than 1.5 for *Coniella*) (van Niekerk *et al*. 2004). Pigmentation alone is difficult to interpret (Table 1), although *P. tibouchinae* has hyaline conidia that become smoky brown at maturity and a l:b ratio of 1.7. Additionally the results of the phylogenetic analysis place *P. tibouchinae* in the *Pilidiella* clade, distinct from *Coniella* (Figs 1–2). Considering the combination of morphological and molecular data, we prefer to place this fungus in the genus *Pilidiella.* Nevertheless, several species in the group still need to be re-examined, as is evident from Fig. 2, where *Pilidiella eucalyptorum* clusters in the *Coniella* clade, and *C. musariensis* clusters in the *Pilidiella* clade.

Pycnidia in *P. tibouchinae* are small when compared to the species of *Schizoparmaceae* treated by Sutton (1980), Nag Raj (1993), and van Niekerk *et al*. (2004). Morphologically, conidia of *P. tibouchinae* show some similarity to that of *P. eucalyptorum* and *P. petrakioidea.* However, conidia of *P. tibouchinae* lack conidial germ slits (present in *P. eucalyptorum*) and mucoid appendages (present in *P. petrakioidea*). It also has thinner pycnidial walls (7–12 µm), than those in *P. eucalyptorum* (to 25 µm thick), and has hyaline to pale smoky-brown conidia, whereas those of *P. eucalyptorum* are medium to dark reddish brown. Furthermore, *P. tibouchinae* also differs from *P. petrakioidea* in conidial morphology (narrowly ellipsoidal with acutely rounded apices in *P. petrakioidea*) and a l:b ratio larger than 1.9. Five species of *Coniella* have been described in association with members of the *Myrtaceae*: *C. australiensis*, *C. castaneicola*, *C. costae*, *C. fragariae,* and *C. minima.* Considering the close morphological similarity of *Pilidiella* and *Coniella,* the conidial morphology of these species is also provided here for comparison with that of *P. tibouchinae* (Table 1).

Pilidiella tibouchinae is the first species of the genus to be described on a host belonging to *Melastomataceae,* on which it appears to be associated with a rather serious foliar and dieback disease. Further investigations aimed at clarifying the pathological status of the fungus on *Tibouchina granulosa,* and evaluating potential disease control measures are now urgently required, and will be reported elsewhere.

Table 1. Conidial morphology of selected *Coniella* and *Pilidiella* species recorded from members of *Myrtales*.

Species	Size	l:b rate	Shape	Appendage	Germ slit	Reference
Coniella australiensis	(9–)10–11(–14) × (6–)7–8(–10) µm	1.4	Broadly ellipsoidal	+	-	van Niekerk *et al.* (2004)
C. castanaeicola	13–29 × 2.5–3.5 µm	7.3	Fusoid to falcate	+	-	Nag Raj (1993)
C. costae	19–28 × 7–7.5 µm	3.2	Fusoid to ellipsoid	-	-	Dianese *et al.* (1993)
C. delicata	7–9 × 2.5–3 µm	2.9	Ellipsoid	-	-	Sutton (1980)
C. fragariae	(8–)9–10(–12.5) × (5–)6–7(–8) µm	1.5	Ellipsoid	+	+ in older conidia	van Niekerk *et al.* (2004)
C. macrospora	(18.3–)25–29(–32.5) × (13–)16–20(–21.5) µm	1.5	Ovoid, ellipsoid, pyriform, globoid	+	-	van der Aa (1983)
C. minima	6.5–7.5 × 3.5–4.5 µm	1.5	Globoid to subgloboid	-	-	Sutton (1969)
C. terminaliae	2–8 × 2–3.5 µm	2:01	Globose to subglobose	-	-	Firdousi *et al.* (1994)
Pilidiella crousii	(6–)7–12(–13.5) × (2.5–)3–5 µm	2.2	Narrowly ellipsoid to ellipsoid	-	-	Rajeshkumar *et al.* (2011)
P. diplodiella	(10–)12–15(–19) × (4–)5–6 µm	2.3	Narrowly ellipsoid	+	-	van Niekerk *et al.* (2004)
P.eucalyptorum	(9–)10–12(–14) × (6–)7–8 µm	1.6	Broadly ellipsoid or limoniform	uncommon	+	van Niekerk *et al.* (2004)
P. granati	9–16 × 3–4.5 µm	2.8	Ellipsoid	+	-	Nag Raj (1993)
P. jambolana	19–22 × 3.5–4 µm	5.7	Elongate-fusoid	-	-	Ahmad (1967)
P. petrakioidea	12–14.5 × 6.5–8 µm	1.9	Narrowly ellipsoid	+	-	Nag Raj (1993)
P. tibouchinae	10–13 × 6–8 µm	1.7	Broadly ellipsoid	-	-	This publication

ACKNOWLEDGEMENTS

We thank the technical staff of CBS, Arien van Iperen (cultures), Marjan Vermaas (photographic plates), and Mieke Starink-Willemse (DNA isolation, amplification and sequencing) for their invaluable assistance.

REFERENCES

Aa HA van der (1983) A new species of *Coniella*. *Proceedings of the Koninklijke Nederlandse Akademie van Wetenschappen, C* **86**: 121–125.

Ahmad S (1967) Contributions to the fungi of West Pakistan–VI. *Biologica* **13**: 15–42.

Bensch K, Groenewald JZ, Dijksterhuis J, Starink-Willemse M, Andersen B, Summerell BA, Shin H-D, Dugan FM, Schroers H-J, Braun U, Crous PW (2010) Species and ecological diversity within the *Cladosporium cladosporioides* complex (*Davidiellaceae*, *Capnodiales*). *Studies in Mycology* **67**: 1–94.

Bremer B, Bremer K, Chase MW, Reveal JL, Soltis DE, *et al*. (2003) An update of the angiosperm phylogeny group classification for the orders and families of flowering plants: APG II. *Botanical Journal of the Linnean Society* **141**: 399–436.

Crous PW, Gams W, Stalpers JA, Robert V, Stegehuis G (2004) MycoBank: an online initiative to launch mycology into the 21st century. *Studies in Mycology* **50**: 19–22.

Crous PW, Groenewald JZ, Risède J-M, Simoneau P, Hyde KD (2006) *Calonectria* species and their *Cylindrocladium* anamorphs: species with clavate vesicles. *Studies in Mycology* **55**: 213–226.

Crous PW, Groenewald JZ, Summerell BA, Wingfield BD, Wingfield MJ (2009b) Co-occurring species of *Teratosphaeria* on *Eucalyptus*. *Persoonia* **22**: 38–48.

Crous PW, Schoch CL, Hyde KD, Wood AR, Gueidan C, Hoog GS De, Groenewald JZ (2009a) Phylogenetic lineages in the *Capnodiales*. *Studies in Mycology* **64**: 17–47.

Crous PW, Verkley GJM, Groenewald JZ, Samson RA (eds) (2009c) *Fungal Biodiversity*. [CBS Laboratory Manual Series **1**.] Centraalbureau voor Schimmelcultures, Utrecht.

Castlebury LA, Rossman AY, Jaklitsch WJ, Vasilyeva LN (2002) A preliminary overview of the *Diaporthales* based on large subunit nuclear ribosomal DNA sequences. *Mycologia* **94**: 1017–1031.

Dianese JC, Medeiros RB, Santos LTP, Sutton BC (1993) *Coniella costae* sp. nov. on leaves of *Myrcia tomentosa* from Brazilian cerrado. *Mycological Research* **97**: 1234–1236.

FAO (1996) *International Standard for Phytosanitary Measures.* Rome: Secretariat of the International Plant Protection Convention.

Farr DF, Rossman AY (2010) Fungal databases. Beltsville, MD: Systematic Mycology and Microbiology Laboratory, ARS, USDA; http://nt.ars-grin.gov/fungaldatabasews/.

Ferreira FA, Alfenas AC, Coelho L (1997) Portas-de-entrada para *Coniella fragariae* em folhas de eucalipto. *Revista Árvore* **21**: 307–311.

Firdousi SA, Sharma CD, Vyas KM (1994) A new species of *Coniella* from India. *Acta Botanica Indica* **22**: 134–135.

Hoog GS de, Gerrits van den Ende AHG (1998) Molecular diagnostics of clinical strains of filamentous basidiomycetes. *Mycoses* **41**: 183–189.

Lamprecht SC, Crous PW, Groenewald JZ, Tewoldemedhin YT,

Marasas WFO (2011) *Diaporthaceae* associated with root and crown rot of maize. *IMA Fungus* **2**: 13–24.

Lorenzi H (2002) *Árvores brasileiras: manual de identificação e cultivo de plantas arbóreas do Brasil*. Vol. 1. 4a edição. Nova Odessa, SP: Instituto Plantarum.

Nag Raj TR (1993) *Coelomycetous Anamorphs with Appendage-bearing Conidia*. Waterloo: Mycologue Publications.

Niekerk JM van, Groenewald JZ, Verkley GJM, Fourie PH, Wingfield MJ, Crous PW (2004) Systematic reappraisal of *Coniella* and *Pilidiella*, with specific reference to species occurring on *Eucalyptus* and *Vitis* in South Africa. *Mycological Research* **108**: 283–303.

Rayner RW (1970) *A Mycological Colour Chart*. Kew: Commonwealth Mycological Institute.

Rossman AY, Farr DF, Castlebury LA (2007) A review of the phylogeny and biology of the *Diaporthales. Mycoscience* **48**: 135–144.

Sutton BC (1969) Type studies of *Coniella*, *Anthasthoopa*, and *Cyclodomella*. *Canadian Journal of Botany* **47**: 603–608.

Sutton BC (1980) *The Coelomycetes: fungi imperfecti with pycnidia, acervuli and stromata.* Kew: Commonwealth Mycological Institute.

USDA/Fungal Database. Available at http://nt.ars-grin.gov/fungaldatabases/index.cfm. (accessed 20 March 2010).

Vilgalys R, Hester M (1990) Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *Journal of Bacteriology* **172**: 4238–4246.

White TJ, Bruns T, Lee J, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In*: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds), *PCR Protocols: a guide to methods and applications*: 315–322. San Diego: Academic Press.

doi:10.5598/imafungus.2012.03.01.02

IMA FUNGUS · VOLUME 3 · NO 1: 9–14

ARTICLE

Reappraisal and neotypification of *Phyllachora feijoae*

Lilian C. Costa, Davi M. Macedo, and Robert W. Barreto

Universidade Federal de Viçosa, Departamento de Fitopatologia, 36570-000, Viçosa, MG, Brazil; corresponding author e-mail: Davi M. Macedo, dmmesk@yahoo.com.br

Abstract: *Acca sellowiana* (*Myrtaceae*), feijoa (in Brazil, goiaba da serra), is a native southern South America tree that produces edible fruits which, although only occasionally cultivated in South America, became a significant fruit crop in New Zealand. Recently, during surveys for fungal pathogens of feijoa in southern Brazil, several plants were found bearing tar-spot symptoms caused by a species of *Phyllachora.* A literature search enabled us to identify the fungus as *Phyllachora feijoae*, a little-known species originally described in the 19th century by H. Rehm and later transferred to the genus *Catacauma*. The name *Catacauma feijoae*, although now regarded as a later synonym of *P. feijoae* is still mistakenly in use (as, for instance, in the Brazilian list of fungi on plants). The type specimen was most probably deposited in the Botanisches Garten und Museum Berlin-Dahlem (B) and lost or destroyed during World War II, and could not be located. The recent recollection of abundant material of this fungus in the vicinity of Pelotas (Rio Grande do Sul, Brazil) allowed its re-examination and neotypification. *Phyllachora feijoae* is also illustrated here for the first time.

Key words:
Ascomycota
Brazil
fruit crop
Myrtaceae
Neotropics
nomenclature
Phyllachoraceae

Article info: Submitted: 6 November 2011; Accepted: 13 March 2012; Published: 5 April 2012.

INTRODUCTION

The plant family *Myrtaceae* includes approximately 150 genera with over 5 500 species (Heywood *et al.* 2007), amongst which are some important forestry species (e.g. *Eucalyptus* spp.) and several fruit crops such as guava (*Psidium guajava*). Some, such as *Acca sellowiana* (common name feijoa; in Brazil, goiabeira da serra) are only minor fruit crops. *Acca sellowiana* is a shrub or small tree native to southern South America (southern Argentina, Brazil, Paraguay, and Uruguay) and, although only occasionally cultivated in South America, it has become more significant as a fruit crop in New Zealand (Al-Harthy 2010). There are few published records of fungal pathogens associated with feijoa (Farr & Rossman 2011, Mendes & Urben 2011). However, during a recent search for pathogens of feijoa in the southern Brazilian state of Rio Grande do Sul, individuals of *A. sellowiana* in rural areas in the vicinity of Pelotas had foliage with intense tar-spot symptoms. Such symptoms were typical of those caused by fungi belonging to the genus *Phyllachora*. Examination of specimens collected and a literature and herbarium search were performed in order to clarify the identity of the fungus on feijoa, and the results of these investigations are presented here.

MATERIAL AND METHODS

Samples of diseased foliage of *Acca sellowiana* were collected in two localities. These were dried in a plant press and taken to the laboratory for further examination. Representative specimens were deposited in the local herbarium (Herbarium Universidade Federal de Viçosa, VIC). Examination of selected leaves bearing tar-spot symptoms with the help of an Olympus SXZ7 stereoscopic microscope revealed that fungal structures were immersed in the leaf tissue and sections were prepared and mounted in lactophenol and lactofucsin for further examination. Additionally, sections were also prepared with a freezing microtome (Cryostat Microm® HM 520). Observations, photographs, and line drawings were prepared with a light microscope Olympus BX51, fitted with a digital camera (Olympus E-volt 330) and a drawing tube.

TAXONOMY

Phyllachora feijoae Rehm, *Hedwigia* **36**: 370 (1897).
Synonym: *Catacauma feijoae* (Rehm) Theiss & Syd., *Ann. Mycol.* **13**: 397 (1915).
(Fig. 1)

Type: **Brazil**: *Rio Grande do Sul*: Pelotas, Chácara da Brigada, Cerro da Buena, on leaves of *Acca sellowiana* (*Myrtaceae*), 18 Aug. 2010, *R. W. Barreto* (VIC 31476 – **neotype designated here;** B 70 0015054 – isoneotype).

Other specimen examined: **Brazil**: *Rio Grande do Sul*: Pelotas, Capão do Leão, on leaves of *Acca sellowiana* (*Myrtaceae*), 18 Aug. 2010, *R. W. Barreto* (VIC 31766).

Lesions on living leaves, adaxially on all leaves at various developmental stages, initially punctiform, becoming irregular tar-spots, raised, with age surrounded by yellowish to reddish peripheral necrotic haloes, widely distributed and leading to foliage distortions, 0.2–0.3 × 2.1–3.0 mm diam, indistinct

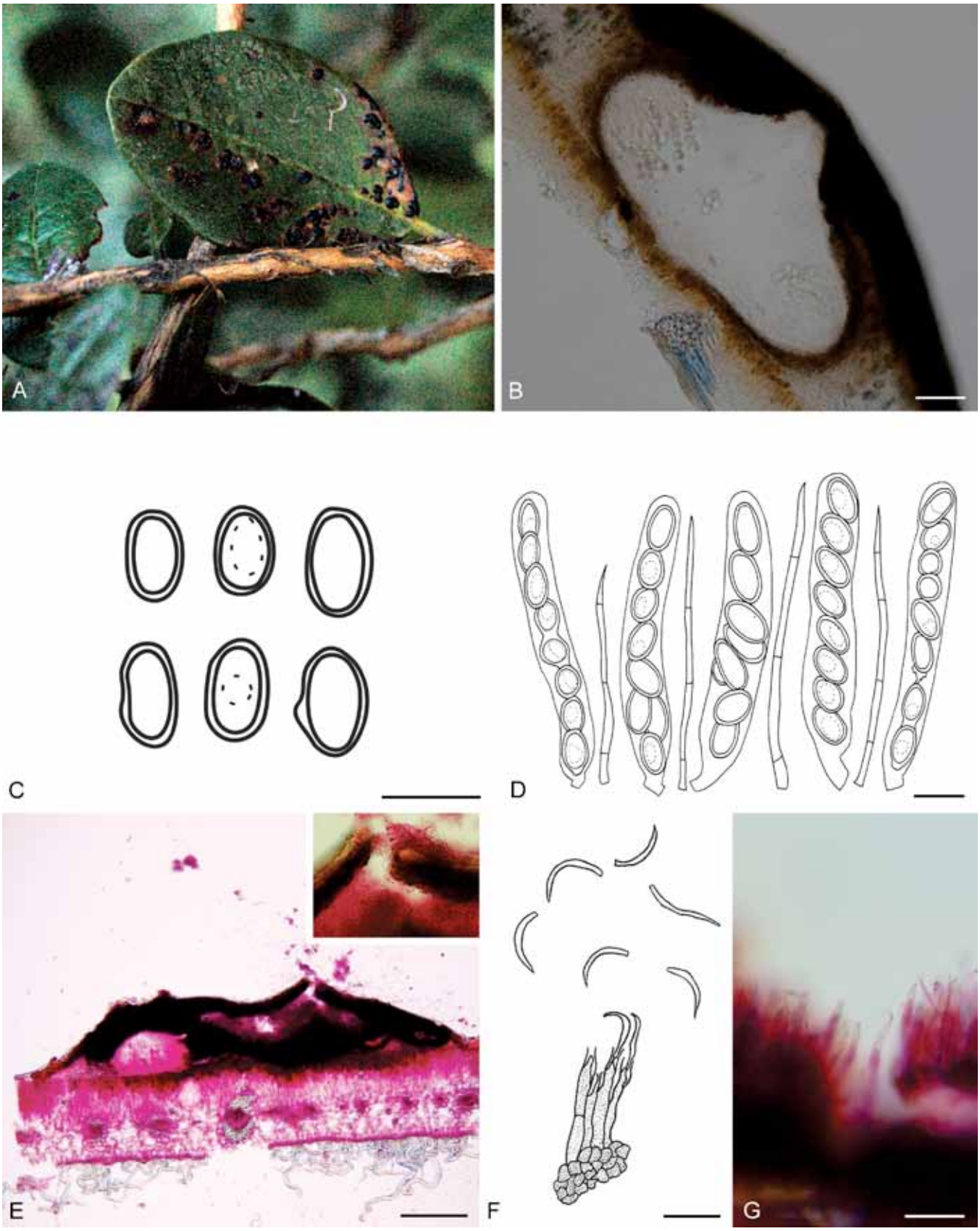


Fig. 1. *Phyllachora feijoae* (VIC 31766). **A.** Tar-spots on leaves of *Acca sellowiana*. **B**. Perithecium and clypeus. **C.** Ascospores. **D.** Paraphyses and asci with ascospores. **E**. Ascomata (*left*) and conidioma (*right*) and close up part of the conidioma. **F–G.** Conidiogenous cell and conidia. Bars: B = 80 µm, C = 20 µm, D = 20 µm, E = 275 µm, F–G = 10 µm.

abaxially. *Internal mycelium* intra- and intercellular, hyphae 2.0–3.0 µm diam, branched, septate, hyaline to pale brown. *External mycelium* absent. *Stromata* adaxial, clypeate, shield-like, merged with the upper wall of the ascoma. *Conidia* formed within stromata externally indistinguishable from teleomorph stomata; flattened, lenticular to irregular (in section), epigenous, subepidermal, single or in combination with ascomata, sometimes very broad occupying nearly the whole breadth of the stroma, 615–1729 x 100–184 µm walls of dark brown *textura angularis*, 38.5–69 µm thick, smooth; *conidiogenous cells* subcylindrical, straight, 15–25(–40) x 2–3 µm, 0–1-septate, pale brown; *conidia* mucilaginous, enteroblastic, acicular, curved, lunate or sigmoid, 13–19 x 1.5 µm, aseptate, thin-walled, hyaline, smooth. *Ascomata* perithecial, epigenous, immersed, solitary, spherical to subsphaerical, somewhat to strongly depressed, short papillate, 41–218 µm diam, inconspicuously ostiolate, composed of thin-walled brown *textura angularis*, walls 6.5–44 µm, 7–11 cells thick, outer layers dark brown, inner layers pale brown to subhyaline. *Interascal tissue* of paraphyses, 2.5–3 µm diam, longer than the asci, filiform, septate, hyaline, thin-walled, constricted at the septae; periphyses well-developed, filiform, hyaline, thin-walled. *Asci* unitunicate, cylindrical to clavate, short-stalked, 70.5–104 x 13–27 µm, apex broadly rounded to nearly flat, thin-walled, 8-spored. *Ascospores* at first uniseriate but sometimes partially biseriate, 15.5–22 x 8–14 µm, ellipsoidal to cylindric-ellipsoidal, rounded at the ends, walls 2–3 µm thick, aseptate, hyaline, smooth, without a mucous sheath or appendages.

Notes: Very little information is available on *Phyllachora feijoae*. Only a very brief description is given in the original publication of Rehm (1897). Later, Theissen & Sydow (1915) prepared a more complete description of the fungus when combining it into *Catacauma*. This is, nevertheless, somewhat incomplete and no illustrations were provided. Furthermore, the description was apparently based on Rehm's material collected in "Serra Geral, Minas Gerais – Brazil". The last publication dealing with this fungus was that of Jimenez & Hanlin (1992), where names of fungi described in *Catacauma* were listed. Although the authors acknowledged that after Petrak's (1924) work it became widely accepted that the distinction of *Catacauma* from *Phyllachora* was artificial, they prudently did not propose that names in *Catacauma* should be immediately rejected or recombined into *Phyllachora* without a careful re-examination of types. Since that publication, mycologists have shown little interest in the names of fungi referred to *Catacauma,* but some earlier fungal names in *Phyllachora* have been reinstated. That is the case of the name *C. feijoae*, presently listed in MycoBank and *Index Fungorum* as a later synonym of *P. feijoae*. Nevertheless, this name is still being used in other instances (e.g. the Brazilian list of fungi on plants; Mendes & Urben 2011).

An expanded description based on the material recently collected in Brazil is provided above. This is also the first time illustrations of *P. feijoae* have been published. The original material of the species studied by Rehm would almost certainly have been deposited in the collections of the Botanisches Garten und Museum Berlin-Dahlem (B), but if so it appears to have been lost or destroyed during World War II as it could not now be found (H.J.M. Sipman, pers. comm.). We therefore designate one of the recent collections as a neotype to fix the application of the name.

DISCUSSION

The fungus on *Acca sellowiana* exhibits all the typical features, both in terms of symptoms produced on the host and in its morphology, to members of the genus *Phyllachora* (*Phyllachoraceae*, *Phyllachorales*). *Phyllachora* is a large genus including approx. 1000 named species (Kirk *et al.* 2008). All species of *Phyllachora* are biotrophic plant pathogens, causing tar-spots on members of numerous plant families, but are particularly common on *Fabaceae* (Cannon 1991) and *Poaceae* (Parbery 1971). Besides the presence of a well-developed, dark brown to black clypeus, other features such as the formation of the perithecia within the plant tissues, and hyaline, thin-walled, smooth and aseptate ascospores, are typical for the genus (Cannon 1991). Around 70 species of *Phyllachora* have been described on members of *Myrtaceae* worldwide (Farr & Rossman 2011), with 21 species recorded on this host-family in Brazil (Mendes & Urben 2011). Species of *Phyllachora* associated with *Myrtaceae* have never been monographed.

Some of the older records of *Phyllachora* on *Myrtaceae* were later recognized as mistakenly placed in that genus. Some were found to belong to other genera, - such as *P. pululahuensis* (now regarded as a synonym of *Vestegrenia multipunctata*; von Arx & Müller 1954), and *P. eucalypti* (now recognized as a synonym of *Clypeophysalospora latitans*; Crous *et al.* 1990). Other species were recombined into genera such as *P. peribebuyensis* which is now treated as *Coccodiella peribebuyensis* (Katumoto 1968). Several names in *Phyllachora* that are listed on members of *Myrtaceae* were found to be later synonyms of already known species names: *P. conspurcata* (syn. *P. tropicalis*; Saccardo 1883), *P. phylloplaca* (syn. *P ipirangae*; Theissen & Sydow 1915b), *P. pseudostromatica* (syn. *P. melaleuca*; Sydow & Sydow 1904), and *P. semillunata* (syn. *P. selenospora*; Petrak & Ciferri 1930). Additionally, *P. langdonii* is now treated as a subspecies of *P. callistemonis*, *P. callistemonis* subsp. *langdonii* (Pearce & Hyde 1994).

In the case of species of *Phyllachora* recorded from Brazil, an issue to be taken into consideration is that numerous species names are included in Mendes *et al.* (1998), and have also been kept in the database of fungi on plants in Brazil (Mendes & Urben 2011) but quoted as being "in press". These names, for which Medeiros & Dianese are given as authors, have never been validly published and include the following species designations associated with members of *Myrtaceae*: *P. eugenii-complicatae, P. eugenii-punctifolie, P. myrciae-decrescentis, P. myrciae-guianensis, P. myrciae-multiflorae, P. myrciae-multiflorae, P. myrciae-pallescentis, P. myrciae-tematae, P. myrciae-tortae,* and *P. pampulhae*. Although all these designations are not validly published, most may well represent good taxonomic species which are still awaiting formal description. Most were collected in the Brazilian cerrado, an area rich in endemic organisms of all kinds.

A study of the 48 published descriptions of taxa (including three varieties) of *Phyllachora* described from hosts belonging

Table 1. Data on *Phyllachora* spp. described on hosts belonging to *Myrtaceae*.

Species	Asci (µm)	Ascospores (µm)	Host plants	References
P. ambígua	50–60 x 8–12	9–11 x 6	*Syzygium cumini* (syn. *Eugenia jambolana*)	Theissen & Sydow (1915b)
P. angustispora	80–90 x 12–14	30 x 8–9	*Eugenia* sp.	Saccardo (1916)
P. bella	60–70 x 5–7	7.5–9 x 3–4	*Syzygium australe* (syn. *E. australis*)	Sydow (1937)
P. biareolata	90–95 x 6–9	12 x 5	*Eugenia rhombea*	Saccardo (1891)
P. biguttulata	50–65 x 8–10	10–12 x 5–5.5	*Campomanesia rhombea*	Saccardo (1913)
P. brenesii	70–80 x 10–15	12–17 x 8–10	*Eugenia guayaquilensis*	Sydow & Petrak (1929)
P. callistemonis	115–210 x 12.5–16	18–27.5 x 7.5–10	*Callistemon pallidus*	Pearce & Hyde (1994)
P. callistemonis subsp. *Langdonii*	100–154 x 12–20	18–25 x 6–9	*Callistemon* sp.	Pearce & Hyde (1994)
P. callistemonis subsp. *Similis*	117–173 x 18–27.5	18–29 x 7.5–12.5	*Callistemon viminalis*	Pearce & Hyde (1994)
P. capensis	100–120 x 13–14	60–70 x 5–6	*Eugenia zuluensis*	Doidge (1942)
P. cayennensis	68–75 x 12–14	20–24 x 7–8	*Psidium* sp.	Theissen & Sydow (1915b)
P. clavata	110–140 x 15–18	39–45 x 3–6	*Myrcia* sp.	Garces Orejuela (1944)
P. curvulispora	60–80 x 10–20	17–20 x 5–7	*Myrtaceae* sp.	Saccardo (1925–1928)
P. distinguenda	60–70 x 18	18–20 x 4.5	*Myrtaceae* sp.	Saccardo (1899)
P. egenula	70–85 x 7–8	10–13 x 5-6	*Leptospermum lanigerum*	Sydow (1938)
P. emarginata	80–130 x 18–28	16–20 x 10–12	*Eugenia* sp.	Petrak (1948)
P. eugeniae	60–75 x 7–9	8–10 x 4–4.5	*Eugenia rhombea*	Chardón (1927)
P. feijoae	60 x 25	18 x 10	*Acca sellowiana*	Rehm (1915)
P. gentilis	120 x 4–12	18–20 x 8–9	*Eugenia* sp.	Saccardo (1895)
P. goyazensis	70–90 x 17–18	12–14 x 8–12	*Myrtaceae* sp.	Hennings (1895)
P. guavira	100–110 x 6–8	12 x 5	*Psidium* sp.	Theissen & Sydow (1915b)
P. ipirangae	30–90 x 10–12	15–16 x 8	*Eugenia* sp.	Theissen & Sydow (1915b)
P. lindmanii	80–90 x 13–16	16–24 x 13–16	*Myrtaceae* sp.	Theissen & Sydow (1915a)
P. maculata[*]		22–25	*Eucalyptus* sp.	Cooke (1891)
P. manuka		10.5–13 × 6.5–8	*Leptospermum scoparium*	Johnston & Cannon (2004)
P. melaleucae	66–84 x 8–11		*Melaleuca spinosa*	Theissen & Sydow (1915a)
P. myrciae[*]			*Eugenia bimarginata*	Saccardo (1883)
P. myrciae-rostratae	100–120 x 6–8	14–17 x 5–6	*Myrcia splendens* (syn. *M. rostrata*)	Viégas (1944)
P. muelleri	95–120 x 13–15	28–32 x 6–7	*Eugenia dodonaeifolia*	Chardón *et al.* (1940)
P. myrrhinii	50–72 x 12–16	14–16 x 5	*Myrrhinium atropurpureum* var. *octandrum*	Theissen & Sydow (1915a)
P. nigerrimum	100–130 x 9	10–16 x 5	*Campomanesea adamantium* (syn. *C. caerulea*)	Viégas (1944)
P. opaca	80–85 x 6–8	10 x 4–4.5	*Myrtaceae* sp.	Berlese & Voglino (1886)
P. peglerae	120–140 x 17–20	20–23 x 12–13	*Eugenia capensis*	Doidge (1942)
P. pettimenginii	85–105 x 14–18	2–8-32 x 8.5–11	*Myrtaceae* sp.	Maire (1908)
P. rhytismoides		14–19.5 × 12–15.5	*Melaleuca cajuputi*	Cannon (1991)
P. rickiana	68–78 x 14–15	10–13 x 6	*Myrtaceae* sp.	Theissen (1918)
P. rimulosa	85–100 x 10	14 x 8	*Eugenia* sp.	Saccardo (1925–1928)
P. samanensis	70–83 x 13–16.5	32–40 x 6–7.5	*Eugenia* sp.	Petrak & Ciferri (1932)
P. shivasii	136–225 x 10–15	15–22 x 6–8.5	*Melaleuca viridiflora*	Pearce & Hyde (1995)
P. subcircinans	80–90 x 10–16	14–16 x 8–10	*Psidium grandifolium*	Viégas (1944)
P. subopaca	75 x 10–15	12–14 x 7	*Myrtaceae* sp.	Saccardo (1899)
P. tachirensis	109–166 x 9.5–12	13–17 x 7–8	*Eugenia* sp.	Chardón & Toro (1934)
P. tropicalis	70–75 x 10–14	15–18 x 7–8	*Psidium grandifolium*	Saccardo (1883)
P. truncatispora	70–90 x 16–24	22–26 x 7–8	*Myrtaceae* sp.	Viégas (1944)
P. urbaniana	70–90 x 16–18	14–15 x 6–8	*Myrtaceae* sp.	Saccardo (1899)
P. verrucosa	78–105 x 15–19	14–20 x 9–13	*Melaleuca leucadendra*	Arx & Müller (1954)

Table 1. (Continued).

Species	Asci (µm)	Ascospores (µm)	Host plants	References
P. whetzelii	87–109 x 8–10.5	11.5–13 x 3–4	*Eugenia* sp.	Chardón (1921)
P. woodiana	80–100 x 6–7.5	12.5–15.0 x 5–6	*Eugenia capensis*	Doidge (1942)

to *Myrtaceae*, is summarized in Table 1. This shows that there are three species of *Phyllachora* with close morphological similarity to *P. feijoae* on *A. sellowiana*: *P. brenesii*, *P. emarginata,* and *P. subcircinans.* Each of those species was found to have morphological differences from *P. feijoae. Phyllachora. brenesii* has perithecia with narrower walls (5 µm thick), and asci which are also narrower (10–15 µm wide). *Phyllachora emarginata* has thinner ascospore walls (2 µm). And *P. subcircinans* has much wider perithecia (250–500 µm diam). Additionally, *P. feijoae* can be recognized as distinct from the other species known on *Myrtaceae* (Table 1) by a combination of morphometric features; differences in perithecial diameter, ascus width, and the absence of a mucilaginous sheath on the ascospores. Although no comparison of the morphology of *P. feijoae* with other species on *Myrtaceae* was attempted in previous publications, our results indicate that this species is distinct from other *Phyllachora* species on this host-family, and so deserves recognition as a separate species. No significant discrepancies were found between the morphology of the neotype and the description provided in Theissen & Sydow (1915).

ACKNOWLEDGEMENTS

We acknowledge financial support from the Fundação de Amparo a Pesquisa de Minas Gerais (FAPEMIG) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and also would like to thank Harrie J. M. Sipman for providing information on material now held in the herbarium Botanisches Garten und Museum Berlin-Dahlem.

REFERENCES

Al-Harthy AS (2010) *Postharvest treatments to stend storage life of feijoa (Acca sellowiana*). PhD thesis, Massey University, New Zealand.

Arx JA von, Müller E (1954) Die Gattungen der amerosporen Pyrenomuceten. *Beitrage zur Kryptogamenflora der Schweiz* **11**(1): 1–434.

Berlese AN, Voglino P (1886) *Sylloge Fungorum. Additamenta ad Volumina I-IV.* Patavia: P. A. Saccardo.

Cannon PF (1991) A revision of *Phyllachora* and some similar genera on the host family *Leguminosae*. *Mycological Paper* **163**: 1–302.

Chardón CE (1921) A contribution to our knowledge of the pyrenomycetes of Porto Rico. *Mycologia* **13**: 279–300.

Chardón CE Toro RA (1934) Mycological Explorations of Venezuela. Monographs of the University of Puerto Rico. *Physical and Biological Sciences* **2**: 1–353.

Chardón CE, Miller JH, Muller AS (1940) Ascomycetes from the State of Minas Gerais (Brazil*). Mycologia* **32**: 172–204.

Cooke MC (1891) Australian fungi. *Grevillea* **20**: 4–7.

Crous PW, Wingfield MJ, Koch SH (1990) New and interesting records of South African fungi. X. New records of *Eucalyptus* leaf fungi. *South African Journal of Botany* **56**: 583–586.

Cooke MC (1891) Australian fungi. *Grevillea* **20**: 4–7.

Doidge EM (1942) Revised descriptions of South African species of *Phyllachora* and related genera. *Bothalia* **4**: 421–463.

Farr DF, Rossman AY (2011) *Fungal Databases*. Beltsville, MD: Systematic Mycology and Microbiology Laboratory, ARS, USDA; http://nt.ars-grin.gov/fungaldatabases/

Garces Orejuela CG (1944) New or heretofore unreported species of the higher ascomycetes from Colombia and Venezuela. *Mycologia* **36**: 429–459.

Hennings P (1895) Fungi Goyazensis. *Hedwigia* **34**: 88–112.

Heywood VH, Brummitt RK, Culham A, Seberg O (2007) *Flowering Plant Families of the World.* Revd edn. Richmond Hill, ON: Firefly Books.

Jimenez B, Hanlin RT (1992) A list of species names assigned to the genus *Catacauma*. *Mycotaxon* **49**: 219–233.

Johnston PR, Cannon PF (2004) New *Phyllachora* species from *Myrsine* and *Rostkovia* from New Zealand. *New Zealand Journal of Botany* **42**: 921–933.

Katumoto K (1968) On the genus *Coccodiella* Hara. *Journal of Japanese Botany* **43**: 277–283.

Kirk PM, Cannon PF, Minter JA, Stalpers JA (2008) *Ainsworth & Bisby's Dictionary of the Fungi*. 10th edn. Wallingford: CAB International.

Maire R (1908) Champignons de São Paulo. *Annales Micologici* **6**: 144–153.

Mendes MAS, Silva, VL, Dianese, JC, Ferreira MASV, Santos CEN, Neto EG, Urben, AF, Castro C (1998) Fungos em plantas no Brasil. Embrapa/Cenargem. Brasilia.

Mendes MAS, Urben AF (2011) *Fungos relatados em plantas no Brasil.* Brasilia: Laboratório de Quarentena Vegetal, Embrapa Recursos Genéticos e Biotecnologia; http://pragawall.cenargen.embrapa.br/aiqweb/michtml/fgbanco01.asp

Parbery DG (1971) Studies on graminicolous species of *Phyllachora* Nke. in Fckl. *Australian Journal of Botany* **19**: 207–235.

Pearce CA, Hyde KD (1994) The genus *Phyllachora* from Australia: observations on taxa from *Callistemon* species. *Mycological Research* **98**: 1393–1401.

Pearce CA, Hyde KV (1995) *Phyllachora* from Australia. Observations on *P. pseudostromatica*, *P. melaleucae* and new species. *Mycological Research* **99**: 1253–1260.

Petrak F (1924) Mykologische notizen No. 301. Über die phylogeneteischen beziehungen der gattung *Phyllachora* und ihre bedeutung für das System der dothidealean Pilze. *Annales Mycologici* **22**: 1–10.

Petrak F (1948) Pilze aus Ekuador. *Sydowia* **2**: 317–386.

Petrak F, Ciferri R (1930) Fungi Dominicani. *Annales Mycoligici* **28**: 377–420.

Petrak F, Ciferri R (1932) Fungi Dominicani II. *Annales Mycologici* **30**: 149–356.

Rehm H (1897) Beiträge zur Pilzflora von Südamerika III. *Dothideaceae* Gesammelt von Herrn E. Ule in Brasilien. *Hedwgia* **36**: 366–380.

Saccardo PA (1883) *Sylloge fungorum ominium hucusque cognitorum*. Vol 2. Padova: P. A. Saccardo.

Saccardo PA (1891) *Sylloge fungorum ominium hucusque cognitorum*. Vol 9. Padova: P. A. Saccardo.

Saccardo PA (1895) *Sylloge fungorum ominium hucusque cognitorum*. Vol 11. Padova: P. A. Saccardo.

Saccardo PA (1899) *Sylloge fungorum ominium hucusque cognitorum*. Vol 14. Padova: P. A. Saccardo.

Saccardo PA (1913) *Sylloge fungorum ominium hucusque cognitorum*. Vol 22. Padova: P. A. Saccardo.

Saccardo PA (1916) *Sylloge fungorum – Ominium hucusque cognitorum*. Vol 24. Padova: P. A. Saccardo.

Sydow H (1937) Neue oder bemerkenswerte Australische Micromyceten – I. *Annales Micologici* **35**: 22–49.

Sydow H (1938) Neue oder bemerkenswerte Australische Micromyceten - III. *Annales Mycologici* **36**: 295–313.

Sydow H, Petrak F (1929) Fungi Costaricenses a cl. Prof. Alberto M. Brenes collecti. *Annales Mycologici* **27**: 1–86.

Sydow H, Sydow P (1904) Novae fungorum species. *Annales Micologici* **2**: 162–174.

Theissen F, Sydow H (1915) Die *Dothideales*. *Annales Mycologici* **13**: 149–746.

Theissen F (1918) Mycologische Mitteilungen. *Annales Mycologici* **16**: 175–188.

Viégas AP (1944) Alguns fungos do Brasil II - Ascomicetos. *Bragantia* **4**: 5–391.

doi:10.5598/imafungus.2012.03.01.03

IMA FUNGUS · VOLUME 3 · NO 1: 15–24

ARTICLE

Managing and coping with names of pleomorphic fungi in a period of transition[1, 2]

David L. Hawksworth

Departamento de Biología Vegetal II, Facultad de Farmacia, Universidad Complutense de Madrid, Plaza Ramón y Cajal, E-28040 Madrid, Spain; and Department of Life Sciences, Natural History Museum, Cromwell Road, London SW7 5BD, UK; corresponding author e-mail: d.hawksworth@nhm.ac.uk

Abstract: An explanation is provided of the recent changes in the *International Code of Nomenclature for algae, fungi and plants* relating to the ending of the separate naming of different states of fungi with a pleomorphic life-cycle. Issues relating to their implementation are discussed, including problems of defining "widely used", author citations, proofs of holomorphy, typification, the preparation of "Lists of accepted and rejected names" (with a possible timetable), relationship to the existing processes of sanctioning and conservation or rejection, and steps to be considered for the future. This material is presented here to stimulate debate on the actions that should be taken by individuals, and responsible committees, in the current period of transition to a system of fungal nomenclature fit for the 21st century.

Key words:
anamorph
Ascomycota
Basidiomycota
conidial fungi
coelomycetes
hyphomycetes
International Code of Nomenclature
nomenclature
Sneath
teleomorph

Article info: Submitted: 27 March 2012; Accepted: 5 April 2012; Published: 10 April 2012.

"The whole process is evolving, slower than some would like, and too fast for others (Scott A. Redhead, 26 January 2012)"

INTRODUCTION

On 30 July 2011, the long-established practice of allowing separate names to be used for different morphs of the same fungus, dual nomenclature, was ended. On that day, the XVIIIth International Botanical Congress, meeting in Melbourne, Australia, adopted a resolution accepting the decisions of the Nomenclature Section of the Congress that had been reached on 18–22 July 2011 (McNeill *et al.* 2011). Decisions became immediately effective from the date the resolution was adopted, unless a date on which particular provisions become effective was included in the decisions of the Nomenclature Section. These are the effective dates, and not the date of publication of the *International Code of Nomenclature for algae, fungi, and plants* (ICN); the final edited version of the new *Code* is expected in mid-2012 (McNeill *et al*. 2012a). Summaries of the changes relevant to mycologists have, however, been provided elsewhere (Hawksworth 2011, Lendemer 2011, Norvell 2011).

The issue of permitting dual nomenclature for non-lichenized ascomycete and basidiomycete fungi has been a source of continuing controversy, especially since the 1950s. As a consequence, changes in the system have been made at several of the subsequent International Botanical Congresses, the most dramatic being at the Sydney Congress in 1981. However, it was in the early 1990s, when molecular methods were just becoming available, that some mycologists realized that molecular phylogenetic methods could render the dual system redundant. A fungus could be placed in its appropriate phylogenetic position, regardless of the kind of spore-producing structure expressed – even if it were sterile with no spores of any kind being produced. The desirability, and inevitability, of reaching a position of "one name for one fungus" became increasingly recognized amongst mycologists, and the way in which that might be achieved with a minimum of pain started to be discussed. At the same time some mycologists, impatient with a lack of common assent as to what should be done, started to adopt different practices. Debates and discussions ensued during

[1]This article was first published in *Mycosphere* **3**(2): 52–64 (2012), DOI 10.5943/mycosphere/3/2/4/ and is reproduced here with the permission of the publishers and with minor changes, especially in Table 1.

[2]Dedicated to the memory of the numerical taxonomist and bacteriologist Peter H A Sneath (1923–2011), one of my mentors while a student at the University of Leicester in 1964–69, who already tried to convince me in the 1980s that the "approved lists" model was that to follow for fungal and plant names; he died on 9 September 2011, but probably unaware that the first steps along that route had just been approved.

recent International Mycological Congresses (e.g. Seifert 2003, Norvell *et al*. 2010). The matter was also considered by various committees (e.g. Redhead 2010a). Now, stimulated by a special meeting, held under the auspices of the International Commission on the Taxonomy of Fungi (ICTF) in Amsterdam in April 2011 (Hawksworth *et al*. 2011), decisive action was taken at the Melbourne Congress.

As a result of the Melbourne decision, the nomenclature of non-lichenized, pleomorphic fungi has entered a phase of transition. We are now in a period when the actual name to be used, in each case, needs to be unequivocally resolved. Furthermore, when made, the decisions on those names need to be promulgated throughout the mycological community, and indeed to all who use fungal names.

The issue has moved on from "One Name = One Fungus", to "One Fungus = Which Name?"

The number of generic and species names that might be affected is unclear. However, I suspect it may prove necessary to reassess around 2,000–3,000 names of genera, and 10,000–12,000 names of species. In many cases, and probably most, the reassessments will not necessitate changes to familiar well-established names. Recognizing the need to minimize the potential disruption that could ensue, the Congress made some special provisions to mitigate the possible effects of the changes. However, the agreed procedures will take some years to implement fully as, in some cases, deciding on which names to adopt is likely to require protracted discussions. The issue then arises as to what mycologists should do in this period of transition? The aim of this note is to: (1) explain what can be done immediately; (2) detail the changes that come into effect on 1 January 2013; (3) discuss the proposed mechanism to move towards "Lists of accepted and rejected names"; and (4) suggest some options on how to proceed.

THE NEW SITUATION

The separate nomenclatural status afforded to anamorph-typified and teleomorph-typified names ended on 30 July 2011. Regardless of the life-history state represented by their types, all legitimate fungal names are now treated equally for the purposes of establishing priority. The special rules permiting dual nomenclature no longer apply. This has two major consequences:

(1) The correct name is now the earliest published legitimate name; i.e. the principle of priority applies regardless of the sexual stage represented by the name-bearing type (but see also below).

(2) The removal of the special provision for dual nomenclature means that, where names had been introduced for different morphs of a single taxon, those names would strictly be either (a) alternative names (and so not validly published, if proposed at the same time), or (b) nomenclaturally superfluous and illegitimate (if proposed for a taxon where one morph already had a legitimate name). In view of the potential disruption this would cause, names in those two categories are ruled as validly published and legitimate – provided they were published before 1 January 2013 (Art. 59.1).

In some instances, generic names with type species typified by an anamorphic state, and names of genera, species, and infraspecific taxa with anamorphic name-bearing types, will have priority over currently used teleomorph-typified names. There will be cases where anamorph-typified names will have priority of publication, but be little used, so adopting them could be disruptive. Consequently, mycologists are instructed under Art. 57.2 not to adopt anamorph-typified names in cases where either name was "widely used for a taxon . . . . until retention of the teleomorph-typified name has been considered by the General Committee and rejected" (see below). This is necessarily a lengthy procedure and, in instances where both names are not widely used, mycologists are not constrained from immediately adopting older anamorph-typified names. Even in cases of widespread usage of dual nomenclature, where the anamorph name is much used, some mycologists are already adopting anamorph-typified names as the correct ones for taxa. While that may not be considered good practice under the *Code*, in some cases it may be pragmatic; there are no nomenclatural penalties proscribed for such actions.

The converse situation, is not mentioned as requiring consideration by the General Committee (GCN). This case is where a little used teleomorph-typified name has priority over a more widely used anamorph-typified name of later date. This should not be interpreted as a general approval of taking such actions. Indeed, the responsible approach in such cases would be to propose either the less used teleomorph name for rejection in favour of the anamorph-typified name, or the anamorph name to be included on the "Lists of accepted names" (see below). Any decision involving the General Committee is likely to take a considerable time.

For submitted cases, the key guidance is to maintain "existing usage as far as possible", pending the decision (Rec. 56A.1). However, when a recommendation for either conservation or rejection has been announced by the Committee, that should be followed – even though formal ratification would not occur until the Committee's report was accepted at the next International Botanical Congress (Arts. 14.6 and 56.4), due to be held in China in 2017.

Some publications, introducing separate new names for different states of the same fungus, may already have been in advanced stages of preparation, or in press, when the decision to end the dual nomenclatural system was taken. Art. 59.1 protects those appearing before 1 January 2013 from either being ruled as not validly published (as alternative names), or illegitimate (as superfluous names). Without that safeguard, application of the rules that apply to all other fungal names would mean that such names would not be available for use (without special proposals for their conservation; see below). After 1 January 2013, different names proposed for morphs of a single species no longer have such protection but, until that date, names introduced for different morphs will not be ruled as nomenclaturally invalid or illegitimate on that basis.

In summary: (1) Scientific names of pleomorphic ascomycetes and basidiomycetes published on or after 1 May 1753, whether anamorph-typified or teleomorph-typified, compete on an equal footing in determining the nomenclaturally correct name for a fungus; and (2) Names proposed for different states, prior to 1 January 2013,

which would otherwise be ruled as invalid or illegitimate by the application of the general provisions for fungal names, continue to be available for use.

DEFINING "WIDELY USED"

Whether cases where a single taxon has both anamorph-typified and teleomorph-typified names should be submitted for consideration through the mandated Committees, under Art. 57.2 (see above), relies on the phrase "widely used". There is currently no formal guidance on how "widely used" should be defined or interpreted, although two examples of what the Editorial Committee for the Melbourne *Code* considered to be good practice, are being incorporated into the body of the *Code* itself[3]:

Ex. 2. The teleomorph-typified generic name *Eupenicillium* F. Ludw. (1892) and five other teleomorph-typified generic names were treated as synonyms of the anamorph-typified generic name *Penicillium* Link (1809) by Houbraken & Samson (in Stud. Mycol. 70: 24. 2011), *Penicillium* being the oldest and the most widely used generic name. However, in order to remove any controversy and stabilize this nomenclature, it could be appropriate to propose the rejection of the five teleomorph-typified generic names to the General Committee.

Ex. 3. The anamorph-typified generic name *Polychaeton* (Pers.) Lév. (1846) was not taken up by Chommnunti & al. (in Fungal Div. 51: 116. 2011) in preference to the later teleomorph-typified generic name *Capnodium* Mont. (1849) as the latter is in widespread use, and the authors suggest that the teleomorphic name be considered for inclusion in the planned lists of accepted names to be approved by the General Committee under Art. 14.13.

It would be helpful if mycologists involved in making the changes were provided with further guidance on this matter. This would expedite the necessary changes being made, and would need to be borne in mind when preparing draft lists of accepted or rejected names. This is an issue which the Nomenclature Committee for Fungi (NCF) appointed by the International Botanical Congress, and the IUBS/IUMS International Commission on the Taxonomy of Fungi (ICTF), may wish to address.

In reaching a decision as to whether each of a competing pair of state names is "widely used" or not, it will be important to consider the wider community of biologists who use fungal names, and not only fungal taxonomists. In this connection, it is fortunate that web-based search engines are available. A simple *Google* search on a word, such as a generic name, will give the largest number of "hits", but these may contain duplicates. *Google Scholar* is more restrictive in being confined to scholarly publications, rather than usages in general, but both these will not weed-out non-fungal usages of the same word, or its use at a different rank. For example, a search of *Coryne* resulted in 671,000 hits in *Google* and 13,700 in *Google Scholar* due to the inclusion of coryneform bacteria and coryne-bacteria, whereas *Ascocoryne* yielded 133,000 and 1,070 respectively; *Sphaerellopsis,* without the additional search word "rust", had 70,500 hits in *Google* but only 4,800 with "rust" due to problems of an orthographically identical algal genus; and for an unqualified *Polymorphum,* there were 126,000 hits in *Google* and 3,380 in *Google Scholar,* mainly from the use of "*polymorphum*" as a species epithet in diverse organisms. These are very rough and, in some cases, potentially misleading bibliometrics, but they have merit in being broader in their coverage than databases such as *Web of Science* or *Scopus* which catch only a subset of the scientific output, and so are starting to attract more attention as tools in the biblioinformatics community (e.g. Alcaraz & Morais 2012, Krell 2012). In principle, a better guide for usage in fungal taxonomy would be the *Bibliography of Systematic Mycology,* but in that the detailed indexing of genera only started in 1986. Examples of numbers of hits obtained for 25 genera in three datasets are included in Table 1.

Whatever search is conducted, three problems appear to be impracticable to address: (1) usages of names prior to the advent of widespread computerization of bibliographic databases in the mid-1970s and 1980s will only be picked-up occasionally, but could be very numerous; (2) the commonplace situation where both state names of a pleomorphic fungus are cited in a single work (either as accepted names for the different states, or where one is mentioned as a synonym); and (3) the levels of indexing in the databases themselves, for example, if they are based on a search of the entire text, as words in an abstract, or only as keywords.

While some of the caveats discussed in the previous two paragraphs might be overcome with the help of biblioinformatics specialists, others are unlikely to be surmountable in the foreseeable future. Even if the *Biodiversity Heritage Library* and *CyberLiber* were eventually to cover all the systematic mycology publications since 1753, there would be the so-pertinent usage in applied biological journals, patents, and semi-popular magazines, to address. Nevertheless, the numbers of mentions of generic names recovered by search engines or bibliographic databases may serve as a rough-and-ready indication as to what is "widely used", but only with an awareness of the caveats noted above, and a familiarity with current practices in the group of fungi concerned.

If in doubt whether one or both names of a pleomorphic fungus fall into the "widely used" category, it would be prudent to follow the committee route (see below) before committing to a decision in print. If that is not done, an author may face the prospect of embarrassment if the decision is reversed in one of the protected lists of accepted names, not to mention being responsible for additional confusion in the literature, and for perplexing and frustrating all users of the name(s).

AUTHOR CITATION CORRECTIONS

The pre-Melbourne editions of the *Code* included a special provision that meant, if a teleomorph of an anamorph-typified taxon were discovered, and the anamorph-typified name were transferred to a teleomorph-typified generic name, the combination was to be treated as the name of a new species,

[3]This wording may still be subject to some final editorial changes before the new edition of the *Code* is released.

Table 1. Results of searches on 25 pairs of potentially competing generic names in *Google*, *Google Scholar*, and the *Bibliography of Systematic Mycology* (BSM, 1986 on) on 21 February 2012, and possible actions. Generic names in: **bold** = names suggested to be used, *italic* = names suggested for treatment as synonyms, and normal = names suggested for consideration by committees; v. = versus.

Anamorph-typified	Search results				Teleomorph-typified	Search results		
	Google	Google Scholar	BSM			Google	Google Scholar	BSM
(1) ACCEPT PRIORITY ?								
Basipetospora G.T. Cole & W.B. Kendr. 1968	4,170	184	12	**v.**	**Monascus** Tiegh. 1884	1,670,000	10,500	72
Cladosporium Link 1816	586,000	30,900	555	**v.**	*Davidiella* Crous & U. Braun 2003	31,300	258	37
Cryptococcus Vuill. 1901 nom. cons.	4,950,000	72,800	815	**v.**	*Filobasidiella* Kwon-Chung 1976	151,000	2,000	156
Chrysonilia Arx 1981	89,200	433	24	**v.**	**Neurospora** Shear & B.O. Dodge 1927	1,100,000	107,000	323
Endothiella Sacc. 1906	5,100	139	16	**v.**	**Cryphonectria** (Sacc.) Sacc. & D. Sacc. 1905	172,000	7,070	194
Dendryphiopsis S. Hughes 1953	13,200	74	21	**v.**	*Kirschsteiniothelia* D. Hawksw. 1985	482	155	45
Histoplasma Darling 1906	1,910,000	28,200	226	**v.**	*Ajellomyces* McDonough & A.L. Lewis 1968	216,000	1,010	66
Monocillium S.B. Saksena 1955	4,480	691	15	**v.**	**Niesslia** Auersw. 1869	28,700	145	41
Pseudoidium Y.S. Paul & J. N. Kapoor 1986	6,060	182	11	**v.**	**Erysiphe** R. Hedw. ex DC. 1805	1,080,000	32,600	505
Penicillium Link 1809	682,000	210,000	940	**v.**	*Eupenicillium* F. Ludw. 1892	64,500	3,160	121
Sepedonium Link 1809	40,200	1,440	55	**v.**	*Apiocrea* Syd. & P. Syd. 1921	10,700	125	9
Trichoderma Pers. 1794	1,500,000	129,000	486	**v.**	*Hypocrea* Fr. 1825	362,000	4,640	262
Uredo Pers. 1801	146,000	5,020	212	**v.**	**Puccinia** Pers. 1794	819,000	54,400	1,067
(2) ACCEPT LATER NAME ?								
Cladobotryum Nees 1816	12,100	549	63	**v.**	**Hypomyces** (Fr.) Tul. & C. Tul. 1860	189,000	2,330	142
Hansfordiellopsis Deighton 1960	8,460	13	4	**v.**	*Koordersiella* Höhn. 1909	410	8	5
Phomopsis (Sacc.) Bubák 1905 nom. cons.	585,000	16,200	376	**v.**	*Diaporthe* Nitschke 1870	269,000	7,300	256
Polychaeton (Pers.) Lév. 1846	3,300	70	14	**v.**	**Capnodium** Mont. 1849	26,300	1,340	53
Scopulariopsis Bainier 1907	215,000	6,130	127	**v.**	*Microascus* Zukal 1885	9,640	898	79
Sphaerellopsis Cooke 1883	4820 [1]	260 [1]	22	**v.**	**Eudarluca** Speg. 1908	14,300	190	21
Ugola Adans. 1763	57,000[2]	36	1	**v.**	**Asterophora** Ditmar 1809	95,200	868	72

Table 1. (Continued).

Anamorph-typified	Search results			Teleomorph-typified		Search results		
	Google	Google Scholar	BSM			Google	Google Scholar	BSM
(3) REFER TO COMMITTEE ?								
Cylindrocladium Morgan 1892	93,100	3,890	195	**v.**	Calonectria De Not. 1867	89,400	2,220	137
Hormoconis Arx & G.A. de Vries 1973	26,900	533	10	**v.**	Amorphotheca Parbery 1969	29,300	233	12
Hypocrella Sacc. 1878	31,200	842	53	**v.**	Aschersonia Mont. 1848	24,800	1,450	60
Stemphylium Wallr. 1833	89,400	9,500	176	**v.**	Pleospora Rabenh. ex Ces. & De Not. 1863	168,000	4,630	276
Polymorphum Chevall. 1822	44,700	549	3	**v.**	Ascodichaena Butin 1977	31,200	93	7

[1] Due to confusion with the algal genus *Sphaerellopsis* Koschikov 1925, searches were for *Sphaerellopsis* + rust; acceptance of *Eudarluca* would facilitate conservation of the algal generic name.
[2] Figure inflated due to use of the same term in human anatomy, even with " + fungus" in the search.

and not as a new combination, if, and only if, a valid diagnosis or description were provided. It was then to be attributed to the author making the connection. If no valid diagnosis of the teleomorph were provided, the binomial would remain as a validly published combination, typified by the anamorphic type of the basionym[4].

This situation did not arise very often but, in those cases where it did, the combinations are now again to be treated as just that, and the author citations changed accordingly. An example of this situation is included in the Melbourne *Code*:

Ex. 3. Mycosphaerella aleuritidis (Miyake) S. H. Ou (1940), when published as a new combination, was accompanied by a Latin diagnosis of the newly discovered teleomorph corresponding to the anamorph on which the basionym *Cercospora aleuritidis* Miyake (1912) was typified. Under previous editions of this *Code, M. aleuritidis* was considered to be the name of a new species with a teleomorph type, dating from 1940, and with authorship attributed solely to Ou. Under the current *Code*, the correct citation is as originally published, i.e. as *M. aleuritidis* (Miyake) S. H. Ou, typified by the type of the basionym.

In cases of this type, the correction can simply be made without any formal actions or even a publication though, when encountered, it would be helpful to inform the compilers of *Index Fungorum* that a correction should be made in the database.

PROOFS OF HOLOMORPHY

One of the key drivers for the end of the dual nomenclatural system for pleomorphic fungi was the realization that, on the basis of sequence data alone, even a fungus not forming any spores could be placed with confidence in the sexual system (Reynolds & Taylor 1992). The kind of spores produced by a fungal specimen or culture are irrelevant to its placement in the phylogenetic system for the fungi as a whole. While molecular results can be expected to be definitive in this regard, and have enabled even fungi known only in a non-sporing state to be incorporated into the sexual system, many of the connections reported in the literature have, as yet, not been examined by molecular methods.

An enormous number of connections between anamorphs and teleomorphs were made in the pre-molecular era, and these were painstakingly compiled in Kendrick (1979); this work remains a remarkable resource today. From the mid-19th century, these connections were largely based on detailed observations of the fungi in nature and, most spectacularly, by Tulasne & Tulasne (1861-65). Later, connections seen in culture, the development of sporocarps in or from one only with conidial states, were used as evidence (e.g. de Bary 1887). During the 20th century, increased rigour was used, with the emphasis on establishing connections by examination of the anamorphic fungi developed from single ascospores. Notwithstanding such careful approaches, a considerable

[4] In several editions of the *Code* prior to that adopted by the Sydney Congress in 1981, the epithet in a binomial placed in a teleomorph-typified genus was also ruled as illegitimate if the type did not represent the teleomorphic state.

number of the reported connections in the literature remain based only on co-occurrences in nature.

When uniting names, typified by different states under the new rules to provide the correct name for a species, particular care should be taken to ensure that the evidence is sound. That is especially so when basing decisions on co-occurrences, particularly as fungicolous fungi have sometimes been misinterpreted as anamorphs of their hosts. The *Code* itself provides no guidance as to proofs of holomorphy, and this remains a taxonomic decision parallel to that of treating any two names as synonyms. Similarly, it is a taxonomic decision whether to describe a conidial fungus in the same genus as one in which a teleomorph is known; in that case, the judgment has to be based on the similarity of that conidial fungus to ones already established as being members of the same genus.

In discussion, I have heard it suggested that molecular evidence should be required for proof of holomorphy. I would concur that either molecular sequence data or evidence from single ascospore cultures must be the "gold standard". However, in reality this is not going to be achievable in any conceivable time-frame for the majority of fungi. While desirable, I would also question if that were necessary at all in certain cases, for instance, when there was evidence from physical connections seen in nature (e.g. in many sooty-moulds), or regular co-occurrences (e.g. *Vouauxiomyces* anamorphs of *Abrothallus* species). The burden of presenting cases "beyond reasonable doubt" will remain that of authors who have to satisfy their peer reviewers, editors, and ultimately the mycological community at large; a situation no different from that which already exists when taxonomic novelties are proposed.

There will be many instances where it is uncertain if a particular species should be transferred to a particular anamorph-typified or teleomorph-typified genus, and I would caution against wholesale uncritical transfers in such cases – especially as it is becoming clear that so many fungal genera are polyphyletic. This will also have to remain an issue for taxonomic judgement, either by individuals or committees, but it is to be expected that there will be numerous "orphaned" species names, i.e. ones under generic names now synonymized with others. While this is an undesirable situation, it is no different from numerous names already in the literature under generic names such as *Mycosphaerella*, *Phoma*, *Sphaeria,* and *Sporidesmium*.

While not ideal, it must not be forgotten that the placement of a taxon under a particular generic name is no impediment to the use of the name in identification or inclusion in artificial diagnostic keys, other identification aids, or use in publications. When using a generic name I recognize as probably being wrong for a species, but not having enough evidence to make a transfer, or introduce a new generic name, my personal practice is to place the generic name in quotation marks (e.g. "*Sporidesmium" lichenicola*). The late Martin B. Ellis drilled into me, when a neophyte mycologist in the early 1970s, that the important thing was to give the taxon a label with a good description so that it could be recognized by others and discussed.

TYPIFICATION

An epitype is essentially an interpretative type; a specimen or illustration designated to fix the precise application of a name where the name-bearing type lacks characters necessary for its identification. For example, molecularly-sequenced epitypes are increasingly being designated to fix the application of names where DNA cannot be recovered from the name-bearing types. As an interim step towards the ending of dual nomenclature, the Vienna Congress of 2005 extended the original concept further, and authorized the designation of teleomorph-types as "epitypes" for names already typified by anamorphic material (McNeill *et al.* 2006). This particular extension of the epitype concept was introduced in order to avoid having to introduce a new scientific name when the teleomorph of a species, previously known only in the anamorphic state, was discovered. The term "teleotype" was proposed for this special category of epitypes by Redhead (2010b), but the special terminology was not adopted by the Melbourne Congress in 2011. Nevertheless, with the changes effected at that Congress, there are likely to be numerous instances where it will be desirable to designate epitypes exhibiting a state not evident on the name-bearing type of a name. Epitypes designated for this purpose can represent the anamorph or the teleomorph; there is no longer any restriction of such actions to teleomorphic material.

NAMES OF FAMILIES AND ORDERS

Some mycologists have expressed concern that by allowing anamorph-typified and teleomorph-typified names to compete on an equal basis, this will lead to the loss of some very familiar and long-established suprageneric names, particularly those of families and orders. However, while family names must be based on a legitimate generic name (Art. 18.3), that generic name does not have to be that currently accepted as the correct name for a genus. For example, the treatment of *Eurotium* as a synonym of *Aspergillus* does not in itself prevent the use of *Eurotiaceae* and *Eurotiales*, nor would the adoption of *Trichoderma* as the correct name for *Hypocrea* preclude the continued use of either *Hypocreaceae* or *Hypocreales*. However, while the principle of priority does not apply to higher categories such as order, class, or subphylum, it does to that of family. Consequently, *Cladosporiaceae* (Sacc.) Nann. 1934 would have priority over *Davidiellacae* C.L. Schoch *et al*. 2007 and, in order to retain *Hypocreaceae* de Not. 1844, that name would have to be conserved (see below) against the earlier *Trichodermataceae* Fr. 1825 to remain in use.

INFORMAL DESIGNATIONS

Some mycologists have expressed concern over the loss of data that can be of practical importance, for example, in referring to a particular state that is the causal agent of a plant disease. This was already recognized by Seifert *et al.* (2000) who proposed the adoption of lower-case non-italic names, such as "acremonium-anamorph" and "trichoderma-

anamorph". I can see no objection to these or similar phrases being included in the titles of publications or associated with species names, either outside or inside brackets, where it is appropriate to refer to a particular state. However, in such expressions, it might be simpler to use "morph" rather than "anamorph" or "teleomorph" as the last two terms are not familiar to non-mycologists. In due time, I would like to see a recommendation to encourage this practice included in a future edition of the *Code,* even though such a proposal made to the Vienna Congress in 2005 (Hawksworth 2004) was not accepted.

LISTS OF ACCEPTED AND REJECTED NAMES

The *Code* has various appendices dealing with lists of conserved and rejected names and suppressed publications, and also accords special protection to names adopted in certain mycological works that are deemed to be "sanctioned" (see below). Prior to the Melbourne Congress, there was no mechanism whereby additional lists of names might be adopted for protection or rejection *en bloc*. This changed for all non-lichenized fungi on 30 July 2011 when procedures for the adoption of lists of accepted (Art. 14.13) or rejected names (Art. 56.3) were approved. In the case of names on the new Accepted Lists, the competing synonyms over which another is preferred would remain available for use in a different taxonomy (Art. 14.6), provided that they do not compete with the accepted name. However, in the case of the Rejected Lists, the names cannot be resurrected except by conservation (Art. 56.3; see below). For this reason, I suspect that many mycologists will embrace the concept of Accepted Lists more favourably than that of the Rejected Lists.

It is important to be aware that while the motivation of the concept of these Lists was the changes in the former special rules relating to the names of pleomorphic fungi, the Lists can cover any fungal names except those of "lichen-forming fungi and those fungi traditionally associated with them taxonomically, e.g. *Mycocaliciaceae*". Reasons for this exception, which I personally find unconvincing, are addressed by Lendemer (2011).

There is no restriction on who might produce a List, its taxonomic scope, or the ranks that can be covered. Initial Lists for consideration can be prepared by individuals or small groups, as well as formally constituted committees or subcommittees of international or national mycological organizations. However, when a List has been produced, the *Code* requires it to be submitted to the General Committee on Nomenclature (GCN). The GCN will pass it to the Nomenclature Committee for Fungi (NCF), who in turn will refer it to a subcommittee, which it has established in consultation with the GCN "and appropriate international bodies". It is anticipated that the "appropriate international bodies" will include the International Commission on the Taxonomy of Fungi (ICTF) as well as similar bodies, such as the International Commission on Yeasts (ICY), and their subcommittees. Where possible, the subcommittees should include users of names other than taxonomists for reasons noted below.

Following review and refinement of a List by the subcommittee tasked with this work, it is then to be submitted to the NCF. After a period of discussion within the NCF, a vote would be taken; a 60 % majority is adopted by the NCF when considering individual name conservation and rejection proposals but, the NCF would have to consider whether it wished to follow that system for these special Lists. When approved by the NCF, the List will in turn pass to the GCN. Following approval by the GCN, the List would await formal adoption by the following International Botanical Congress.

The Melbourne *Code* does not require a period of open consultation, but it is anticipated that a procedure, parallel to that already well established for the conservation and rejection of particular names (see below), would be followed, i.e., the Lists would be published and open for comment prior to any voting by the NCF. The Lists would ideally be made available through a particular website, with a commenting facility, as that would maximize the involvement of mycologists at large. It is imperative that the process is transparent, and open to inputs from those working in applied and non-taxonomic aspects of mycology, as well as to taxonomists. This is necessary in order to avoid the mycological community as a whole feeling Lists have been imposed upon them, for if they are not seen to be to the benefit of the entire subject, there will be those who decide not to follow what they consider the dictates of some clique.

It is imperative that Lists are meticulously prepared, and the bibliographic details and type information are verified. Names on the Accepted Lists "are to be listed with their types together with those competing synonyms (including sanctioned names) against which they are to be treated as conserved" (Art. 14.13). While every effort should be made to make even the earliest drafts as accurate as possible, this is not critical. When preparing the *Lists of Names in Current Use* for genera of all groups of organisms covered by the *Code*, experience was that if "quick and dirty" drafts were first drawn up and widely circulated, numerous mycologists would critically assess and correct entries for groups in which they had a particular interest. That procedure took five years (Greuter *et al.* 1993), but does mean that a considerable amount of checking has already been done for fungal names at the rank of genus. In addition, there is a variety of other substantial data sets that also are available for use in compiling entries for Lists. These include the *Outline of Ascomycota* (Lumbsch & Huhndorf 2010), *Ainsworth & Bisby's Dictionary of the Fungi* (Kirk *et al*. 2008), the *Species Fungorum* database (www.speciesfungorum.org/Names/Names.asp), *The Genera of Hyphomycetes* (Seifert *et al.* 2011), compilations of reported anamorph-teleomorph connections in Kendrick (1979) and, most significantly, the listing of 739 non-teleomorph-typified generic names linked to teleomorph genera by Hyde *et al.* (2011).

Allowing an adequate period of consultation will be imperative, as the Lists will become a cornerstone of fungal nomenclature for the future. One possible time-line that could be achievable, at least for generic names, would be to:

(1) Release "quick and dirty" (hopefully not too dirty!) drafts for comment on the internet by the end of 2012.

(2) Invite mycologists to express interest in either serving on or helping committees or subcommittees mandated by the NCF, with preparing Lists by the end of 2012.

(3) Encourage comments and corrections on the Lists by the end of June 2013, and have the NCF mandated committees and subcommittees consider inputs received, and prepare a revision of the Lists.
(4) Issue revised versions of the Lists by the end of December 2013, after consideration by committees or subcommittees mandated by the NCF to perform that task.
(5) Debate and conduct a poll on acceptance of the Lists open to all participants during the 10th International Mycological Congress (IMC10) in August 2014.
(6) Have the NCF mandated committees and subcommittees make further revisions and corrections by December 2014, place the updated versions on the internet, and submit them to the NCF for approval.
(7) Discuss and approve the Lists within the NCF by December 2015 and submit them to the GCN.
(8) Have the GCN consider and approve the Lists by January 2016.
(9) Present the Lists for formal adoption at the International Botanical Congress in 2017.
(10) Include the Lists as Appendices in the 2018 edition of the *International Code of Nomenclature for algae, fungi, and plants.*

What is imperative is that the NCF, in consultation with the ICTF and other international bodies, determines and publicizes the schedules. Species lists for some families or genera (e.g. *Saccharomycetaceae, Trichocomaceae*), where much work has already been done, could well be integrated into this time-scale, but others would undoubtedly take much longer. Particular time-lines would need to be developed and advertised on an ordinal, familial, or generic basis for species names, depending on how mandated infrastructure is developed by the NCF. I suspect that it will be difficult to have all in a sufficiently mature state for adoption by 2017 Congress.

The Lists are not restricted to names affected by the changes in the rules relating to pleomorphic fungi. The preparation of these Lists will consequently also provide an opportunity for larger scale protection of currently accepted non-lichenized fungal names whether pleomorphism is known or not. Lists could, therefore, cover all accepted taxa within particular orders, families, or genera. This is an issue for consideration by those involved in the preparation and revisions of particular Lists, and the matter merits serious consideration at the "One Fungus = Which Name?" symposium to be held under the auspices of the ICTF in Amsterdam on 12–13 April 2012.

That the process will inevitably be lengthy will be found frustrating by some but, as the consequences will have to be embraced by future generations of mycologists, this seems unavoidable. In the case of the preparation of the *Approved Lists of Bacterial Names*, which includes around 300 generic and 1,800 specific names, the first draft was made available in 1976, the revised List was published in 1980, and this was formally accepted at the 1982 International Congress of Bacteriology (Sneath 1986). That process took six years, which is similar to the time-line suggested above. However, in mycology, there are many more names to be handled, although the precise numbers on which decisions will be necessary are unknown. Fortunately, today, we have the huge advantage of the internet and nomenclatural databases which were not available to the bacteriologists of the 1970s.

The actual format of entries in the Lists will need to follow that used in the current Appendices of the *Code* which list conserved and rejected names. In the case of species names, it will also be advantageous, wherever possible, to cite references to deposited molecular sequence data when available for the name-bearing type; in some cases, it could be helpful to designate a sequenced epitype in the List.

Once approved by the GCN and the subsequent International Botanical Congress, the extent to which a List may be added to or revised is not made explicit in the Melbourne *Code*. Indeed, it seems to be somewhat ambiguous on this point. While listed names are to be "treated as conserved" (Art. 14.13) and "entries of conserved names may not be deleted" (Art. 14.14.), the accepted names on the Lists are not in the same category as conserved names. This matter will need to be considered by the NCF, but it would clearly be advantageous to have the Lists open. This would enable them to be added to as detailed treatments of families and genera become available.

The issue of how to prepare approved lists of names, which have specially protected status, is currently a matter undergoing discussion in the zoological community, and it is anticipated that proposals from the International Commission on Zoological Nomenclature (ICZN) will be released for general discussion shortly. It will be important for mycologists to monitor those discussions as they may be helpful in suggesting how best to develop and seek approval for fungal Lists.

SANCTIONED NAMES

The inclusion of a fungal name on an Accepted List over-rides the specially protected status of the sanctioned names of ascomycetes and basidiomycetes (Art. 15). This is evident as sanctioned names are mentioned as "competing synonyms" to be included in the Lists in Art. 14.13. However, a sanctioned status should be one issue for those preparing lists to take into account when deciding which of two competing names should be commended for acceptance.

CONSERVATION AND REJECTION

The long established system for the conservation and rejection of names of families, genera, and species is independent from that of the new Lists. The system provides a mechanism for avoiding the displacement of well-established names for purely nomenclatural reasons, such as priority of publication, and also permits typification with a type other than that previously designated. Guidance on preparing proposals under these provisions is provided by McNeill *et al.* (2012b).

In the new Lists, the names are "treated as conserved" Art. 14.13) or "treated as rejected" (Art. 56.3), but are not formally conserved or rejected. This is an important distinction as conservation and rejection procedures grant a more final solution, since names once ruled upon cannot be deleted and, in the case of rejected names, are not to be used (Art. 56.1). Names listed as not to be used in favour

of conserved names, however, are still available for use in a different taxonomy provided they do not compete with a conserved name.

Conservation and rejection over-ride inclusion in the new Lists but, at the same time, some names that now compete are already conserved, for example *Cryptococcus* and *Phomopsis* (Table 1). Were such already conserved names not to be those preferred in the Accepted Lists, formal proposals for the conservation of the preferred name, over that which had been previously conserved, would have to be made.

Where the adoption of the earliest legitimate generic name or species name for a pleomorphic fungus would result in the change of long-established and widely used names, the mechanisms for the conservation and rejection of names are available for use now. Such proposals would strictly be independent from the planned Lists of accepted and rejected names (see above). However, whether the NCF, established by the Melbourne Congress, would wish to vote on them separately, and pre-empt any treatment in an adopted List, is uncertain. It would be helpful if the NCF could provide guidance on its approach to such proposals. However, for particularly controversial cases, as the Lists will take a considerable time to prepare and be approved, use of these procedures may be the most expedient course of action to remove uncertainties in a timely manner, especially for fungi of particular economic or medical importance.

NEXT STEPS

Here, to provide some background for the discussions now commencing regarding their implementation, I have endeavored to explain what is involved in the new arrangements for the naming of pleomorphic fungi adopted at the Melbourne Congress in 2011. I have also suggested a possible timetable of actions as a basis for wider discussion – and without prejudice to the result of the decisions of the NCF.

The new provisions are already in force, and mycologists preparing their work for publication will need to make decisions on what names to use while the preparation of Accepted and Rejected Lists of names progresses. This is already recognized in the *Code* through the examples given in Art. 14.13 (see above) and not only is, but was, already happening prior to the Melbourne Congress. To make a decision now over competing names is not contrary to the *Code,* provided its general provisions for all names are met – except that where an anamorph-typified name has priority by date over a widely used teleomorph-typified name. However, it would be unwise to rush into making any formal nomenclatural changes that may prove controversial until at least draft Lists have been made available. In Table 1, I have indicated some examples of different situations and actions that might be taken in those cases as a basis for discussion.

The problem over the large numbers of cases that would need to be addressed in mycology, and the appreciation that many would not be controversial, led to the inclusion in the Amsterdam Declaration on Fungal Nomenclature (Hawksworth *et al*. 2011: para 5) of the Principle of the First Reviser, a concept borrowed from the International Code of Zoological Nomenclature (ICZN 1999: Art. 24.2). This is essentially that the author(s) first making a choice between generic names should be followed, and that those choices should be registered in a nomenclatural depositary (e.g. *MycoBank*, *Index Fungorum*). It was suggested that such cases only needed referral to an internationally mandated committee if a case to overturn the choice of the first reviser was prepared. This provision was not, however, amongst the proposals presented to the Melbourne Congress, but may merit consideration as a way of expediting decisions on numerous cases. This is a topic which could merit discussion at the upcoming "One Fungus = Which Name?" symposium.

Transition can be a painful process, but this new dawn of fungal nomenclature promises to deliver a system truly fit-for-purpose for mycology in the 21st century. I trust that all mycologists will work constructively towards the realization of that goal.

CAVEATS

The interpretations and views presented here are personal, and those involved in fungal nomenclature should consult the *International Code of Nomenclature for algae, fungi and plants* (McNeill *et al.* 2012a) when it becomes available. Information on the procedures to be used for the development of Lists of accepted and rejected names, or other guidance, prepared by the Nomenclature Committee for Fungi, or the International Commission on the Taxonomy of Fungi, should also be consulted as they become available. The suggestions made as to actions that might be considered appropriate in the particular cases included in Table 1 are presented here merely as a basis for discussion, and are without prejudice to final decisions on those cases.

ACKNOWLEDGEMENTS

This contribution would not have been prepared without the persistence of Kevin D. Hyde, and was completed while I was in receipt of funding from project CGL2011-25003 of the Ministerio de Economía y Competitividad (MECC) of Spain. I am grateful to my wife, Dr Patricia E.J. Hawksworth for striving to make the text intelligible to non-nomenclaturalists.

References

Alcaraz C, Morais S (2012) Citations: results differ by databases. *Nature* **483**: 36.

de Bary A (1887) *Comparative Morphology and Biology of the Fungi, Mycetozoa and Bacteria.* Oxford: Clarendon Press.

Greuter W, Brummitt RK, Farr E, Killian N, Kirk PM, Silva PC (1993) *NCU–3: names in current use for extant plant genera.* [Regnum Vegetabile no. 129.] Königstein: Koeltz Scientific Books.

Hawksworth DL (2004) Limitation of dual nomenclature for pleomorphic fungi. *Taxon* **53**: 596–598.

Hawksworth DL (2011) A new dawn for the naming of fungi: impacts of decisions made in Melbourne in July 2011 on the future

publication and regulation of fungal names. *MycoKeys* **1**: 7–20*; IMA Fungus* **2**: 155–162.

Hawksworth DL, Crous PW, Redhead SA, Reynolds DR, Samson RA, Seifert KA, Taylor JW, Wingfield MJ [& 69 signatories] (2011) The Amsterdam Declaration on Fungal Nomenclature. *IMA Fungus* **2**: 105–112; *Mycotaxon* **116**: 91–500.

Hyde KD, McKenzie EHC, KoKo TW (2011) Towards incorporating anamorphic fungi in a natural classification – checklist and notes for 2010. *Mycosphere* **2**:1–88.

International Commission on Zoological Nomenclature (ICZN) (1999) *International Code of Zoological Nomenclature.* 4th edn. London: International Trust for Zoological Nomenclature.

Kendrick B (ed.) (1979) *The Whole Fungus: the sexual-asexual system*. 2 vols. Ottawa: National Museums of Canada.

Kirk PM, Cannon PF, Minter DW, Stalpers JA (2008) *Ainsworth & Bisby's Dictionary of the Fungi.* 10th edn. Wallingford: CAB International.

Krell F-T (2012) The journal impact factor as a performance indicator. *European Science Editing* **38**: 3–5.

Lendemer JC (2011) Changes to the International Code for Botanical Nomenclature passed in Melbourne: a lichenological perspective. *Opuscula Philolichenum* **10**: 6–13.

Lumbsch HT, Huhndorf SM (2010) Outline of *Ascomycota* – 2009. *Fieldiana, Life and Earth Sciences* **1**: 1–42.

McNeill J, Barrie FR, Burdet HM, Demoulin V, Hawksworth DL, Marhold K, Nicolson DH, Prado J Silva PC, Skog JE, Wiersema JH, Turland NJ (eds) (2006) *International Code of Botanical Nomenclature (Vienna Code) adopted by the Seventeenth International Botanical Congress Vienna, Austria, July 2005*. [Regnum Vegetabile no. 146.] Ruggell: R.G. Ganter Verlag.

McNeill J, Turland NJ, Monro A, Lepschi BJ (2011) XVIII International Botanical Congress: preliminary mail vote and report of Congress action on nomenclature proposals. *Taxon* **60**: 1507–1520.

McNeill J, Barrie FR, Buck WR, Demoulin V, Greuter W, Hawksworth DL, Herendeen PS, Knapp S, Marhold K, Prado J, Prud'homme van Reine WF, Smith GE, Wiersema JH, Turland NJ (eds) (2012a) *International Code of Nomenclature for algae, fungi, and plants (Melbourne Code) adopted by the Eighteenth International Botanical Congress Melbourne, Australia, July 2011.* [Regnum Vegetabile, in press.] Ruggell: A.R.G. Ganter Verlag.

McNeill J, Redhead SA, Wiersma JH (2012b) Guidelines for proposals to conserve or reject names. *Taxon* **61**: 248–251.

Norvell LL (2011) Fungal nomenclature. 1. Melbourne approves a new Code. *Mycotaxon* **116**: 481–490.

Norvell LL, Hawksworth DL, Petersen RH, Redhead SA (2010) IMC9 Edinburgh Nomenclature Sessions. *Mycotaxon* **113**: 503–511; *IMA Fungus* **1**: 143–147; *Taxon* **59**: 1867–1868.

Redhead SA (2010a) Report on the Special Committee on the Nomenclature of Fungi with a Pleomorphic Life Cycle. *Taxon* **59**: 1863–1866.

Redhead SA (2010b) Proposals to define the new term 'teleotype', to rename Chapter VI, and to modify Article 59 to limit dual nomenclature and to remove conflicting examples and recommendations. *Taxon* **59**: 1927–1929.

Reynolds DR, Taylor JW (1992) Article 59: reinterpretation or revision? *Taxon* **41**: 91–98.

Seifert KA (ed.) (2003) Has dual nomenclature for fungi run its course? The Article 59 debate. *Mycotaxon* **88**: 493–508.

Seifert KA, Gams W, Crous PW, Samuels GJ (eds) (2000) Molecules, morphology and classification: towards monophy-letic genera in the ascomycetes. *Studies in Mycology* **45**: 1–230.

Seifert KA, Morgan-Jones G, Gams W, Kendrick B (2011) *The Genera of Hyphomycetes*. [CBS Biodiversity Series no. 2.] Utrecht: CBS-KNAW Fungal Biodiversity Centre.

Sneath PHA (1986) Nomenclature of bacteria. In: *Biological Nomenclature Today* (eds WDL Ride, T Younés): 36–48. [IUBS Monograph no. 2.] Eynsham, Oxford: IRL Press.

Tulasne LR, Tulasne C (1861–65) *Selecta Fungorum Carpologia*. 3 vols. Paris: Imperial Press.

doi:10.5598/imafungus.2012.03.01.04

IMA FUNGUS · VOLUME 3 · NO 1: 25–38

ARTICLE

Afrocantharellus gen. stat. nov. is part of a rich diversity of African *Cantharellaceae*

Donatha D. Tibuhwa[1,2*], Sanja Savić[2], Leif Tibell[2], and Amelia K. Kivaisi[1]

[1]Department of Molecular Biology and Biotechnology, University of Dar es Salaam, P.O. Box 35179, Dar es Salaam, Tanzania (Permanent address); corresponding author e-mail: dtibuhwa@yahoo.co.uk

[2]Department of Systematic Biology, Institute for Organismal Biology, Uppsala University, Norbyvägen 18D, 75236 Uppsala, Sweden

Abstract: A new genus in the *Cantharellaceae*, *Afrocantharellus*, is recognized based on results from phylogenetic analyses of rDNA LSU and concatenated LSU/5.8-ITS2/ATP6 data. It was previously recognized as a subgenus, but comprehensive fieldwork and the acquisition of numerous sequences for previously neglected African *Cantharellus* species formed the basis for a reappraisal of generic and species delimitations. *Afrocantharellus* is characterized morphologically by the basidiomes having thick, distantly spaced diverging folds of variegated colour. In contrast to most of *Cantharellus*, *Afrocantharellus* mostly lacks clamp connections. Phylogenies of *Cantharellus* and *Afrocantharellus* based on LSU and a concatenated data set are provided, along with descriptions of and a key to the four species and one form of *Afrocantharellus* recognized. Six new combinations are made.

Key words:
Africa
ATP6
Cantharellus
ITS
LSU
Molecular phylogeny
Tanzania

Article info: Submitted: 25 February 2012; Accepted: 16 May 2012; Published: 21 June 2012.

INTRODUCTION

Cantharellaceae comprise mycorrhizal and saprobic fungi, which in most cases have a vase-shaped or funnel-shaped basidiome and a spore-bearing smooth, wrinkled, veined or folded lower side. *Cantharellus*, as presently delineated, includes about 23 species in North America, seven in South America, seven in Australia, nine in Europe, three in New Zealand, 46 in Africa, and 19 in Asia (Eyssartier 2003, Tibuhwa *et al*. 2008, Buyck & Hofstetter 2011, Buyck *et al*. 2011, Eyssartier *et al*. 2009, Shao *et al*. 2011). *Cantharellus* includes several well-known and highly esteemed edible species. In Africa, *Cantharellus* species are widely collected and sold on local markets. A revision of African *Cantharellus* from the Belgian Congo was given by Heinemann (1958), who later (Heinemann 1966) also treated species from Katanga, describing *C. platyphyllus* and *C. symoensii* as new. In a review of edible mushrooms from Burundi (Buyck 1994), a further species, *C. splendens,* was described, and others are mentioned in a list of *Cantharellus* species from the same country (Buyck & Nzigidahera 1995). Further notes on *Cantharellus* from Africa, including detailed investigations of some type specimens, were published by Eyssartier & Buyck (1998). A list of and key to *Cantharellus* species known from Tanzania was provided by Buyck *et al.* (2000). Nomenclatural notes and descriptions of new subgenera and sections in *Cantharellus* were published by Eyssartier & Buyck (2001).

Molecular studies of the 'cantharelloid clade'

The phylogeny of the 'cantharelloid clade', including *Cantharellus* and the closely related *Craterellus*, has recently been investigated using molecular data, and reviewed by Moncalvo *et al.* (2006). Incongruence was noted between relationships as reconstructed from different genes, particularly with respect to the placement of *Tulasnella*. *Cantharellus* and *Craterellus* consistently were monophyletic and sister-groups in analyses based on LSU, SSU, mtSSU, and *RPB*2 sequences. Large subunit nuclear encoded rDNA (LSU) and or ITS sequences have been used for elucidating the phylogeny of or in *Cantharellales* in several papers (Feibelman *et al*. 1994, Feibelman *et al.* 1997, Hibbett *et al*. 1997, Pine *et al*. 1999, Li *et al*. 1999, Dahlman *et al*. 2000, Hibbett *et al*. 2000, Binder & Hibbett 2002, Moncalvo *et al*. 2006, Olariaga *et al*. 2009). In *Cantharellaceae*, according to Feibelman *et al*. (1994), the ITS region is unusually long and highly variable in length, especially in the chanterelles (see also Dunham *et al*. 2003). Additionally, significant length variability in ITS and morphology of North America *Cantharellus cibarius*-like chanterelles has been demonstrated, suggesting a species complex masked by a common morphology (Feibelman *et al*. 1994, Dunham *et al*. 2003, Pilz *et al.* 2003). Moncalvo *et al*. (2006) recommended the use of protein-coding genes such as *RPB*2 for the reconstruction of evolutionary relationships in the cantharelloid clade. This, however, primarily had a background in incongruent placement of *Tulasnella* with different datasets, whereas LSU still seems to efficiently resolve relationships, also in *Botryobasidium* and *Tulasnella*. Problems in using LSU datasets include long-branch attraction in some types of analyses, particularly in distance and parsimony-based analyses (Moncalvo *et al.*

2006). Alignment problems are also sometimes encountered. These, however, are much more pronounced at the order or family level, but are manageable and cause much less data loss within the genera (Moncalvo *et al*. 2006).

Although LSU- and mtSSU-based analyses previously have been shown to efficiently resolve phylogenetic relationships in *Cantharellaceae* (Moncalvo *et al.* 2006), here data from additional regions was utilized. ATP6 (which codes for ATP-ase subunit 6) has so far not been used for phylogenetic inference in *Cantharellaceae*, but Kretzer & Bruns (1999) successfully resolved phylogenetic relationships in *Boletales* using this protein-coding gene. Recently, a maximum likelihood analysis was employed on a dataset for the protein coding gene tef-1*,* leading to the recognition of a new North American *Cantharellus* species (Buyck *et al*. 2011) and including discussions of species delimitation in the *Cantharellus cibarius* complex in the southeastern USA (Buyck & Hofstetter 2011). Buyck & Hofstetter (2008) presented preliminary results of a four gene phylogeny for *Cantharellus*, employing mtSSU, LSU, and two protein-coding loci, tef-1 and *RPB*2, where *ca.* 45 species from four continents were sampled suggesting the recognition of at least six different clades. However, in conclusion those authors stated that more studies on a larger data set were needed for the recognition of further taxa. Although several molecular studies have investigated relationships of the 'cantharelloid clade' (Hibbett *et al.* 1997, 2000, Pine *et al.* 1999, Hibbett & Donoghue 2001, Binder & Hibbett 2002, Larsson *et al.* 2004, Binder *et al*. 2005, Mathney 2005, Moncalvo *et al.* 2006) and *Cantharellus* (Feibelman *et al.* 1997, Dahlman *et al.* 2000, Dunham *et al.* 2003, Thacker & Henkel 2004, Henkel *et al.* 2005), to our knowledge just a few sequences from African species have been published. Considering the high diversity of the genus in Africa, this might well have hampered our understanding of the phylogeny of *Cantharellus* and the 'cantharelloid clade' as a whole.

Thus, the main criticism that can be levelled against the molecular analyses so far published of phylogenetic relationships of *Cantharellus s. lat.* is that the taxon sampling has been quite limited. The species sampled have been almost exclusively from the Northern Hemisphere, despite the rich diversity of *Cantharellus* in other parts of the world. The diversity of *Cantharellus* in Africa is particularly exceptional, and the inclusion of data on African *Cantharellus* may thus be expected to contribute substantially to alleviate the lack in comprehensiveness and phylogenetic relationships in current analyses.

Current species recognition in *Cantharellus*

In *Cantharellus*, as currently circumscribed, the distinction between the species still often remains extremely subtle given the few and variable morphological characters available for species recognition (Buyck & Hofstetter 2011). For example the name *C. cibarius* (or '*C*. cf. *cibarius'*) often refers to any yellowish chanterelle, and *C. cibarius* is no doubt the most commonly misapplied name for a chanterelle. When the status of nominal species and morphological variability within the species was not clear, sometimes these 'ambiguous species' were included in species groups or species complexes. *Cantharellus cibarius*, considered to contain 'several cryptic geographic species' by Moncalvo *et al*. (2006), is the type of *Cantharellus* and this complicates the circumscription of *Cantharellus s. str.* Additionally, Buyck & Hofstetter (2011) stated that many morphologically similar species and infraspecific taxa had been included under *C. cibarius*.

However, with the use of molecular information, there is evidence that a substantial number of unrecognized fungal species are hidden under traditional phenotype-based species names (e.g. Carriconde *et al*. 2008). However, the outcome of recent studies of basidiomycetes based on molecular data varies. In some cases the recognition of morphologically circumscribed species and infrageneric taxa, as monophyletic groups, is not supported (e.g. Geml *et al*. 2006, Frøslev *et al*. 2007, Nagy *et al*. 2012). Thus, species recognition based on molecular data should be adopted when a morphological species concept is inapplicable in the sense that it is not consistent with the genetic information. Not wanting to argue a general, criterion-based 'species concept' (see also Hey 2006), we have for this study searched for congruence between molecular phylogenies and morphological features evaluated *a posteriori* in recognizing taxa.

The aim of this study is to contribute to a better understanding and reassessment of the phylogeny of *Cantharellus* based on the inclusion of molecular data derived from the rich diversity of African *Cantharellus* species based on partial LSU, 5.8-ITS2, and ATP6 sequences.

MATERIALS AND METHODS

Taxon and sequence sampling

All *Cantharellus* samples were collected by the first author both in the northern and southern parts of Tanzanian miombo woodlands (Fig. 1) in April–June and September–December during four consecutive years (2004–2007). Specimens were preserved either by immediate freezing in saturated brine solution, in CTAB until investigated, or dried overnight at 60 °C for herbarium deposition and further analysis. Microscopic characters were examined as in Tibuhwa *et al*. (2008). This involved recording 40 measurements of each feature from both fresh specimen preserved in CTAB, and dry specimens observed in 10 % ammonium solution in an aqueous solution of Congo red. The estimated size of the measured feature was obtained statistically and presented as: (min) min-SD – AV – max-SD (max) Q, in which min = lowest value recorded for the measured feature, max = highest value, AV = arithmetic mean and SD standard deviation; Q the ratio length/width (Eyssartier *et al.* 2001, Tibuhwa *et al*. 2008). Spore shapes were described according to Bas (1969).

For molecular characterization 5.8S–ITS2 and ATP6 were sequenced for 21 and 20 specimens of *Cantharellus* respectively*,* and LSU for 36 specimens, including three *Craterellus* species. In total, 77 new sequences were produced. GenBank numbers and voucher specimen information for sequences we generated are listed in Table 1, together with sequences obtained from GenBank. To estimate the phylogenetic position of African *Cantharellus* species as represented by the Tanzanian material, we worked with two

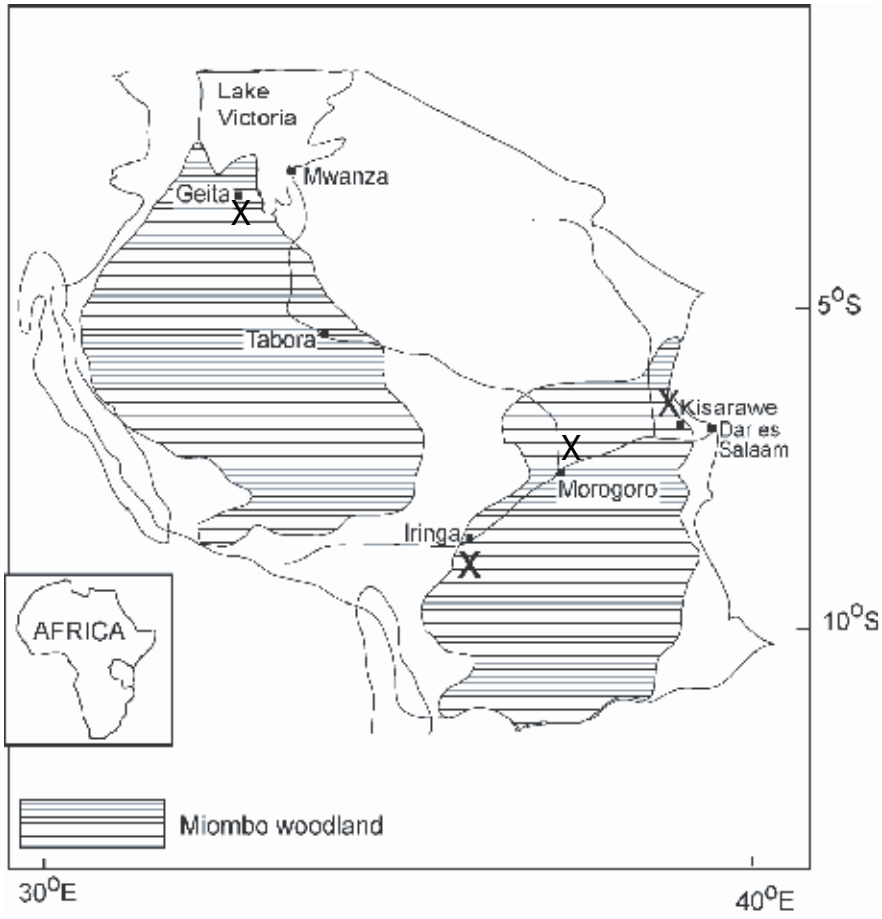


Fig. 1. Map showing the distribution of Miombo-woodlands in Tanzania. Approximate positions of collecting sites are marked with 'X'.

datasets: (1) a large LSU dataset; and (2) a more restricted dataset of concatenated LSU/5.8-ITS2/ATP6.

The first dataset: The larger dataset LSU comprised 92 taxa of *Cantharellus* and related genera selected for this study. Sequences from GenBank were selected so that if possible at least two sequences representing each species were included. In the selection of representatives of the 'cantharelloid clade' and choice of outgroup we were guided by the results presented by Moncalvo *et al.* (2006). In the large LSU sampling, representatives of *Craterellus*, *Hydnum*, and *Multiclavula* were included representing more remote relatives of *Cantharellus*. *Multiclavula mucida* was used as outgroup.

The second dataset: A concatenated data set included LSU/5.8-ITS2/ATP6, forming 28 sets of sequences representing 17 species. We tried to include the same representatives for all three regions; however, the concatenated matrix was not entirely complete, missing three sequences for 5.8-ITS2 and four for ATP6. The ATP6 sampling was limiting this selection. In the ATP6 partition, however, no *Craterellus* sequence was available, and of Northern Hemisphere *Cantharellus* species only two, *viz*. *C. cibarius*, and *C. cinnabarinus* were included. Considering that *C. cibarius* is a frequently misapplied name, it is problematic to combine different sequences available from GenBank under this name. Thus we decided not to include it in our second data set. Moreover, we failed to obtain additional ATP6 sequences from twelve Northern Hemisphere *Cantharellus* species and two *Craterellus* species because of amplification problems and the potential occurrence of paralogs. Interestingly, the same issue did not arise during the amplification of ATP6 from African species. In addition, we used an amalgamated set for *Clavulina* sequences, combining from GenBank for LSU and 5.8-ITS2 from *Cl. cinerea* with *Clavulina* sp. for ATP6; *Dacrymyces chrysospermus* served as outgroup.

The alignments, together with the trees from the Bayesian analyses (Figs 2–3), have been deposited in TreeBASE (http://purl.org/phylo/treebase/phylows/study/TB2:S12709).

Molecular study

DNA extraction, amplification, and sequencing

Total DNA was extracted from the inner part of the basidiomes, preferentially from the hymenium to avoid contamination, following the protocol of the Plant Genomic DNA extraction Kit (VIOGEN). Diluted (10^{-1} – 10^{-3}) or undiluted DNA was used for PCR amplifications. The 5' end of the LSU, and 5.8-ITS2 and ATP6 were amplified. Primers used were: (a) for the 5' part of LSU: LR3 and LR5 (Vilgalys & Hester 1990), and forward primer LROR (http://www.biology.duke.edu/fungi/mycolab/primers.htm#Large subunit RNA (25-28S) primer sequences) or LCa1 (primer designed for this study: 5'–GTCCGAGTTGTAGATGAG–3'); (b) for amplification of 5.8S-ITS2 part of ITS region see Table 2; (c) for the ATP6: ATP6-2 and ATP6-3 (Kretzer & Bruns 1999).

For PCR amplification of all three regions (LSU, 5.8-ITS2, and ATP6) we used the AccuPower® PCR PreMix (Bioneer, Daejeon, Korea), adding 3 µL diluted or undiluted DNA, 1.5 µL of each primer (10 µM), and water to a total volume

Table 1. Specimens and sequences used in this study, with their respective voucher information. GenBank accession numbers in bold represent sequences published here for the first time; corresponding voucher and collector numbers are provided. Other GenBank ID numbers represent sequences already published.

No	Species	Voucher	Locality	Collection no. (UPS)	LSU-GB	5.8-ITS2 GB	ATP6-GB
1	*Afrocantharellus fistulosus*	DDT31	TANZANIA: Kisarawe	Tibuhwa 31.2006	**JQ976959**	—	—
2	*A. fistulosus*	DDT43	TANZANIA: Kisarawe	Tibuhwa 43.2007	**JQ976965**	—	—
3	*A. platyphyllus* f. *cyanescens*	DDT63	TANZANIA: Morogoro	Tibuhwa 1063.2007	**JQ976970**	—	—
4	*A. platyphyllus* f. *platyphyllus*	DDT78	TANZANIA: Iringa	Tibuhwa 1078.2007	**JQ976978**	**JQ976947**	**JQ976926**
5	*A. platyphyllus* f. *platyphyllus*	DDT03	TANZANIA: Morogoro	Tibuhwa 1003.2004	**JQ976950**	**JQ976929**	—
6	*A. platyphyllus* f. *platyphyllus*	DDT41	TANZANIA: Kisarawe	Tibuhwa 1041.2006	**JQ976964**	—	—
7	*A. splendens*	DDT57	TANZANIA: Morogoro	Tibuhwa 1057.2007	**JQ976967**	**JQ976937**	**JQ976916**
8	*A. splendens*	DDT17	TANZANIA: Geita	Tibuhwa 1017.2005	**JQ976956**	**JQ976932**	**JQ976911**
9	*A. symoensii*	DDT36	TANZANIA: Kisarawe	Tibuhwa 1036.2005	**JQ976961**	**JQ976934**	**JQ976914**
10	*A. symoensii*	DDT04	TANZANIA: Morogoro	Tibuhwa 1004.2005	**JQ976951**	—	—
11	*A. symoensii*	DDT66	TANZANIA: Iringa	Tibuhwa 1066.2007	**JQ976971**	**JQ976940**	**JQ976919**
12	*A. symoensii*	DDT11	TANZANIA: Morogoro	Tibuhwa 1011.2005	**JQ976953**	—	—
13	*A. symoensii*	DDT67	TANZANIA: Iringa	Tibuhwa 1067.2007	**JQ976972**	**JQ976941**	**JQ976920**
14	*A. symoensii*	DDT14	TANZANIA: Geita	Tibuhwa 1014.2004	**JQ976955**	—	—
15	*Botryobasidium isabellinum*				AF393047	—	DQ534597.1
16	*C. appalachiensis*				DQ898690	—	—
17	*C. appalachiensis*				HM750916	—	—
18	*C. cascadensis*				AY041159	—	—
19	*C. cascadensis*				AY041158	—	—
20	*C. cascadensis*				AY041161	—	—
21	*C. cascadensis*				AY041160	—	—
22	*C. cibarius* var. *cibarius*				AY041156	—	—
23	*C. cibarius* var. *cibarius*				AY041155	—	—
24	*C. cibarius* var. *cibarius*				AY041157	—	—
25	*C. cibarius* var. *roseocanus*				AY041152	—	—
26	*C. cibarius* var. *roseocanus*				AY041153	—	—
27	*C. cibarius* var. *roseocanus*				AY041154	—	—
28	*C. cibarius* var. *roseocanus*				AY041151	—	—
29	*C. cibarius* var. *multiramis*				HM750920	—	—
30	*C. cibarius*	SS574	SWEDEN: Uppland	Olariaga & Felipe 2005/503752	**JQ976981**	—	—
31	*C. cibarius*				EU522825	—	—
32	*C. cibarius*				AJ406428	—	—
33	*C. cibarius*				HM750927	—	—
34	*C. cibarius*				AY745708		
35	*C. cibarius*				DQ898693	—	—
36	*C. cibarius* var. *longipes*				HM750924	—	—
37	*C. cinnabarinus*				AY041168	—	—
38	*C. cinnabarinus*				DQ898692	—	—
					—	—	DQ120944
					—	DQ898649	—
39	*C. congolensis*	DDT77	TANZANIA: Morogoro	Tibuhwa 1077.2007	**JQ976977**	**JQ976946**	**JQ976925**
40	*C. congolensis*	DDT76	TANZANIA: Iringa	Tibuhwa 1076.2007	**JQ976976**	**JQ976945**	**JQ976924**

Table 1. (Continued).

No	Species	Voucher	Locality	Collection no. (UPS)	LSU-GB	5.8-ITS2 GB	ATP6-GB
41	*C. densifolius*	DDT40	TANZANIA: Kisarawe	Tibuhwa 1040.2006	**JQ976963**	**JQ976935**	**JQ976915**
42	*C. densifolius*	DDT58	TANZANIA: Morogoro	Tibuhwa 1058.2006	**JQ976968**	**JQ976938**	**JQ976917**
43	*C. floridulus*	DDT33	TANZANIA: Morogoro	Tibuhwa 1033.2006	**JQ976960**	—	**JQ976913**
44	*C. floridulus*	DDT38	TANZANIA: Morogoro	Tibuhwa 1038.2005	**JQ976962**	—	—
45	*C. formosus*				AY041166	—	—
46	*C. formosus*				AY041164	—	—
47	*C. formosus*				AY041165	—	—
48	*C. garnierii*				AY392767	—	—
49	*C. garnierii*				AY392768	—	—
50	*C. isabellinus*				HM750931	—	—
51	*C. isabellinus*	DDT30	TANZANIA: Morogoro	Tibuhwa 1030.2006	**JQ976958**	—	—
52	*C. isabellinus* var. *parvisporus*	DDT12	TANZANIA: Morogoro	Tibuhwa 1012.2004	**JQ976954**	**JQ976931**	**JQ976910**
53	*C. isabellinus* var. *parvisporus*	DDT22	TANZANIA: Geita	Tibuhwa 1022.2005	**JQ976957**	**JQ976933**	**JQ976912**
54	*C. lateritius*				DQ898694	—	—
55	*C. minor*				DQ898691		
56	*C. minor*				HM750923	—	—
57	*C. pallens*	SS577	SWEDEN: Uppland	Danell & Olariaga 2005 (503727)	**JQ976984**	—	—
58	*C. persicinus*				AY041169	—	—
59	*C. pseudocibarius*	DDT02	TANZANIA: Morogoro	Tibuhwa 1002.2004	**JQ976949**	**JQ976928**	**JQ976908**
60	*C. pseudocibarius*	DDT05	TANZANIA: Geita	Tibuhwa 1005.2004	**JQ976952**	**JQ976929**	**JQ976909**
61	*C. pseudoformosus*				GU237071	—	—
62	*C. rhodophyllus*				HM750925	—	—
63	*C. ruber*	DDT60	TANZANIA: Iringa	Tibuhwa 1060.2007	**JQ976969**	**JQ976939**	**JQ976918**
64	*C. ruber*	DDT45	TANZANIA: Kisarawe	Tibuhwa 1045.2007	**JQ976966**	**JQ976936**	—
65	*C. subalbidus*				AY041148	—	—
66	*C. subalbidus*				AY041150	—	—
67	*C. subalbidus*				AY041146	—	—
68	*C. subalbidus*				AY041147	—	—
69	*C. subalbidus*				AY041149	—	—
70	*C. tomentosus*	DDT68	TANZANIA: Morogoro	Tibuhwa 1068.2007	**JQ976973**	**JQ976942**	**JQ976921**
71	*C. tomentosus*	DDT69	TANZANIA: Morogoro	Tibuhwa 1069.2007	**JQ976974**	**JQ976943**	**JQ976922**
72	*Cantharellus* sp.				HM750917	—	—
73	*Cantharellus* sp.				HM750922	—	—
74	*Cantharellus* sp.				HM750928	—	—
75	*Cantharellus* sp.				HM750930	—	—
76	*Cantharellus* sp.				HM750926	—	—
77	*Cantharellus* sp.				HM750918	—	—
78	*Cantharellus* sp.				HM750921	—	—
79	*Cantharellus* sp.				AJ271192	—	—
80	*Cantharellus* sp.				AY041167	—	—
81	*Cantharellus* sp.				HM750929	—	—
82	*Cantharellus* sp. 2	DDT70	TANZANIA: Morogoro	Tibuhwa 1070.2007	**JQ976975**	**JQ976944**	**JQ976923**
83	*Cantharellus* sp. 2	DDT79	TANZANIA: Morogoro	Tibuhwa 1079.2007	**JQ976979**	**JQ976948**	**JQ976927**
84	*Clavulina cinerea*				AM259211	AF185974	—
	Clavulina sp.						DQ120947
85	*Craterellus chantarellus.* var. *intermedius*				HM750919	—	—

Table 1. (Continued).

No	Species	Voucher	Locality	Collection no. (UPS)	LSU-GB	5.8-ITS2 GB	ATP6-GB
86	*Craterellus cornucopioides*				AY700188	—	—
						JF907967	
87	*C. cornucopioides*				AJ279572	—	—
88	*C. lutescens*	SS575	SWEDEN: Uppland	Olariaga 2005 (503703)	**JQ976982**	—	—
89	*C. lutescens*				EU522746	—	—
90	*C. melanoxeros*	SS576	SWEDEN: Uppland	Aronsson 2008 (441865)	**JQ976983**	—	—
91	*C.* sp.				HM113529	—	—
92	*C. tubaeformis*				AF287851	—	—
						AF385632	
93	*C. tubaeformis*	SS572	SWEDEN: Uppland	Lindau 2010	**JQ976980**	—	—
94	*C. tubaeformis*				DQ898741	—	—
95	*Dacrymyces chrysospermus*				AF287855	—	EU339249
96	*Hydnum rufescens*				AY293187	—	—
97	*Multiclavula mucida*				AF287875	—	—

of 20 µL. For LSU, and 5.8-ITS2 the PCR thermal cycling parameters were as described in Savić & Tibell (2009) for LSU. Amplification and thermal cycling parameters for PCR of the ATP6 followed, with the modifications, the protocol of Kretzer & Bruns (1999): five cycles of 35 s at 94 °C, 55 s at 37 °C, 1 min at 72 °C, followed by 30 cycles of 35 s at 94°C, 55 s at 45 °C, and 1 min at 72 °C, and final elongation for 10 min at 72 °C. Amplification products were visualized on 0.5 % agarose gels stained with ethidium bromide and the PCR product was purified using Millipore plates (MultiScreen™ PCR, Danvers, MA). Sequencing, automated reaction clean up, and visualization were carried out as described by Macrogen (www.macrogen.com).

Alignments and phylogenetic analyses

To evaluate the phylogenetic relationship in a sample of African taxa, all four data sets (larger dataset of LSU, smaller dataset of LSU, 5.8S-ITS2, and ATP6) were aligned separately using MAFFT (Katoh *et al*. 2002, 2005) on the online server (v. 6), which was used to create alignments that utilized the L-INS-i (for LSU and ATP6) and E-INS-i (5.8-ITS2) MAFFT algorithm. All four alignments were generated using the default settings (gap opening penalty = 1.53 and offset value = 0.00).

The first LSU dataset was submitted to the Cyberinfrastructure for Phylogenetic Research (CIPRES Science Gateway: http://www.phylo.org/) for preliminary analysis with RAxML v. 7.2.8 (Stamatakis 2006, Stamatakis *et al*. 2008). Before the final alignment, regions where positional homology was doubtful were excluded from the final alignment.

Using the AIC implemented in JModeltest v. 0.1.1 (Guindon & Gascuel 2003, Posada 2008), the Bayesian analysis employed the GTR+G model for the first dataset (larger LSU matrix), 5.8-ITS2 and ATP6; GTR+G+I was employed for smaller LSU partition (however its likelihood score was also very close to that of the GTR+G model). Before concatenation of the sequences for the second dataset (LSU/5.8-ITS2/ATP6), single-gene analyses were performed to detect significant conflicts among datasets and partitions. A conflict was considered significant if a well-supported monophyletic group, for example MLb ≥ 70 % (Mason-Gamer & Kellogg 1996), was found not to be well supported as non-monophyletic when different loci were used. Each single-locus alignment was analyzed separately employing rapid bootstrap heuristics in RAxML v. 7.2.8 (Stamatakis *et al*. 2008) via a Web server available at the Vital-IT Unit at Swiss Institute of Bioinformatics (http://phylobench.vital-it.ch/raxml-bb/index.php), executing 100 rapid bootstrap replicates employing a GTRMIX model (switching from GAMMA to CAT for rapid bootstrapping); thereafter a thorough ML search was conducted under the GAMMA model. No significant incongruence among datasets was detected (data not shown), hence the three matrices were concatenated. After the exclusion of ambiguously aligned regions and introns,

Table 2. Primers used for amplification of the 5.8S-ITS2 part of ITS region.

Primer		Sequence
forward	ITS3C	5'–GCATCGATGAAGAACGCAGT–3'
reverse	Lcan	5'–GTCCGAGTTGTAGATGAG–3'
forward	5.8Scanf	5'– CGATGAAGAACGCAGCG–3'
forward	5canf	5'–CATCGAGTCTTTGAACGCAAAC–3'
reverse	LcanR	5'– ATCGAGTCTTTGAACGCAAAC–3'

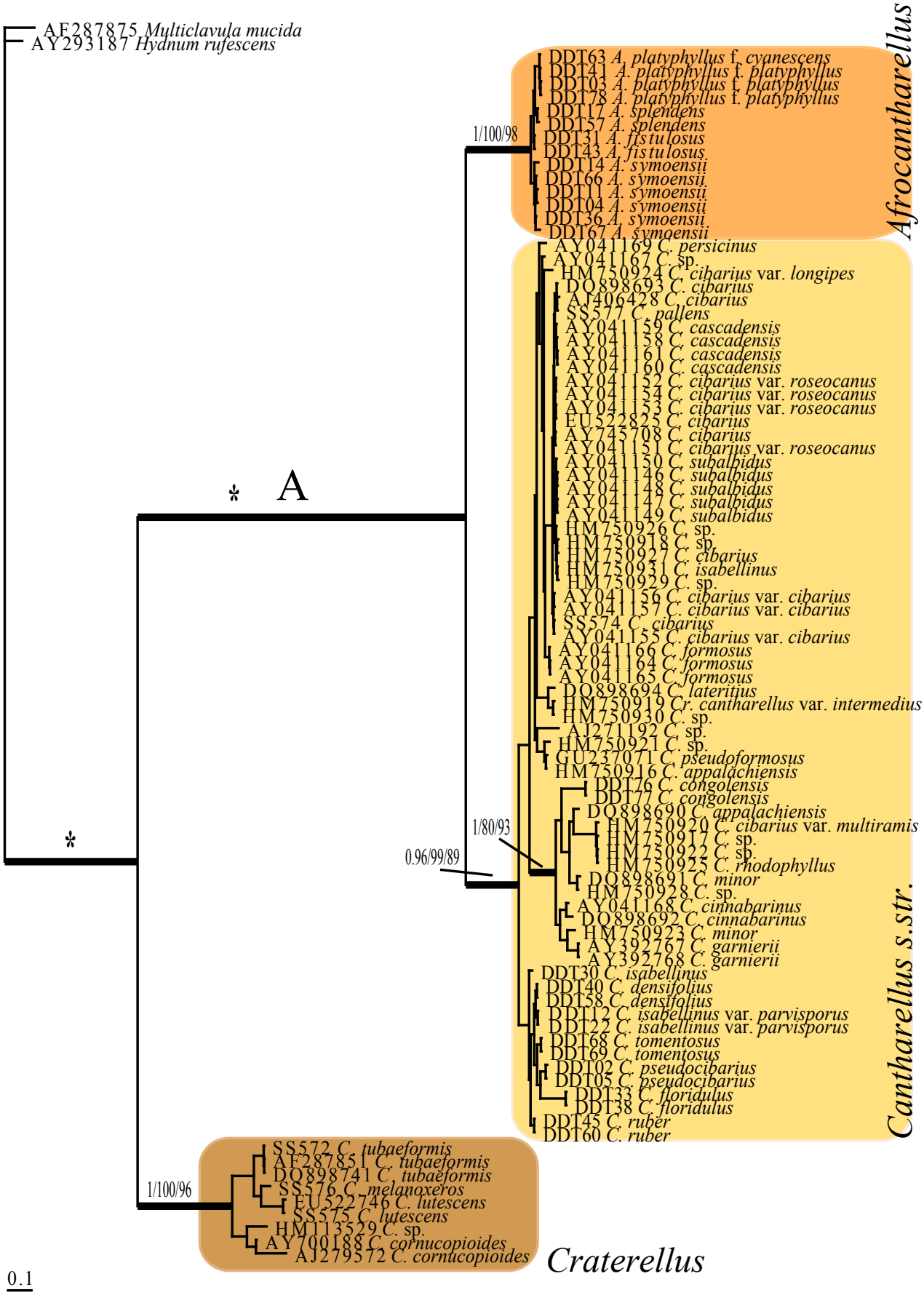


Fig. 2. Phylogenetic relationships among 92 specimens (Table 1) representing 54 taxa of cantharelloid fungi based on a Bayesian analysis of the large LSU dataset. The tree was rooted using *Multiclavula mucida*. The three support values associated with each internal branch correspond to PP, MPbs and MLb proportions, respectively. Branches in bold indicate a support of PP ≥ 95 % and MPbs, MLb ≥ 70 %. An asterisk on a bold branch indicates that this node has a support of 100 % for all support estimates.

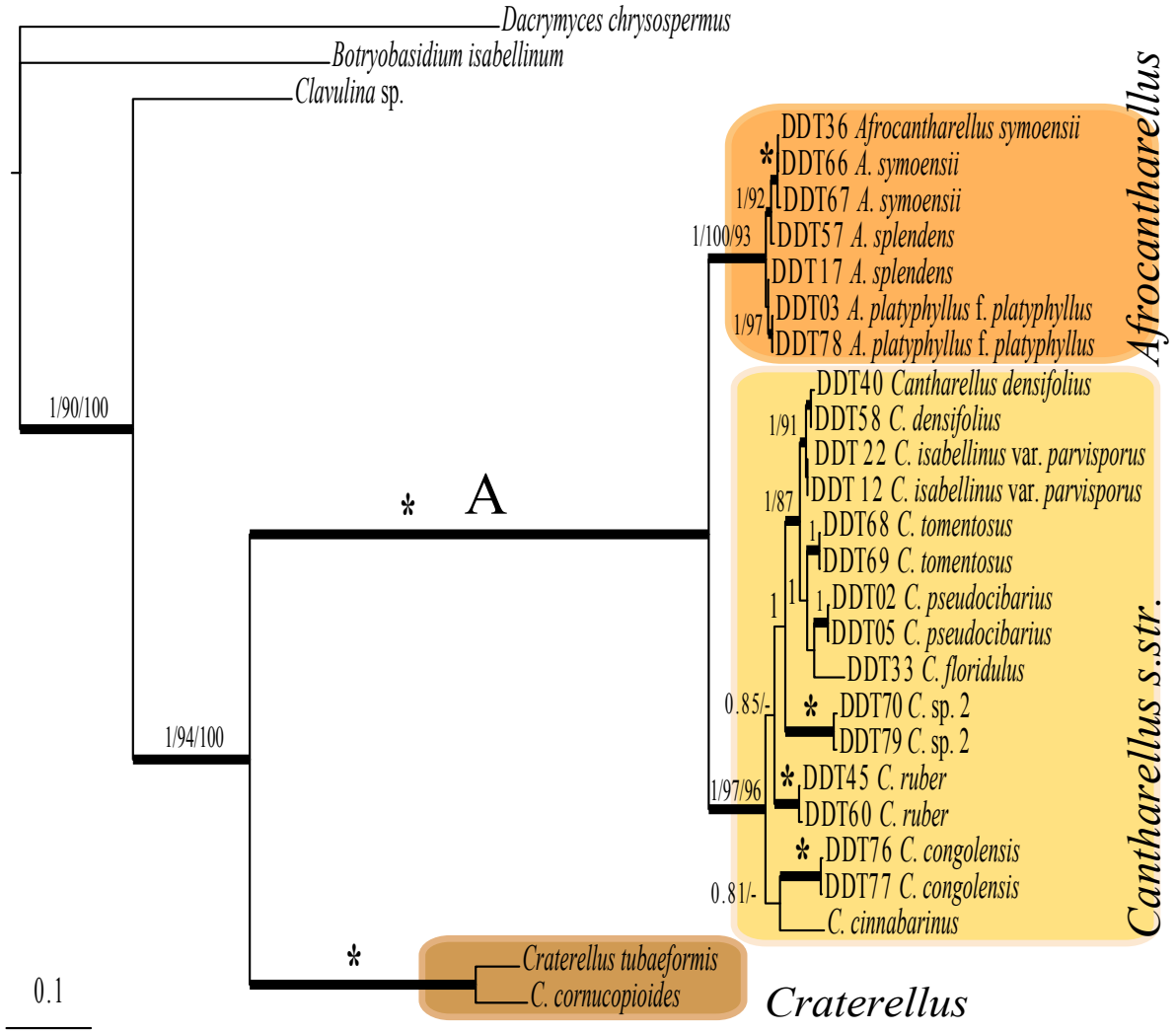


Fig. 3. Phylogenetic relationships among 28 concatenated sequences (Table 1) representing 17 taxa of cantharelloid fungi based on a Bayesian analysis of a LSU/5.8-ITS2/ATP6 dataset. The tree was rooted using *Dacrymyces chrysospermus*. The support values associated with each internal branch correspond to PP, MPbs and MLb proportions, respectively. Branches in bold indicate a support of PP ≥ 95 % and MPbs, MLb ≥ 70 %. An asterisk on a bold branch indicates that this node has a support of 100 % for all support estimates.

the concatenated data matrix contained 1906 unambiguously aligned sites.

Phylogenetic relationships were inferred separately for both data sets, the first larger LSU dataset and the second concatenated LSU/5.8-ITS2/ATP6 dataset, based on Bayesian analysis. Using MrBayes v. 3.2.1 (Ronquist & Huelsenbeck 2005) for each analysis two parallel runs were carried out for two million generations. Each run included four chains, and trees were sampled every 100 generations; we stopped the runs when the average standard deviation of split frequencies (across different runs) was ≤ 0.01. Using relative burn-in the first 25 % of sampled trees were discarded.

In order to obtain additional support values, Maximum parsimony (MP) analyses as well as MP bootstrapping (MPbs) of both data were conducted with PAUP* v. 4.0b10 for Windows (Swofford 2002). The most parsimonious trees from analyses applied a heuristic search using 1000 random addition sequences (RAS), TBR branch swapping algorithm, save multiple trees, collapse zero length branches when maximum length is zero, gaps treated as a fifth character state, characters given equal weight. A bootstrap analysis of 1000 replicates with five RAS per replicate, TBR branch swapping was then conducted. Additional support values for first and second data set were further estimated with maximum likelihood rapid bootstraping (MLb), employing rapid bootstrap heuristics in RAxML v. 7.2.8 as described above (Stamatakis *et al*. 2008).

Bayesian posterior probabilities (PP) ≥ 95 %, and MPbs and ML bootstrapping (MLb) ≥ 70 % were considered to be significant.

RESULTS

The LSU phylogeny

The LSU alignment (the first data set) contained 92 sequences with 853 total and 269 conserved sites. A Bayesian analysis yielded the phylogeny presented in Fig. 2.

Cantharellus s. lat. (clade A) is strongly supported on a long branch (PP=1.0; MPbs=100; MLb=100), and *Craterellus* is the sister-group of clade A (PP=1.0; MPbs=100; MLb=96). In clade A there are two distinct and strongly

Table 3. Morphological features of *Afrocantharellus* and *Cantharellus.*

	Afrocantharellus	*Cantharellus*
Basidiome colour	always variegated	Mostly uniformly coloured
Hymenophore	well-developed with thick diverging folds	Poorly-developed, without folds or with thin folds but never with thick diverging folds
Folds	thick, blunt, always decurrent and distantly spaced	Relatively thin, sharp, subdecurrent or decurrent and not distantly spaced
Clamp connections	Mostly absent	Mostly present

supported branches, one containing only African species (*Afrocantharellus*; PP=1.0; MPbs=100; MLb=98) and another with both Northern Hemisphere, African, and one New Caledonian species, *Cantharellus s. str.* (PP=0.96; MPbs=99; MLb=89). Species relationships within *Cantharellus s. str.* and *Afrocantharellus* were mostly resolved, although with very low support.

The combined data set phylogeny

The three-locus Bayesian phylogeny is presented in Fig. 3. *Craterellus*, despite missing ATP6 (the third data set) in the concatenated matrix, was again strongly supported (PP=1.0; MPbs=100; MLb=100) as the sister-group of clade A, *Cantharellus s. lat.* (PP=1.0; MPbs=100; MLb=100). All species in our sampling traditionally placed in *Cantharellus* (*Cantharellus s. lat.*) were recovered as two sister clades, *Cantharellus s. str.* and *Afrocantharellus*, with high support values (PP=1.00, MPbs=97; MLb=96 and PP=1.00, MPbs=100; MLb=93 respectively).

In the phylogenies based on the first and second datasets (large LSU and concatenated LSU/5.8-ITS2/ATP6) *Cantharellus s. lat.* includes two strongly supported subclades, *Cantharellus s. str.* and *Afrocantharellus* for all three support estimates (Figs 2– 3).

Afrocantharellus, the sister-clade of *Cantharellus s. str*. in both phylogenies obtained high support, and this, in conjunction with the rather distinctive morphological characteristics of having a well-differentiated hymenophore with diverging folds, the variegated colour of the basidiomes and sometime also the stipe (Table 3, Fig. 4) support the recognition of *Afrocantharellu*s at generic level. Based on molecular evidence and morphological features, we suggest emendation revised circumscription of *Cantharellus* to exclude the species closely related to *C. symoensii*, and the elevation of *Cantharellus* subgen. *Afrocantharellus* to generic level.

TAXONOMY

Afrocantharellus (Eyssart. & Buyck) Tibuhwa, **gen. stat. nov.**

MycoBank MB518687

Basionym: *Cantharellus* subgen. *Afrocantharellus* Essyart. & Buyck, *Docums Mycol*. **121**: 55 (2001).

Type: *Cantharellus symoensii* Heinem., *Bull. Jard. bot. État Brux*. **36**: 343 (1966).

Basidiomata fleshy, variegated, vividly coloured, red to orange or yellowish, rarely pale; cap 3.5–18 cm diam, hymenophore with very well-differentiated, thick, blunt, distantly spaced and diverging folds, clamp connections mostly absent.

Key to the species of *Afrocantharellus*

1 Basidiomata small to large, cap 3.5–18 cm diam, stipe not compressed laterally, stuffed or solid, clamps absent 2
Basidiomata small, cap 1.5–2.5 cm diam, stipe laterally compressed and hollow, clamps present ..... 1. **A. fistulosus**

2 (1) Basidiomata large and robust, cap 6–18 cm diam; uniformly orange-red; staining hands upon handling; folds yellowish orange; pileipellis a trichoderm ..... 4. **A. splendens**
Basidiomata medium-sized to large; cap 3.5–12 cm diam; orange-red, but irregularly speckled with other tinges, never staining the hands when handled; folds bright yellow or pale yellow; pileipellis a cutis 3

3 (2) Basidiospores ellipsoid (Q = 1.6–2.3); folds bright yellow; cap orange-red, disrupted by pinkish tinges towards the margin ..... 5. **A. symoensii**
Basidiospores subglobose (Q = 1.2–1.5); folds pale yellowish, no pinkish tinges towards the margin 4

4 (3) Stipe, cap margin, and folds with glaucous or bluish tinges ..... 3. **A. platyphyllus f. cyanescens**
Stipe, cap margin and folds without glaucous or bluish tinges ..... 2. **A. platyphyllus f. platyphyllus**

Species of *Afrocantharellus*

1. **Afrocantharellus fistulosus** (Tibuhwa & Buyck) Tibuhwa, **comb. nov.**
MycoBank MB800280
(Fig. 4B)
Basionym: *Cantharellus fistulosus* Tibuhwa & Buyck, *Cryptogamie, Mycol.* **29**: 133 (2008).

Type: **Tanzania**: *Coast region*, Kazimzumbwi forest reserve, Kisarawe, 06°04'32'' S, 039°15'56'' E, miombo dominated by *Brachystegia*, *Combretum* and *Julbernardia*, April 2007, *Tibuhwa D 43.2007* (UPS – holotype; isotypes: PC, UDSM – isotypes).

Description: Tibuhwa *et al*. (2008).

Distribution: Known only from Tanzania.

Comments: This species is easily recognized in the field by its small size, yellow colour, cap with clearly brown matted centre, pink hymenophore composed of widely spaced folds, and by the smooth hollow stipe, which is slightly twisted or compressed.

Other material examined: **Tanzania**: *Coast region*: Kazimzumbwi forest reserve, Kisarawe, 06°04'32'' S, 039°15'56'' E, *Tibuhwa D 31.2006* (UPS, UDSM). *Iringa region*: Madibira forest,08°15'08'' S and 35°17'21'' E, alt. 1847 m, in Uapaca woodland, May 2007, *Tibuhwa D 59.2007* (UPS, UDSM).

2. **Afrocantharellus platyphyllus** (Heinem.) Tibuhwa, **comb. nov. f. platyphyllus**
MycoBank MB518693
Basionym: *Cantharellus platyphyllus* Heinem., *Bull. Jard. bot. État Brux*. **36**: 342 (1966).

Type: **Democratic Republic of Congo**: Elisabethville, 1932, *De Loose 31* (BR – holotype).

Vernacular names: Tanzania (Bena dialect): Bunyamalagata, Wifindi (Hehe dialect): Wisogolo.

Basidiomata medium-sized to large. *Cap* 3.5–9.5 cm wide, deep orange crimson towards the cap centre. *Folds* well-developed**,** yellow, thick and distantly spaced, forking or with numerous cross–veins. *Stipe* 1.5–6.5 × 1–1.5 cm, solid, slightly attenuated toward the base and pale yellow in colour. *Basidia* clavate (44.1–)55.4(–70.0) × (5.2–)7.2(–9.2) µm (Q = 6.6–9.2). *Basidiospores* subglobose, (6.3–)7.5(–8.6) × (5.0–)6.2(–7.1) µm (Q = 1.1–1.5). *Suprapellis* a cutis of 10–12 µm wide hyphae. *Clamps* none.

Distribution: Reported from Burundi (Buyck 1994), the Democratic Republic of Congo (Heineman 1966), Tanzania (Härkönen *et al.* 1995, Buyck *et al.* 2000), and Zimbabwe (Sharpe & Wursten, http://www.vumba-nature.com).

Comments: This species is quite distinct in the deep orange to crimson colour, especially towards the cap centre, which clearly contrasts with the pale bright yellow folds. In the field it resembles *A. symoensii* but lacks the pink tinge on the basidiomes of *A. symoensii*; it also differs in subglobose basidiospores, rather than the ellipsoid ones of *A. symoensii*.

*Descriptions and illustrations:*Heinemann (1966) and Härkönen *et al.* (1995, 2003).

Other material examined: **Tanzania**: *Coast region*: Kisarawe, 06°04'32'' S, 039°15'48'' E, *Tibuhwa 1041.2006* (UPS, UDSM); *Morogoro region:* SUA forest reserve, 06°52'34'' S, 37°67'29' E, *Tibuhwa 1003.2004* (UPS, PC, UDSM); *Iringa region:* Madibira forest, 08°15'08'' S, 35°17'21'' E, *Tibuhwa 1078.2007* (UPS, UDSM); Vigama village, *Buyck 98.126* (PC), *Buyck 98.127* (PC), *Buyck 98.130* (PC).

3. **Afrocantharellus platyphyllus f. cyanescens** (Buyck) Tibuhwa, **comb. nov.**
MycoBank MB518693
(Fig. 4D)
Basionym: *Cantharellus cyanescens* Buyck, *Ubwoba: Champ. Comest. l'Ouest Burundi* [Publ. Agricole no. 34]: 112 (1994).

Type: **Burundi**: Nyamirambo, 1994, *Buyck* (BR – holotype).

Vernacular names: Tanzania (Hehe dialect): Wisogolo; (Bena dialect): Wifindi, Bunyamalagata. Burundi (Kirundi dialect): Peri Itukura.

Basidiomata medium-sized to large. *Cap* 5–10 cm wide, in the field with conspicuous glaucous or bluish tinges on the orange-red cap, margin and folds especially in young stages, but later fading. *Folds* deeply decurrent, thick, blunt, diverging, distantly spaced, strongly meshed, bright yellow speckled with bluish grey tinges. *Stipe* 3–6 × 0.9–1.3 cm, smooth, solid, cylindrical, the same colour as the folds in the upper half while fading to grey-cream towards the base. *Basidia* clavate (45.0–)55.0(–75.0) × (5.0–)7.0(–7.5) µm (Q = 6.3–9.8), with 2–4 spores. *Basidiospores* (7.5–)10.0(–10.6) × (5.2–)6.1(–6.5) µm (Q = 1.3–1.5), smooth, broadly ellipsoid to subglobulose. *Suprapellis* a cutis of 8.0–15 µm wide hyphae. *Clamps* none.

Distribution: Burundi (Buyck 1994) and Tanzania (newly reported here).

Comments: This taxon is recognized in the field by its fleshy deep orange cap interrupted by blue or glaucous tinges and folds which are strongly meshed and not purely yellow but with orange–grey tinges. These unique tinges on the cap, stipe and folds distinguish it from the otherwise very similar *A. platyphyllus* f. *platyphyllus*.

Description: Buyck (1994).

Other material examined: **Tanzania**: *Morogoro region:* Ubenazomosi woodland, 06°55'11'' S, 037°35'20'' E, *Tibuhwa 1063.2007* (UPS, UDSM), *Tibuhwa 1056.2007* (UPS, UDSM); *Coast region:* Kisarawe, 06°04'32'' S, 039°15'56'' E, *Tibuhwa 1034.2006* (UPS, UDSM).

Fig. 4. Basidiomes of *Afrocantharellus* and *Cantharellus* species showing morphological differences of the hymenophores: **A.** *Afrocantharellus symoensii* (*Tibuhwa 1011.2005*; UPS). **B**. *A. fistulosus* (holotype). **C.** *A. splendens* (*DDT 1053.2011*; UDSM). **D.** *A. platyphyllus* f. *cyanescens* (*Tibuhwa 1063.2007*; UPS). **E**. *Cantharellus congolensis* (*Tibuhwa 1076.2007*; UDSM). **F.** *C. rufopunctatus* (*Tibuhwa 1010.2004*; UDSM). All photos taken in Tanzania by Donatha D. Tibuhwa.

4. **Afrocantharellus splendens** (Buyck) Tibuhwa, **comb. nov.**
MycoBank MB518692
(Fig. 4C)
Basionym: *Cantharellus splendens* Buyck, *Ubwoba: Champ. Comest. l'Ouest Burundi* [Publ. Agricole no. 34]: 112 (1994).

Type: **Burundi**: under *Brachystegia*, *Buyck 5518* (BR – holotype).

Vernacular names: Tanzania (Nyambo dialect): Binyantuku. Burundi (Kirundi dialect): Peri magufa.

Basidiomata large. *Cap* 8–18 cm wide, bright orange-red. *Folds* thick, blunt diverging, distantly spaced, pale yellow with orange tinges. *Stipe* 2.5–7 × 1.2–3.5 cm, smooth, solid, subcylindrical, slightly attenuated toward the base, of the same colour as the cap but paling to white toward the base. *Basidia* narrowly cylindrical–clavate, (40.0–)49.7(–57.4) × (5.4–)6.6(–7.7) µm (Q = 6.7–9.1). *Basidiospores* ellipsoid (8.1–)9.9(–12.0) × (3.7–)4.2(–4.7) µm (Q = 2.0–2.7). *Suprapellis* a trichoderm of more or less ramified, hyphae 5.5–8.0 µm wide. *Clamps* none.

Distribution: Burundi (Buyck 1994), and Tanzania (Buyck *et al.* 2000).

Comments: This species is easily recognized in the field by the large, fleshy and bright orange-red basidiomes, which recall those of *A. symoensii* and *A. platyphyllus*. The pigmentation of the cap stains the hands upon handling, and microscopically a trichoderm pileipellis distinguishes it from these other two species.

Description: Buyck (1994).

Other material examined: **Tanzania**: *Morogoro region:* Ubenazomosi woodland, 06°55'11'' S, 037°34'20'' E, *Tibuhwa 1057.2007* (UPS, UDSM); *Mwanza region:* Geita-Rwamgasa forest reserve, 03°09'50'' S, 32°04'52'' E, *Tibuhwa 1017.2005* (UPS, UDSM).

5. **Afrocantharellus symoensii** (Heinem.) Tibuhwa, **comb. nov.**
MycoBank MB518691
(Fig. 4A)
Basionym: *Cantharellus symoensii* Heinem., *Bull. Jard. bot. État. Brux.* **36**: 343 (1966).

Type: **Democratic Republic of Congo**: Kasumbalesa, 1958, *Symoens 6037* (BR – holotype).

Vernacular names: Tanzania (Nyamwezi dialect): Mkukwe. (Bena dialect): Wifindi, (Hehe dialect): Wisogolo. Burundi (Kirundi dialect): Peri nyakeke, Peri itukura.

Basidiomata medium-sized to large. *Cap* 3.5–8 cm wide, smooth, orange-red disrupted with pale pink and yellow patches especially towards the margin. *Folds* thick, blunt, diverging, distantly spaced, yellow or slightly pale. *Stipe* 2.5–4 × 0.9–2 cm, smooth, solid or rarely somewhat lax at maturity, cylindrical but slightly wider towards the cap, of the same colour as the folds. *Basidia* clavate (38.2–)48.7(–59.3) × (5.0–)6.5(–8) µm (Q = 6.3–10.0). *Basidiospores* (7.4–)9.0(–10.6) × (4.5–)4.9(–5.2) µm (Q = 1.6–2.3), ellipsoid. *Suprapellis* a cutis of 7.5–10 µm wide hyphae. *Clamps* none.

Distribution: Reported from Burundi (Buyck 1994), the Democratic Republic of Congo (Heineman 1966), Tanzania (Buyck *et al.* 2000, Härkönen *et al.* 1995), and Zambia (Eyssartier & Buyck 1998).

Comments: This is one of the most common *Afrocantharellus* species in tropical Africa. It is easily recognized in the field by the fleshy orange-red cap with yellow and pink patches towards the margin, and the bright yellow, distantly spaced, thick folds. It has often been confounded with *C. longisporus*, but differs in the differently shaped spores, and in lacking clamp connections (Eyssartier & Buyck 1998, Buyck *et al.* 2000).

Descriptions and illustrations: Eyssartier & Buyck (1998) give a detailed description of the holotype, and more descriptions and/or illustration are found in Buyck (1994), Heinemann (1966), and Härkönen *et al.* (1995, 2003).

Other material examined: **Tanzania**: *Morogoro region*: SUA forest reserve, 06°51'22'' S, 37°39'23'' E, *Tibuhwa 1004.2005* (UPS, PC, UDSM); Ubenazomosi woodland, 06°55'11'' S, 37°34'20'' E, *Tibuhwa 1011.2005* (UPS, PC, UDSM); *Coast region:* Kazimzumbwi forest reserve, S 06°04'32'' S, 039°15'56'' E, *Tibuhwa 1007.2005* (UPS, PC, UDSM), *Tibuhwa 1036.2005* (UPS, UDSM), *Tibuhwa 1037.2006* (UPS, UDSM); *Mwanza region:* Geita-Polepole forest reserve, 02°52'29'' S, 32°07'27'' E, *Tibuhwa 1014.2004* (UPS, PC, UDSM); *Tabora region:* Masange forest reserve, 04°59'22'' S, 032°40'20'' E, *Tibuhwa 1021.2005* (UPS, UDSM); *Iringa region:* Madibira forest, *Tibuhwa 1067.2007* (UPS, UDSM), *Tibuhwa 1066.2007* (UPS, UDSM); *Dar e Salaam District*: bought in a market, *Buyck 98.113* (PC, UDSM); *Coast region:* Msanga area, near Chanika village, *Buyck 98.011* (PC, UDSM).

DISCUSSION

There are no major strongly supported species group subclades in the LSU- phylogeny of *Cantharellus s. str.*, except for a well-supported clade containing *Cantharellus congolensis* (PP=1.00; MPbs=80; MLb=93) that almost exclusively (apart from *C. congolensis* and *C. garnierii*) contains Northern Hemisphere species. *Cantharellus congolensis* (Fig. 4E) was placed in subgen. *Afrogomphus* by Eyssartier & Buyck (2001), and *C. floridulus*, which was placed in subgen. *Rubrinus* (Eyssartier & Buyck 2001), have relatively long branch-lengths, but with low support. That the name of the generic type species, *C. cibarius,* is present on several subclades in the LSU analysis of *Cantharellus s. str.* supports the opinion that this name may either embrace several cryptic species, or that many morphologically similar species and infraspecific taxa have been included under that name. Only by combining extensive molecular data with critical morphological studies will further elucidate the taxonomy and systematics of this group.

Afrocantharellus was a strongly supported clade in the LSU phylogeny (Fig. 2) with only a limited variation among the species in the LSU region investigated. *Afrocantharellus* is, however, strongly supported in the three-gene phylogeny (Fig. 3) and species are reasonably well resolved, the only exception being *A. splendens.* For both specimens of *A. splendens* (DDT17 and DDT57) we managed to obtain all three regions (LSU/5.8-ITS2/ATP6), with ATP6 being slightly shorter in one, however, *A. splendens* is monophyletic in the large LSU phylogeny (Fig. 2).

Afrocantharellus, as represented recently by *C. platyphyllus* and *C. symoensii* in a one-gene phylogeny (tef-

1) by Buyck & Hofstetter (2011) and Buyck *et al*. (2011), the clade was also distinct. In this phylogeny, which was basically the same in both papers, the systematic arrangement follows Eyssartier & Buyck (2001) although there was no support in the phylogeny for the lower branches. This might be due to the tef-1 seeming to be a slow-evolving gene, for example in comparison to *RPB*2 (Matheney *et al*. 2007). In our LSU phylogeny (Fig. 2), *C. fistulosus* is within *Afrocantharellus*. Although recently described from Tanzania as *Cantharellus fistulosus* (Tibuhwa *et al*. 2008), and also morphologically reported as best fitting in subgenus *Parvocantharellus* as defined by Eyssertier & Buyck (2001) and based on characters such as the abundance of clamp connections. However, molecular data place this species in *Afrocantharellus*, and thus the absence of clamp connection is not a synapomorphy for *Afrocantharellus*. The species of *Afrocantharellus* are morphologically reasonably well-characterized (Table 3), and a short description of the species is given in the taxonomic part above. It consists of species closely related to *A. symoensii*, e.g. *A. platyphyllus* f. *platyphyllus*, which in the field is difficult to distinguish from *A. symoensii*. Other taxa included are *A. platyphyllus* f. *cyanescens, A. splendens,* and *A. fistulosus.* Eyssartier & Buyck (2001) referred these species to *Cantharellus* subgen. *Afrocantharellus,* except for *A. fistulosus*. However, *Afrocantharellus* is characterized by having a well-differentiated hymenophore with diverging folds, and all species apart from *A. fistulosus* lack clamp connections.

Relying only on morphological characters may be misleading in the study of these difficult taxa (Buyck & Hofstetter 2011, Buyck *et al*. 2011). It was obvious in our analyses that some species names used for sequences in GenBank had been misapplied, such as *Cantharellus cibarius* and *C. minor*. Combining morphological and molecular data, is clearly the best approach to make progress in the study of genera with a rather uniform morphology where few characters are available for morphological study. Moreover, that we do not have clear morphological synapomorphies for all monophylethic groups within former *Cantharellus s. lat.* should not discourage the recognition of further taxa in the future.

ACKNOWLEDGEMENTS

We are grateful to Sida-SAREC, through the International Science Programme at Uppsala University and the Molecular Biology project of the University of Dar es Salaam, for financial support. We are also indebted to the curators of BR, PC, and UPS for placing material at our disposal. We are further grateful to Bart Buyck for some specimen identifications, and also wish to express our gratitude to two anonymous reviewers, and the helpful work of the editors.

REFERENCES

Bas C (1969) Morphology and subdivision of *Amanita* and amonograph on its section *Lepidella*. *Persoonia* **5**: 285–579.

Binder M, Hibbett DS (2002) Higher-level phylogenetic relationships of *Homobasidiomycetes* (mushroom-forming fungi) inferred from four rDNA regions. *Molecular Phylogenetics and Evolution* **22**: 76–90.

Binder M, Hibbett DS, Larsson KH, Larsson E, Langer E, Langer G (2005) The phylogenetic distribution of resupinate forms across the major clades of mushroom-forming fungi (*Homobasidiomycetes*). *Systematics and Biodiversity* **3**: 113–157.

Buyck B (1994) *Ubwoba: Les Champignons Comestibles de l'Ouest du Burundi*. [Publicacion Agricole no. 34.] Bruxelles: Administration Generale de la Cooperation au Developpement.

Buyck B, Nzigidahera B (1995) Ethnomycological notes from western Burundi. *Belgian Journal of Botany* **128**: 131–138.

Buyck B, Hofstetter V (2008) A multigene phylogeny for worldwide *Cantharellus*. *Inoculum* **59**: 22.

Buyck B, Hofstetter V (2011) The contribution of tef-1 sequences to species delimitation in the *Cantharellus cibarius* complex in southeastern USA. *Fungal Diversity* **49**: 35–46.

Buyck B, Cruaud C, Couloux A, Hofstetter V (2011) *Cantharellus texensis* sp. nov. from Texas, a southern lookalike of *C. cinnabarinus* revealed by tef-1 sequence data. *Mycologia* **103**: 1037–1046.

Buyck B, Eyssartier G, Kivaisi A (2000) Addition to the inventory of the genus *Cantharellus* (*Basidiomycota*, *Cantharellaceae*) in Tanzania. *Nova Hedwigia* **71**: 3–4.

Carriconde F, Gardes M, Jargeat P, Heilmann-Clausen J, Mouhamadou B, Gryta H (2008) Population evidence of cryptic species and geographical structure in the cosmopolitan ectomycorrhizal fungus, *Tricholoma scalpturatum*. *Microbial Ecology* **56**: 513–524.

Dahlman M, Danell E, Spatafora JW (2000) Molecular systematics of *Craterellus*: cladistic analyses of nuclear LSU rDNA sequence data. *Mycological Research* **104**: 388–394.

Dunham SM, Kretzer A, Pfrender ME (2003) Characterization of Pacific golden chanterelle (*Cantharellus formosus*) genet size using co-dominant microsatellite markers. *Molecular Ecology* **12**: 1607–1618.

Eyssartier G (2003) *The genus Cantharellus*. PhD thesis, Muséum National d'Histoire Naturelle Paris.

Eyssartier G, Buyck B (1998) Contribution à la systématique du genre *Cantharellus* en Afrique tropicale: étude de quelques espèces rouges. *Belgian Journal of Botany* **131**: 139–149.

Eyssartier G, Buyck B (2001) Nomenclatural and systematic note on the genus *Cantharellus*. *Documents Mycologiques* **121**: 55–56.

Eyssartier G, Buyck B, Courtecuisse R (2001) New species and combinations in cuboid-spored *Entoloma* species from Madagascar. *Mycological Research* **105**: 1144–1148.

Eyssartier G, Stubbe D, Walleyn R, Verbeken A (2009) New records of *Cantharellus* species (*Basidiomycota*, *Cantharellaceae*) from Malaysian dipterocarp rainforest. *Fungal Diversity* **36**: 57–67.

Feibelman, TP, Bayman P, Cibula WG (1994) Length variation in the internal transcribed spacer of ribosomal DNA in chanterelles. *Mycological Research* **98**: 614–618.

Feibelman TP, Doudrick RL, Cibula WG, Bennett JW (1997) Phylogenetic relationships within the *Cantharellaceae* inferred from sequence analysis of the nuclear large subunit rDNA. *Mycological Research* **101**: 1423–1430.

Frøslev TG, Jeppesen TS, Læssøe T, Kjøller R (2007) Molecular phylogenetics and delimitation of species in *Cortinarius* section *Calochroi* (*Basidiomycota*, *Agaricales*) in Europe. *Molecular Phylogenetics and Evolution* **44**: 217–227.

Geml J, Laursen GA, O'Neill K, Nusbaum HC, Taylor DL (2006)

Beringian origins and cryptic speciation events in the Fly Agaric (*Amanita muscaria*). *Molecular Ecology* **15**: 225–239.

Guindon S, Gascuel O (2003) A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic Biology* **52**: 696–704.

Härkönen M, Niemelä T, Mwasumbi L (1995) Edible mushrooms of Tanzania. *Karstenia* **35** (Suppl.): 1–92.

Härkönen M, Niemelä T, Mwasumbi L (2003) Tanzanian mushrooms: edible, harmful and other fungi. *Norrlinia* **10**: 1–200.

Heinemann P (1958) Champignons récoltes au Congo Belge par Madame Gossens-Fontana III. *Cantharellineae*. *Bulletin du Jardin Botanique de l'Etat Bruxelles* **28**: 385–438.

Heinemann P (1966) Cantharellineae du Katanga. *Bulletin du Jardin Botanique de l'Etat Bruxelles* **36**: 335–352.

Henkel TW, Meszaros R, Catherine AM, Kennedy A (2005) New *Clavulina* species from the Pakaraima Mountains of Guyana. *Mycolological Progress* **4**: 343–350.

Hey J (2006) On the failure of modern species concepts. *Trends in Ecology & Evolution* **21**: 447–450.

Hibbett DS, Donoghue MJ (2001) Analysis of character correlations among wood decay mechanisms, mating systems, and substrate ranges in homobasidiomycetes. *Systematic Biology* **50**: 215–242.

Hibbett DS, Gilbert LB, Donoghue MJ (2000) Evolutionary instability of ectomycorrhizal symbioses in basidiomycetes. *Nature* **407**: 506–508.

Hibbett DS, Pine EM, Langer E, Langer G, Donoghue MJ (1997) Evolution of gilled mushrooms and puffballs inferred from ribosomal DNA sequences. *Proceedings of the National Academy of Sciences, USA* **94**: 12002–12006.

Katoh K, Kuma K, Toh H, Miyata T (2005) MAFFT version 5: improvement in accuracy of multiple sequence alignment. *Nucleic Acids Research* **33**: 511–518.

Katoh K, Misawa K, Kuma K, Miyata T (2002) MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research* **30**: 3059–3066.

Kretzer AM, Bruns TD (1999) Use of atp6 in fungal phylogenetics: an example from the *Boletales*. *Molecular Phylogenetics and Evolution* **13**: 483–492.

Larsson KH, Larsson E, Köljalg U (2004) High phylogenetic diversity among corticioid homobasidiomycetes. *Mycological Research* **108**: 983–1002.

Mason-Gamer R, Kellogg E (1996) Testing for phylogenetic conflict among molecular data sets in the tribe *Triticeae* (*Graminae*). *Systematic Biology* **45**: 524–545.

Matheny PB (2005) Improving phylogenetic inference of mushrooms with *RPB*1 and *RPB*2 nucleotide sequences (*Inocybe*, *Agaricales*). *Molecular Phylogenetics and Evolution* **35**: 1–20.

Matheny PB, Wang Z, Binder M, Curtis JM, Lim YW, Nilsson RH, Hughes KW, Hofstetter V, Ammirati JF, Schoch CL, Langer GE, McLaughlin DJ, Wilson AW, Frøslev T, Ge ZW, Kerrigan RW, Slot JC, Vellinga EC, Liang ZL, Baroni TJ, Fischer M, Hosaka K, Matsuura K, Seidl MT, Vaura J, Hibbett DS (2007) Contributions of rpb2 and tef1 to the phylogeny of mushrooms and allies (*Basidiomycota*, *Fungi*). *Molecular Phylogenetics and Evolution* **43**: 430–51.

Moncalvo JM, Nilsson RH, Koster B, Dunham SM, Bernauer T, Matheny PB, McLenon T, Margaritescu S, Weiß M, Garnica S, Danell E, Langer G, Langer E, Larsson E, Larsson KH, Vilgalys R (2006) The cantharelloid clade: dealing with incongruent gene trees and phylogenetic reconstruction methods. *Mycologia* **98**: 937–948.

Nagy LG, Házi J, Vágvölgyi C, Papp T (2012) Phylogeny and species delimitation in the genus *Coprinellus* with special emphasis on the haired species. *Mycologia* **104**: 254–275.

Olariaga I, Jugo BM, García-Etxebarria K, Salcedo I (2009) Species delimitation in the European species of *Clavulina* (*Cantharellales*, *Basidiomycota*) inferred from phylogenetic analyses of ITS region and morphological data. *Mycological Research* **113**: 1261–1270.

Pilz D, Norvell L, Danell E, Molina R (2003) *Ecology and Management of Commercially Harvested Chanterelle Mushrooms.* [USDA Forest Service Technical Report no. PNW-GTR-576.] Portland, OR: Pacific Northwest Research Station, USDA Forest Service.

Pine EM, Hibbett DS, Donoghue MJ (1999) Phylogenetic relationship of cantharelloid and clavarioid homobasidiomycetes based on mitochondrial and nuclear rDNA sequences. *Mycologia* **91**: 944–963.

Posada D (2008) jModelTest: Phylogenetic Model Averaging. *Molecular Biology and Evolution* **25**: 1253–1256.

Ronquist F, Huelsenbeck JP (2005) *MrBayes.* Version 3.1 (Bayesian analysis of phylogeny); www.mrbayes.net

Savić S, Tibell L (2009) Taxonomy and species delimitation in *Sporodictyon* (*Verrucariaceae*) in Northern Europe and the adjacent Arctic—reconciling molecular and morphological data. *Taxon* **58**: 585–605.

Shao S-C, Tian X-F, Liu P-G (2011) *Cantharellus* in southwestern China: a new species and a new record. *Mycotaxon* **116**: 437–446.

Sharpe C, Wursten B (2009) *A preliminary list of the macrofungi recorded in the Vumba.*www.vumba-nature.com.

Stamatakis A (2006) RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* **22**: 2688–2690.

Stamatakis A, Hoover P, Rougemont J (2008) A Rapid Bootstrap algorithm for the RAxML Web-Servers. *Systematic Biology* **75**: 758–771.

Swofford DL (2002) *PAUP: phylogenetic analysis using parsimony (and other methods).* Version 4.0b10. Sunderland, MA: Sinuaer Associates.

Thacker JR, Henkel TW (2004) New species of *Clavulina* from Guyana. *Mycologia* **96**: 650–657.

Tibuhwa D, Buyck B, Kivaisi A, Tibell L (2008) *Cantharellus fistulosus* sp. nov. from Tanzania. *Cryptogamie Mycologie* **29**: 129–135.

Vilgalys R, Hester M (1990) Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *Journal of Bacteriology* **172**: 4238–4246.

doi:10.5598/imafungus.2012.03.01.05

IMA FUNGUS · VOLUME 3 · NO 1: 39–43

ARTICLE

The identity of *Cintractia disciformis*: reclassification and synonymy of a southern Asian smut parasitic on *Carex* sect. *Aulocystis*

Marcin Piątek

Department of Mycology, W. Szafer Institute of Botany, Polish Academy of Sciences, Lubicz 46, PL-31-512 Kraków, Poland; corresponding author e-mail: m.piatek@botany.pl

Abstract: The identity of a neglected smut fungus, *Cintractia disciformis*, described from *Carex hirtella* in the Western Himalaya, India is reassessed. The species is excluded from *Cintractia* and is confirmed as a distinct species of *Anthracoidea*. Two smuts, *A. nepalensis* on *Carex nakaoana* in Nepal, and *A. haematostomae* on *Carex haematostoma* in China, are similar morphologically and considered to be later heterotypic synonyms of *Cintractia disciformis*. The appropriate nomenclatural combination for this species, *Anthracoidea disciformis* comb. nov., is validated.

Key words:
Anthracoidea
Carex
Cintractia
Historical Collections
Smut Fungi
Taxonomy

Article info: Submitted: 16 March 2012; Accepted: 29 May 2012; Published: 21 June 2012.

INTRODUCTION

The smut fungus *Cintractia disciformis* was originally described from a plant identified as *Carex hirtella* (sect. *Aulocystis*) collected at Nipchang in Western Himalaya, India. *Cintractia disciformis* was first invalidly introduced without the mandatory Latin description that was required from 1 January 1935 until 31 December 2011 (Liro 1935). A few years later Liro (1939) provided the missing Latin diagnosis. The species has only occasionally been reported in the literature, for instance in connection with a second finding on Mt. Sawi in Indian Kashmir on a new host, *Carex haematostoma* (Ling 1949), or in monographic studies on smut fungi (Zundel 1953, Zambettakis 1978, Piepenbring 2000, Vánky 2007a, 2012, Gandhe 2011). Ling (1949) and Zambettakis (1978) prepared descriptions of the Kashmiri specimen of *Cintractia disciformis*. Other authors have either repeated the description from the protologue (Zundel 1953, Gandhe 2011), or not included one (Piepenbring 2000, Vánky 2007a, 2012). In a monograph of *Anthracoidea*, Zambettakis (1978) proposed a new combination "*Anthracoidea disciformis* (Liro) Zambett.", but without giving any indication of the basionym or any reference to the place of its valid publication, rendering the combination invalid (ICN, Art. 33.4), like all new combinations introduced in that work.

Vánky (2012) examined the type material of *Cintractia disciformis* in H, and concluded that it was an *Anthracoidea*. However, he did not accept the species or make any transfer to that genus as he noted that the host *Carex* was not *C. hirtella* according to an annotation by I. Kukkonen on the specimen. Vánky commented that "without the correct name of the host plant it cannot be identified".

Two smuts with similar phenotypic characteristics on related hosts in *Carex* sect. *Aulocystis* in the same geographical area of southern Asia (Himalaya Mts) were described several decades later, *Anthracoidea nepalensis* on *Carex nakaoana* in Nepal (Kakishima & Ono 1988) and *Anthracoidea haematostomae* on *Carex haematostoma* in China (Guo 2006). These two smuts were found to be conspecific by Vánky & Piątek (in Vánky 2007b) and that treatment is followed in the monograph of Vánky (2012). This work aims to clarify the taxonomic status of *Cintractia disciformis* and ascertain whether it is distinct from or conspecific with *Anthracoidea nepalensis* (incl. *A. haematostomae*).

MATERIALS AND METHODS

Sori and spore characteristics were studied using dried herbarium material deposited in H, IBAR and "H.U.V."[1]. The specimens were examined either by light microscopy (LM) and scanning electron microscopy (SEM) or only by light microscopy (LM).

For light microscopy (LM), small pieces of sori were mounted in lactic acid, heated to boiling point and cooled, and then examined under a Nikon Eclipse 80i light microscope. LM micrographs were taken with a Nikon DS-Fi1 camera. Fifty spores were measured from each collection, using NIS-Elements BR 3.0 imaging software. Spore size ranges were assigned to one of the three groups distinguished by Savile (1952): (1) small-sized spores – 13–21(–23) × 9–17(–20) µm;

[1]The personal collection of Kálmán Vánky, "Herbarium *Ustilaginales* Vánky" currently held at his home (Gabriel-Biel-Straße 5, D-72076 Tübingen, Germany).

(2) medium-sized spores – 15–25(–27) × 10–21 µm; (3) large-sized spores – 18–33 × 13–28 µm. Unless otherwise stated, the spores were measured in plane view and measurements are adjusted to the nearest 0.5 µm.

For scanning electron microscopy (SEM), spores taken directly from dried herbarium samples were dusted onto carbon tabs and fixed to an aluminium stub with double-sided transparent tape. The stubs were sputter-coated with carbon using a Cressington sputter-coater and viewed under a Hitachi S-4700 scanning electron microscope, with a working distance of *ca*. 12 mm. SEM micrographs were taken in the Laboratory of Field Emission Scanning Electron Microscopy and Microanalysis at the Institute of Geological Sciences of Jagiellonian University (Kraków).

RESULTS

Anthracoidea disciformis (Liro) M. Piątek, **comb. nov.**
MycoBank MB800481
(Fig. 1)

Basionym: *Cintractia disciformis* Liro, *Myc. Fenn*. fasc. 16, no. 110 (1939).

Synonyms: *Cintractia disciformis* Liro, *Ann. Bot. Soc. zool.-bot. Fenn. "Vanamo"* **6**: 6 (1935); nom. inval. (Art. 36.1).
Anthracoidea disciformis (Liro) Zambett., *Bull. Soc. mycol. France* **94**: 166 (1978); nom. inval. (Art. 33.4).
Anthracoidea nepalensis Kakish. & Y. Ono, *in* Watanabe & Malla, *Crypt. Himal.* **1**: 128 (1988).
Anthracoidea haematostomae L. Guo, *Fungal Diversity* **21**: 83 (2006).

Sorus in one ovary of the inflorescence, black, ovoid, presumably around the achene, about 4 × 2.5 mm diam, composed of agglutinated spores, powdery on the surface, partly hidden by the perigynium and scales. *Spores* small-sized, flattened, disc-shaped, chestnut-brown to reddish brown, regular in shape and size, in plane view globose, subglobose or broadly ellipsoidal, 16.5–18.5(–19.0) × (13.5–)14.0–18.0 µm [av. ± SD, 17.6 ± 0.6 × 15.7 ± 1.2 µm, *n* = 50], in side view broadly ellipsoidal (8.5–)10.0–12.0 µm (measurements without hyaline caps), usually enclosed by prominent mucilaginous sheath visible as hyaline caps on the flattened sides, up to 1.5 µm wide; wall even, 1.5–2.0 µm, darker than the rest of spore; surface finely papillate in LM, spore profile finely serrulate, surface sparsely papillate in SEM, papillae up to 0.3 µm high (from SEM micrographs), interspaces smooth.

Specimens examined: **China**: *Yunnan Province*: Deqen, elev. 2700 m, on *Carex haematostoma*, Sept. 1935, *C.W. Wang 70101* ("H.U.V." 20090, isotype of *Anthracoidea haematostomae*). – **India**: Darma, Nipchang, on *Carex plectobasis* (as "*C. hirtella*"), 31 Aug. 1884, *J. F. Duthie* (H s.n. – holotype of *Cintractia disciformis*). – **Nepal**: *Bagmati Zone*: Langtang, Kyangjin–Langshisa, elev. 3900 m, on *Carex haematostoma* (syn. *C. nakaoana*), 3 Sept. 1986, *Y. Ono 86NE-223* (IBAR 0628 – isotype of *Anthracoidea nepalensis*); elev. 3800 m, on *Carex haematostoma* (syn. *C. nakaoana*), 3 Sept. 1986, *Y. Ono 86NE-214* (IBAR 0619, paratype of *Anthracoidea nepalensis*); Kyangjin, elev. 3800 m, on *Carex haematostoma* (syn. *C. nakaoana*), 4 Sept. 1986, *Y. Ono 86NE-234* (IBAR 0639 – paratype of *Anthracoidea nepalensis*).

Hosts and distribution: On members of *Carex* sect. *Aulocystis*: *Carex digyna*, *C. haematostoma* (syn. *C. nakaoana*), and *C. plectobasis* (syn. *C. hirtella*). Known from China, India, and Nepal.

Observations: While the host of *Cintractia disciformis* is uncertain according to the annotation by Ilkka Kukkonen on the holotype; re-identification of the specimen based on one inflorescence is difficult. However, the sedge definitely belongs to the section *Aulocystis*, and the length of perigynia (5.0–6.5 mm) indicates an affinity with *Carex plectobasis* (syn. *C. hirtella*) according to the available keys and descriptions in the Flora of Pakistan and Flora of China (eFloras, http://www.efloras.org). Guo (1994) has also reported *Anthracoidea nepalensis* on this host (as *Carex hirtella*).

The host of *Anthracoidea nepalensis* was reported as *Carex nakaoana*, but this species is now considered synonymous with *C. haematostoma* (Chlebicki 2002). Yet another host of this smut is *C. digyna* listed in Chinese reports of *A. nepalensis* (Guo 1994, as "*digyne*"). The host of *A. haematostomae* is *C. haematostoma.* Vánky (2007b) included the European sedge *C. sempervirens* in the list of hosts of *A. nepalensis*; this was evidently a mistake, and the species was not cited as a host in his subsequent monograph (Vánky 2012). *Carex sempervirens* has to be excluded from the host range of *A. disciformis*.

DISCUSSION

The internal structure of the sori is one of the main differentiating characteristics between *Anthracoidea* and *Cintractia* (Kukkonen 1963, Piepenbring 2000). Unfortunately this feature could not be examined in the holotype of *Cintractia disciformis* without destroying the specimen. However, the hyaline caps on the spores preclude a placement in *Cintractia* and support an affinity to *Anthracoidea.* Further, species of *Cintractia* are not known to occur on *Carex,* nor even members of the *Cariceae* (Piątek & Vánky 2007). The internal sorus structure of *Anthracoidea nepalensis* (Fig. 2), regarded here as a synonym, is typical of *Anthracoidea* species in that the spores are formed on the outer surface of the achene, and not within the U-shaped pockets embedded in the sterile stroma, a character of the genus *Cintractia* (Kukkonen 1963, Piepenbring 2000). This provides additional indirect evidence that *Cintractia disciformis* is a member of the genus *Anthracoidea* as indicated by Zambettakis (1978) and Vánky (2012).

The characteristics of the holotype of *Cintractia disciformis* are included in the species description presented above and shown in the illustrations (Fig. 1). The morphological details of specimens of *Anthracoidea nepalensis* I examined were: sori globose or ovoid surrounding the achenes, about 1.5–3.0 long and 1.5–2.5 mm wide, spores disc-shaped, chestnut-brown to reddish brown, globose, subglobose, rarely broadly ellipsoidal or somewhat subangulate, (15.0–)15.5–19.5(–20.5) × (12.5–)14.0–18.0(–19.0) µm, the flattened

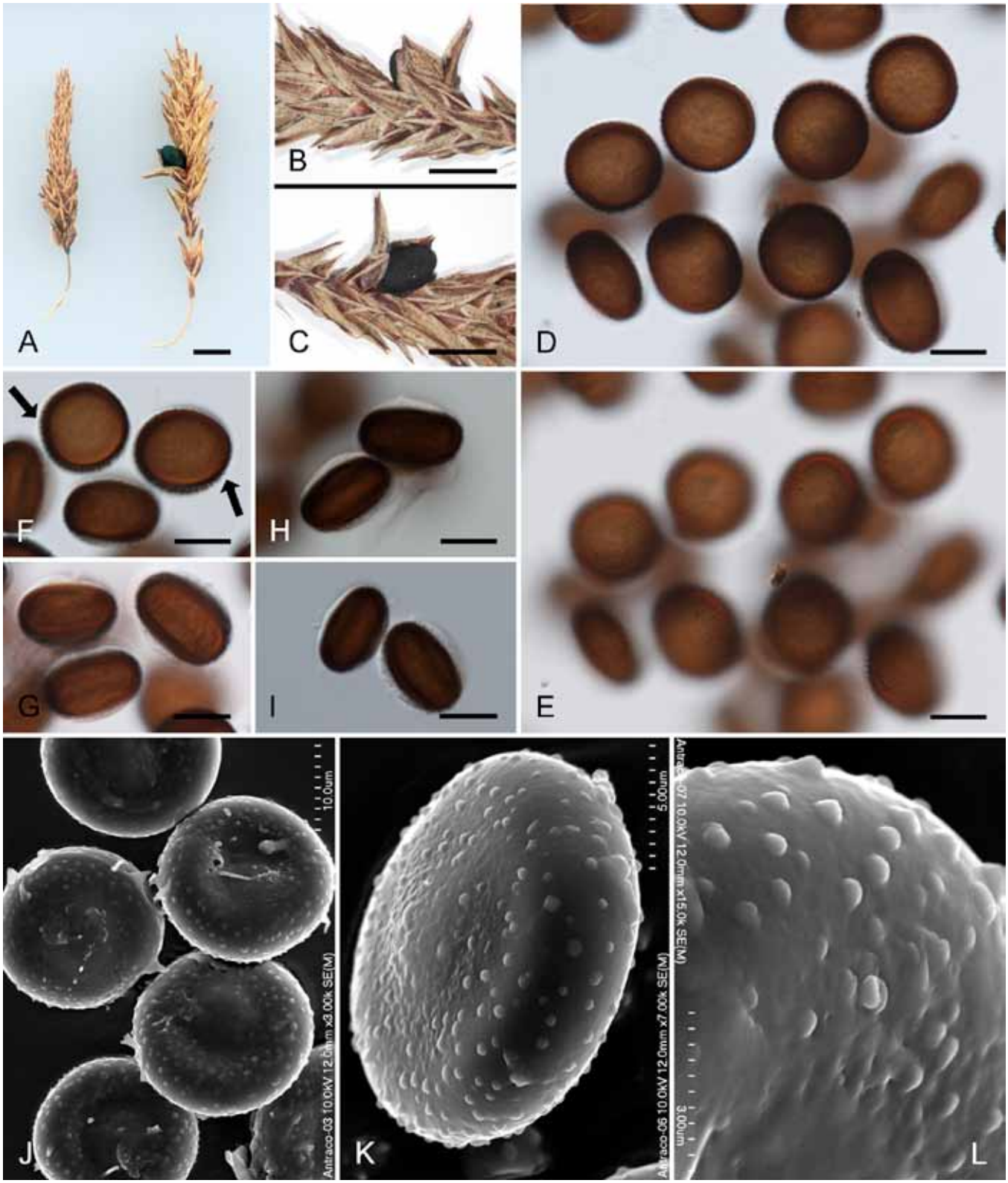


Fig. 1. *Anthracoidea disciformis* (H s.n. – holotype). **A.** The holotype material. **B–C.** Enlarged sorus visible from both sides of inflorescence respectively. **D–E.** Spores in LM, median and superficial views respectively. **F–I.** Spores with prominent hyaline mucilaginous sheath on the flattened sides. Note small papillae on spore surface indicated by arrows on picture F. **J–K.** Spores in SEM. Note remnants of mucilaginous sheath on surface of spores illustrated on picture J and in central part of spore illustrated on picture K. **L.** Ornamentation of spore in SEM. Bars: A–C = 5 mm, D–J = 10 µm, K = 5 µm, L = 3 µm.

sides of spores rarely enclosed by a hyaline mucilaginous sheath, spore wall even, 1.0–1.5(–2.0) µm, spore surface finely papillate, spore profile finely serrulate. The SEM characteristics of spores of *A. nepalensis* (Kakishima & Ono 1988, Chlebicki 2002) agree well with those of *Cintractia disciformis*.

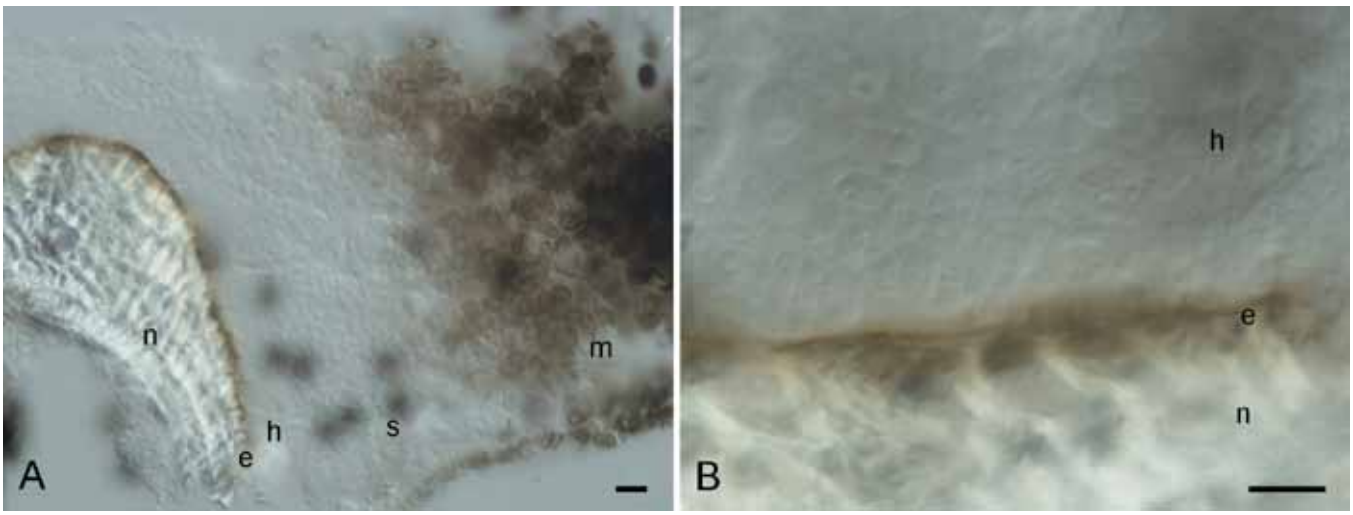


Fig. 2. Internal sorus structure of *Anthracoidea nepalensis* (IBAR 0619). **A.** Transverse section through the sorus. **B.** Enlarged area close to the achene surface. Abbreviations: n – rudimentary achene, e – dark layer of the remnants of the achene epidermis, h – layer of sporogeneous hyphae, s – layer of young hyaline spores, m – layer of gradually maturing dark spores. Bars: A = 20 µm, B = 10 µm.

The morphology of *Anthracoidea haematostomae* was investigated by Vánky & Piątek (in Vánky 2007b) to establish the synonymy between this species and *A. nepalensis*, although only the morphology of *A. nepalensis* was presented in the published results. However, the key morphological features of the material of *A. haematostomae* studied are: spores dark reddish brown, 17.5–22 × 15–20 µm; spore wall even, 1.5–2.5 µm thick, with hyaline caps, spore surface finely papillate, spore profile finely wavy. The spore ornamentation observed in SEM (Guo 2006) also agrees well with that of *Cintractia disciformis*.

The morphology of *Cintractia disciformis*, *Anthracoidea nepalensis* and *A. haematostomae* is very similar, and the only differences concern the hyaline mucilaginous sheath. This sheath was less developed in the material of *A. nepalensis,* and the spores are somewhat larger and the spore wall slightly thicker in *A. haematostomae* compared to *Cintractia disciformis*. However, these minor differences lie within the normal variability of a single *Anthracoidea* species (Kukkonen 1963, Denchev 1991, Piątek & Mułenko 2010, Savchenko *et al.* in press). Consequently, these three species names are considered as synonymous and the oldest available name, *Cintractia disciformis*, is therefore taken up as a new combination, that proposed by Zambettakis (1978) being invalid.

The disc-shaped, papillate spores of *Anthracoidea disciformis* are distinctive and rarely observed in other *Anthracoidea* species that have verruculose or rarely smooth spores. This feature readily differentiates this smut from four other *Anthracoidea* species infecting members of *Carex* sect. *Aulocystis* which all have verruculose spores (*viz*. *A. altera*, *A. misandrae*, *A. sempervirentis*, and *A. stenocarpae*). In the entire genus, only a few other *Anthracoidea* species have disc-shaped and papillate spores, for example *A. bistaminatae* (Guo 2006), *A. lindebergiae* (Vánky 1994), *A. mulenkoi* (Piątek 2006), *A. pygmaea* (Guo 2002), *A. royleanae* (Guo 2006), *A. setschwanensis* (Guo 2007), *A. smithii* (Vánky 2007a), and *A. xizangensis* (Guo 2005), all of which infect *Kobresia*. Interestingly, most of these *Anthracoidea* species occur in eastern and southern Asia. An exception is *A. lindebergiae*, which is widely distributed in arctic and alpine ecosystems of the Northern Hemisphere. Whether these *Anthracoidea* species are closely related and have evolved from a common ancestor is unclear and open to future studies.

This study demonstrates that a critical evaluation of historical names could prevent an unnecessary proliferation of names proposed for the same organism. Such taxonomical expertise appears even more urgent in the light of molecular initiatives, especially DNA Barcoding (Seifert 2008, Begerow *et al.* 2010, Schoch *et al.* 2012). In order to be most effective the molecular studies should be accompanied by a critical reassessment of as many historical names of fungal species as possible that can be linked to freshly collected specimens for use in molecular analyses (Lücking 2008, Hyde *et al.* 2010).

ACKNOWLEDGEMENTS

I thank the curators of H, "H.U.V." and IBAR for the loan of specimens, Anna Łatkiewicz (Kraków, Poland) for her help with the SEM micrographs, and David L. Hawksworth (Madrid, Spain / London, UK) and Roger G. Shivas (Dutton Park, Australia) for helpful comments on the manuscript. This study was supported by the Polish Ministry of Science and Higher Education (grant no. 2 P04G 019 28).

REFERENCES

Begerow D, Nilsson H, Unterseher M, Maier W (2010) Current state and perspectives of fungal DNA barcoding and rapid identification procedures. *Applied Microbiology and Biotechnology* **87**: 99–108.

Chlebicki A (2002) Two cypericolous smut fungi (*Ustilaginomycetes*) from the Thian Shan and their biogeographic implications. *Mycotaxon* **83**: 279–286.

Denchev CM (1991) Genus *Anthracoidea* Bref. (*Ustilaginales*) in Bulgaria – species and morphometric variability of the teliospores. *Fitologija* **41**: 51–60.

Gandhe RV (2011) *Ustilaginales of India*. Dehra Dun: Bishen Singh Mahendra Pal Singh.

Guo L (1994) *Anthracoidea* and allied genera in China (*Ustilaginales*). *Mycosystema* **7**: 89–104.

Guo L (2002) Two new species of *Ustilaginomycetes* and a species new to China. *Mycotaxon* **82**: 147–150.

Guo L (2005) Two new species of *Anthracoidea* (*Ustilaginales*) from China. *Mycotaxon* **94**: 47–50.

Guo L (2006) Six new species of *Anthracoidea* (*Ustilaginales*) from China. *Fungal Diversity* **21**: 81–92.

Guo L (2007) *Anthracoidea setschwanensis* sp. nov. (*Ustilaginales*) and a new record of *Anthracoidea* from China. *Mycotaxon* **99**: 227–230.

Hyde KD, Abd-Elsalam K, Cai L (2010) Morphology: still essential in a molecular world. *Mycotaxon* **114**: 439–451.

Kakishima M, Ono Y (1988) Three species of smut fungi (*Ustilaginales*) from Nepal. In: *Cryptogams of the Himalayas.* Vol. 1. *The Kathmandu Valley* (M Watanabe, SB Malla, eds): 127–132. Tsukuba: National Science Museum.

Kukkonen I (1963) Taxonomic studies on the genus *Anthracoidea* (*Ustilaginales*). *Annales Botanici Societatis Zoologicae Botanicae Fennicae 'Vanamo'* **34**(3): 1–122.

Ling L (1949) Taxonomic notes on Asiatic smuts. – I. *Sydowia* **3**: 123–134.

Liro JI (1935) Über neue, seltene und vermeinte Ustilagineen. *Annales Botanici Societatis Zoologicae Botanicae Fennicae 'Vanamo'* **6**(1): 1–18.

Liro JI (1939) *Mycotheca fennica. Die Etiketten*. No. 301–600. Helsinki.

Lücking R (2008) Taxonomy: a discipline on the brink of extinction. Are DNA barcode scanners the future of biodiversity research? *Archives des Sciences* **61**(2): 75–88.

Piątek M (2006) *Anthracoidea mulenkoi* (*Ustilaginomycetes*), a new cypericolous smut fungus from Pakistan. *Nova Hedwigia* **83**: 109–116.

Piątek M, Mułenko W (2010) The calcareous mires in South-East Poland are home to two rare *Anthracoidea* species. *Acta Mycologica* **45**: 175–184.

Piątek M, Vánky K (2007) *Cintractia bulbostylidicola* sp. nov. (*Ustilaginomycotina*) from North America. *Nova Hedwigia* **85**: 187–194.

Piepenbring M (2000) The species of *Cintractia* s.l. (*Ustilaginales*, *Basidiomycota*). *Nova Hedwigia* **70**: 289–372.

Savchenko KG, Lutz M, Piątek M, Heluta VP, Nevo E. (in press) *Anthracoidea caricis-meadii* is a new North American smut fungus on *Carex* sect. *Paniceae*. *Mycologia*.

Savile DBO (1952) A study of the species of *Cintractia* on *Carex*, *Kobresia*, and *Scirpus* in North America. *Canadian Journal of Botany* **30**: 410–435.

Seifert KA (2008) Integrating DNA Barcoding into the Mycological Sciences. *Persoonia* **21**: 162–166.

Schoch CL, Seifert KA, Huhndorf S, Robert V, Spouge JL, Levesque CA, Chen W, Fungal Barcoding Consortium (2012) Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for *Fungi*. *Proceedings of the National Academy of Sciences, USA* **109**: 6241–6246.

Vánky K (1994) *European Smut Fungi*. Stuttgart: G. Fischer Verlag.

Vánky K (2007a) Smut fungi of the Indian Subcontinent. *Polish Botanical Studies* **26**: 1–265.

Vánky K (2007b) Taxonomic studies on *Ustilaginomycetes* – 27. *Mycotaxon* **99**: 1–70.

Vánky K (2012) *Smut Fungi of the World.* St Paul, MN: American Phytopathological Society Press.

Zambettakis C (1978) Les *Anthracoidea* des *Carex* et les Ustilaginées aberrantes. *Bulletin de la Société Mycologique de France* **94**: 109–260.

Zundel GL (1953) The *Ustilaginales* of the world. *Pennsylvania State College School of Agriculture Department of Botany Contribution* **176**: xi+1–410.

doi:10.5598/imafungus.2012.03.01.06

IMA FUNGUS · VOLUME 3 · NO 1: 45–58

ARTICLE

Ceratocystis eucalypticola sp. nov. from *Eucalyptus* in South Africa and comparison to global isolates from this tree

Marelize van Wyk[1], Jolanda Roux[2], Gilbert Kamgan Nkuekam[2], Brenda D. Wingfield[1], and Michael J. Wingfield[1]

[1]Department of Genetics, Tree Protection Co-operative Programme (TPCP), Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Private Bag X20, Hatfield, Pretoria 0028, Pretoria, South Africa

[2]Department of Microbiology and Plant Pathology, DST/NRF Centre of Excellence in Tree Health Biotechnology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Private Bag X20 Hatfield, Pretoria 0028, Pretoria, South Africa; corresponding author e-mail: Jolanda.roux@fabi.up.ac.za

Abstract: *Eucalyptus* trees, mostly native to Australia, are widely planted in the tropics and Southern Hemisphere for the production of wood and pulp. Worldwide surveys of diseases on these trees have yielded a large collection of *Ceratocystis* isolates from dying trees or from wounds on their stems. The aim of this study was to characterise these isolates and to consider their relatedness to each other. Culture appearance, morphological features and a distinctive fruity odour in all cultures were typical of species in the *Ceratocystis fimbriata sensu lato* (*s. lat.*) complex. Phylogenetic analyses of sequences for the combined ITS, βt-1 and TEF1-α gene regions revealed a genetically diverse group of isolates residing in a single large clade, that were distinct from all other species in the *C. fimbriata s. lat.* complex. Based on morphology and phylogenetic inference, the *Eucalyptus* isolates are recognised as closely related. The South African isolates are described here as a new species, *C. eucalypticola*.

Key words:
canker stain diseases
Microascales
tree pathogens
wounds

Article info: Submitted: 20 March 2012; Accepted: 1 May 2012; Published: 21 June 2012.

INTRODUCTION

Eucalyptus species are mostly native to Australia, but have been widely planted in the tropics and Southern Hemisphere. This is because they are adapted to a wide range of different environments and are typically fast growing. It has further been suggested that the success of these trees as non–natives is due to the separation from their natural enemies (Wingfield *et al.* 2008, Roux & Wingfield 2009). The potential threat of pests and pathogens to the sustainability of eucalypt plantations in areas where they are not native is consequently great and of substantial concern to forestry industries globally (Old *et al.* 2003, Wingfield *et al.* 2008).

In order to understand and manage the threat of pests and pathogens to *Eucalyptus* species grown as non-natives and in plantations, tree health surveys are undertaken regularly. Amongst the pathogens that have been found on these trees, a *Ceratocystis* sp. in the *C. fimbriata s. lat.* complex causes serious disease problems in Brazil, the Republic of Congo, Uganda, and Uruguay (Laia *et al.* 1999, Roux *et al.* 2000, 2001, 2004, Barnes *et al.* 2003a). Various other *Ceratocystis* species in the *C. fimbriata s. lat.* complex have also been found on naturally occurring or artificially induced wounds on the stems of trees, in various parts of the world. Some of these have been shown to be cryptic taxa that have been provided with names (van Wyk *et al.* 2007, 2008, 2010a, Rodas *et al.* 2007, Heath *et al.* 2009, Kamgan Nkuekam *et al.* 2012). Several species are thought to be pathogens, while the role of others in tree health is not known.

The genus *Ceratocystis* comprises a diverse group of fungi, including saprophytes causing blue-stain of lumber and serious pathogens that cause mortality (Kile 1993). The genus is typified by *C. fimbriata s. str.* that is a pathogen restricted to root crops, specifically sweet potato (Engelbrecht & Harrington 2005). *Ceratocystis fimbriata s. lat.* represents a diverse assemblage of isolates, some of which have been treated as distinct taxa defined based on phylogenetic inference, morphological differences, and mating behaviour (Barnes *et al.* 2001, Engelbrecht & Harrington 2005, Johnson *et al.* 2005, van Wyk *et al.* 2007, 2008, Heath *et al.* 2009). However, Ferreira *et al.* (2010) treated some isolates of the *C. fimbriata s. lat.* complex from Brazil as representing a particular population of *C. fimbriata s. str.*, rather than as discrete taxa.

Global surveys of the health of *Eucalyptus* species in plantations have yielded a large collection of isolates that can loosely be accommodated in the *C. fimbriata s. lat.* complex. The aim of this study was to characterise these isolates and to consider patterns in their distribution on *Eucalyptus* species worldwide.

MATERIALS AND METHODS

Isolates

Isolates used in this study were obtained from: (1) artificially induced wounds on the stems of *Eucalyptus* trees in South

Africa, Thailand, and Indonesia (Table 1). The isolates were obtained by directly transferring spore masses from the apices of ascomata produced on the wounded inner bark and wood to agar plates. When sporulating structures were absent, the wood samples were placed in moist chambers to enhance sporulation. Spore masses were transferred to 2 % Malt Extract Agar (MEA) in Petri dishes and incubated at room temperature. Additionally, the carrot baiting technique was used to obtain isolates (Moller & DeVay 1968). (2) cultures were sourced from the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI) at the University of Pretoria, South Africa. These isolates had previously been identified as representing the *C. fimbriata s. lat.* complex and were from diseased *Eucalyptus* trees in various parts of the world including Brazil, Uganda, Congo, and Uruguay (Table 1).

PCR and sequencing reactions

DNA was extracted from all isolates as described by van Wyk *et al.* (2006a). Three gene regions were selected for PCR amplification, including ITS1 and ITS2, including the 5.8S rDNA operon, part of the beta-tubulin (βt-1) gene, and part of the Transcription Elongation Factor-1 alpha (TEF1-α) gene region. The reactions and programme for amplification were as described by van Wyk *et al.* (2006b). The primers utilized were ITS1 and ITS4 (White *et al.* 1990), βt1a and βt1b (Glass & Donaldson 1995), and EF1F and EF1R (Jacobs *et al.* 2004).

Sequencing reactions were set up and run as described by van Wyk *et al.* (2006a). Sequences of the isolates from *Eucalyptus* were analysed with Chromas Lite 2.01 (http://www.technelysium.com.au). These sequences as well as those for all species in the *C. fimbriata s. lat.* species complex (Table 1) were aligned using MAFFT (http://timpani.genome.ad.jp/%7emafft/server/) (Katoh *et al.* 2002). All sequences derived from this study have been deposited in GenBank (Table 1).

Combined gene tree for all described species in the *C. fimbriata s. lat.* complex

Representative isolates of all described species in the *C. fimbriata s. lat.* complex were included in this dataset, including those obtained for this study from CMW. The sequences of three gene regions (ITS, βt-1 and TEF1-α) were combined and a partition homogeneity test (PHT) was used to determine if the data from the three regions could be combined, using the software programme PAUP v. 4.0b10 (Swofford 2002). Settings in PAUP were as described in van Wyk *et al.* (2010a). *Ceratocystis virescens* was selected as the outgroup taxon.

MrModeltest2 (Nylander 2004) was used to determine the most appropriate model of nucleotide substitution for each of the three gene regions, respectively. These models were then included in the Bayesian analyses using MrBayes (Ronquist & Huelsenbeck 2003). The Bayesian analyses were run as described in van Wyk *et al.* (2010a).

Combined and separate gene trees of unnamed *Ceratocystis fimbriata s. lat.* isolates obtained from *Eucalyptus*

This dataset consisted only of *Ceratocystis fimbriata s. lat.* isolates from *Eucalyptus* trees and that have not yet been described as separate species. A closely related and previously described species, *C. colombiana*, also obtained from *Eucalyptus,* was included as an outgroup. This was done to determine whether these isolates represent one group with no separate grouping or whether geographical grouping exists, as has been documented in *C. fimbriata s. lat.* (Engelbrecht & Harrington 2005, Ferreira *et al.* 2010). Models were obtained for each of the ITS, βt-1 and TEF1-α gene regions with the use of MrModeltest2 (Nylander 2004). Consistent with both the first datasets, these models were incorporated into MrBayes (Ronquist & Huelsenbeck 2003) in order to run Bayesian analyses.

Utilising the *C. fimbriata s. lat.* isolates from *Eucalyptus* trees obtained from the CMW culture collection, the Molecular Evolutionary Genetics Analysis software (MEGA) 4 (Tamura *et al.* 2007) was used to determine the amount of variation for each gene region. The three gene regions were inspected to determine the number of fixed alleles between them. Allele trees were drawn using the software TCS (Clement *et al.* 2000) from the combined dataset for the *Eucalyptus* isolates, including the closely related species *C. colombiana,* known only from *Eucalyptus*.

Culture characteristics and morphology

Two isolates of *Ceratocystis fimbriata s. lat.* from *Eucalyptus* were selected from each country, other than Brazil, for which only one *Eucalyptus* isolate was available. These were used to describe morphological characteristics. Isolates were transferred to each of five 2 % Malt Extract Agar (MEA) plates and incubated in the dark. The isolates were incubated at 30 ºC for 7 d, after which the growth was assessed.

Microscopic examinations were made of isolates from Indonesia, Uruguay, Thailand, and South Africa. Isolates from other countries were excluded because the cultures did not produce ascomata. All taxonomically informative structures were measured from 10 d old cultures on 2 % MEA, mounted in lactic acid. Ten measurements were made for each of the two isolates from Indonesia, Uruguay, Thailand, and South Africa.

A preliminary study of isolates representing the larger collection of *C. fimbriata s. lat.* isolates from *Eucalyptus,* and nested together in the same phylogenetic clade, showed that they are morphologically very similar. Consequently, four isolates (CMW 9998, CMW 15054, CMW 10000 and CMW 11536) from *Eucalyptus* in South Africa were selected for more detailed study. These South African isolates were transferred to five 2 % MEA plates each and incubated at seven different temperatures. These temperatures included 4 ºC and six temperatures between 10 ºC and 35 ºC at 5 ºC intervals. Growth was assessed after 7 d of incubation in the dark. Colony colour was assessed for the same isolates used as in the growth studies, grown on 2 % MEA for seven to 10 d at room temperature (25 ºC). The colour charts of Rayner (1970) were used for descriptions of colony colour.

Fifty measurements were made of all taxonomically informative characters for isolate CMW 11536 from *Eucalyptus* in South Africa. An additional ten measurements were made of these structures for isolates CMW 9998 and CMW 10000 and CMW 15054. The minimum, maximum, average and standard deviation (stdv) was calculated for the

Table 1. Isolates of *Ceratocystis fimbriata s. lat.* spp. used in this study.

Species	Isolate no.	GenBank accession no.	Host	Area
C. albifundus	CMW4068	DQ520638, EF070429, EF070400	*Acacia mearnsii*	South Africa
C. albifundus	CMW5329	AF388947, DQ371649, EF070401	*Acacia mearnsii*	Uganda
C. atrox	CMW19383, CBS120517	EF070414, EF070430, EF070402	*Eucalyptus grandis*	Australia
C. atrox	CMW19385, CBS120518	EF070415, EF070431, EF070403	*Eucalyptus grandis*	Australia
C. cacaofunesta	CMW15051, CBS152.62	DQ520636, EF070427, EF070398	*Theobroma cacao*	Costa Rica
C. cacaofunesta	CMW14809, CBS115169	DQ520637, EF070428, EF070399	*Theobroma cacao*	Ecuador
C. caraye	CMW14793, CBS114716	EF070424, EF070439, EF070412	*Carya cordiformis*	USA
C. caraye	CMW14808, CBS115168	EF070423, EF070440, EF070411	*Carya ovata*	USA
C. colombiana	CMW9565, CBS121790	AY233864, AY233870, EU241487	Soil	Colombia
C. colombiana	CMW5751, CBS121792	AY177233, AY177225, EU241493	*Coffea arabica*	Colombia
C. colombiana	CMW9572	AY233863, AY233871, EU241488	Mandarin	Colombia
C. eucalypticola	**CMW9998, CBS124017**	**FJ236721, FJ236781, FJ236751**	***Eucalyptus* sp.**	**South Africa**
C. eucalypticola	**CMW10000, CBS124019**	**FJ236722, FJ236782, FJ236752**	***Eucalyptus* sp.**	**South Africa**
C. eucalypticola	**CMW11536, CBS124016**	**FJ236723, FJ236783, FJ236753**	***Eucalyptus* sp.**	**South Africa**
C. eucalypticola	**CMW12663**	**FJ236724, FJ236784, FJ236754**	***Eucalyptus* sp.**	**South Africa**
C. eucalypticola	**CMW15054, CBS124018**	**FJ236725, FJ236785, FJ236755**	***Eucalyptus* sp.**	**South Africa**
C. fimbriata s. str.	CMW15049, CBS141.37	DQ520629, EF070442, EF070394	*Ipomaea batatas*	USA
C. fimbriata s. str.	CMW1547	AF264904, EF070443, EF070395	*Ipomaea batatas*	Papua New Guinea
C. fimbriatomima	CMW24174, CBS121786	EF190963, EF190951, EF190957	*Eucalyptus* sp.	Venezuela
C. fimbriatomima	CMW24176, CBS121787	EF190964, EF190952, EF190958	*Eucalyptus* sp.	Venezuela
C. larium	CMW25434, CBS122512	EU881906, EU881894, EU881900	*Styrax benzoin*	Indonesia
C. larium	CMW25435, CBS122606	EU881907, EU881895, EU881901	*Styrax benzoin*	Indonesia
C. manginecans	CMW13851, CBS121659	AY953383, EF433308, EF433317	*Mangifera indica*	Oman
C. manginecans	CMW13852, CBS121660	AY953384, EF433309, EF433318	*Hypocryphalus mangifera*	Oman
C. neglecta	CMW17808, CBS121789	EF127990, EU881898, EU881904	*Eucalyptus* sp.	Colombia
C. neglecta	CMW18194, CBS121017	EF127991, EU881899, EU881905	*Eucalyptus* sp.	Colombia
C. obpyriformis	CMW23807, CBS122608	EU245004, EU244976, EU244936	*Acacia mearnsii*	South Africa
C. obpyriformis	CMW23808, CBS122511	EU245003, EU244975, EU244935	*Acacia mearnsii*	South Africa
C. papillata	CMW8857	AY233868, AY233878, EU241483	*Annona muricata*	Colombia
C. papillata	CMW8856, CBS121793	AY233867, AY233874, EU241484	Citrus lemon	Colombia
C. papillata	CMW10844	AY177238, AY177229, EU241481	*Coffea arabica*	Colombia
C. pirilliformis	CMW6569	AF427104, DQ371652, AY528982	*Eucalyptus nitens*	Australia
C. pirilliformis	CMW6579, CBS118128	AF427105, DQ371653, AY528983	*Eucalyptus nitens*	Australia
C. platani	CMW14802, CBS115162	DQ520630, EF070425, EF070396	*Platanus occidentalis*	USA
C. platani	CMW23918	EF070426, EF070397, EU426554	*Platanus* sp.	Greece
C. polychroma	CMW11424, CBS115778	AY528970, AY528966, AY528978	*Syzygium aromaticum*	Indonesia
C. polychroma	CMW11436, CBS115777	AY528971, AY528967, AY528979	*Syzygium aromaticum*	Indonesia
C. polyconidia	CMW23809, CBS122289	EU245006, EU244978, EU244938	*Acacia mearnsii*	South Africa
C. polyconidia	CMW23818, CBS122290	EU245007, EU244979, EU244939	*Acacia mearnsii*	South Africa
C. populicola	CMW14789, CBS119.78	EF070418, EF070434, EF070406	*Populus* sp.	Poland
C. populicola	CMW14819, CBS114725	EF070419, EF070435, EF070407	*Populus* sp.	USA
C. smalleyi	CMW14800, CBS114724	EF070420, EF070436, EF070408	*Carya cordiformis*	USA
C. smalleyi	CMW26383, CBS114724	EU426553, EU426555, EU426556	*Carya cordiformis*	USA
C. tanganyicensis	CMW15991, CBS122295	EU244997, EU244969, EU244929	*Acacia mearnsii*	Tanzania
C. tanganyicensis	CMW15999, CBS122294	EU244998, EU244970, EU244939	*Acacia mearnsii*	Tanzania
C. tsitsikammensis	CMW14276, CBS121018	EF408555, EF408569, EF408576	*Rapanea melanophloeos*	South Africa
C. tsitsikammensis	CMW14278, CBS121019	EF408556, EF408570, EF408577	*Rapanea melanophloeos*	South Africa
C. variospora	CMW20935, CBS114715	EF070421, EF070437, EF070409	*Quercus alba*	USA
C. variospora	CMW20936, CBS114714	EF070422, EF070438, EF070410	*Quercus robur*	USA
C. virescens	CMW11164	DQ520639, EF070441, EF070413	*Fagus americanum*	USA

Table 1. (Continued).

Species	Isolate no.	GenBank accession no.	Host	Area
C. virescens	CMW3276	AY528984, AY528990, AY529011	*Quercus robur*	USA
C. zombamontana	CMW15235	EU245002, EU244974, EU244934	*Eucalyptus* sp.	Malawi
C. zombamontana	CMW15236	EU245000, EU244972, EU244932	*Eucalyptus* sp.	Malawi
***Ceratocystis* sp.**	**CMW4797**	**FJ236733, FJ236793, FJ236763**	***Eucalyptus* sp.**	**Congo**
***Ceratocystis* sp.**	**CMW4799**	**FJ236734, FJ236794, FJ236764**	***Eucalyptus* sp.**	**Congo**
***Ceratocystis* sp.**	**CMW4902**	**FJ236715, FJ236775, FJ236745**	***Eucalyptus* sp.**	**Brazil**
***Ceratocystis* sp.**	**CMW5312**	**FJ236731, FJ236791, FJ236761**	***Eucalyptus* sp.**	**Uganda**
***Ceratocystis* sp.**	**CMW5313**	**FJ236732, FJ236792, FJ236762**	***Eucalyptus* sp.**	**Uganda**
***Ceratocystis* sp.**	**CMW7764**	**FJ236726, FJ236786, FJ236756**	***Eucalyptus* sp.**	**Uruguay**
***Ceratocystis* sp.**	**CMW7765**	**FJ236727, FJ236787, FJ236757**	***Eucalyptus* sp.**	**Uruguay**
***Ceratocystis* sp.**	**CMW7766**	**FJ236728, FJ236788, FJ236758**	***Eucalyptus* sp.**	**Uruguay**
***Ceratocystis* sp.**	**CMW7767**	**FJ236729, FJ236789, FJ236759**	***Eucalyptus* sp.**	**Uruguay**
***Ceratocystis* sp.**	**CMW7768**	**FJ236730, FJ236790, FJ236760**	***Eucalyptus* sp.**	**Uruguay**
***Ceratocystis* sp.**	**CMW14631**	**FJ236744, FJ236804, FJ236774**	***Eucalyptus* sp.**	**Indonesia**
***Ceratocystis* sp.**	**CMW14632**	**FJ236743, FJ236803, FJ236773**	***Eucalyptus* sp.**	**Indonesia**
***Ceratocystis* sp.**	**CMW16008**	**FJ236735, FJ236795, FJ236765**	***Eucalyptus* sp.**	**Thailand**
***Ceratocystis* sp.**	**CMW16009**	**FJ236736, FJ236796, FJ236766**	***Eucalyptus* sp.**	**Thailand**
***Ceratocystis* sp.**	**CMW16010**	**FJ236737, FJ236797, FJ236767**	***Eucalyptus* sp.**	**Thailand**
***Ceratocystis* sp.**	**CMW16034**	**FJ236739, FJ236799, FJ236769**	***Eucalyptus* sp.**	**Thailand**
***Ceratocystis* sp.**	**CMW16035**	**FJ236738, FJ236798, FJ236768**	***Eucalyptus* sp.**	**Thailand**
***Ceratocystis* sp.**	**CMW18572**	**FJ236740, FJ236800, FJ236770**	***Eucalyptus* sp.**	**Indonesia**
***Ceratocystis* sp.**	**CMW18577**	**FJ236742, FJ236802, FJ236772**	***Eucalyptus* sp.**	**Indonesia**
***Ceratocystis* sp.**	**CMW18591**	**FJ236741, FJ236801, FJ236771**	***Eucalyptus* sp.**	**Indonesia**

Table 2. The number of differences observed between the sequences of the isolates from *Eucalyptus* (*C. fimbriata s. lat.*) from Brazil, South Africa, Uruguay, Uganda, Congo, Thailand, Indonesia, and *C. colombiana*.

Country	Brazil	South Africa	Uruguay	Uganda	Congo	Thailand	Indonesia	*C. colombiana*
Gene region								
ITS								
Brazil	–	9	0	6	13	0	0	23
South Africa	9	**8**	6	6	0	4	9	21
Uruguay	0	6	**4**	7	9	0	0	21
Uganda	6	6	7	**0**	9	0	7	28
Congo	13	0	9	9	**0**	6	11	25
Thailand	0	4	0	0	6	**7**	0	20
Indonesia	0	9	0	7	11	0	**1**	22
C. colombiana	23	21	21	28	25	20	22	**1**
βt								
Brazil	–	0	0	0	0	0	0	3
South Africa	0	**1**	0	0	0	0	0	3
Uruguay	0	0	**0**	0	0	0	0	3
Uganda	0	0	0	**0**	0	0	0	3
Congo	0	0	0	0	**0**	0	0	3
Thailand	0	0	0	0	0	**0**	0	3
Indonesia	0	0	0	0	0	0	**0**	3
C. colombiana	3	3	3	3	3	3	3	**0**
TEF								
Brazil	–	13	9	12	12	12	12	21

Table 2. (Continued).

Country / Gene region	Brazil	South Africa	Uruguay	Uganda	Congo	Thailand	Indonesia	*C. colombiana*
South Africa	13	**7**	0	0	0	0	0	8
Uruguay	9	9	**9**	0	0	0	0	7
Uganda	12	0	0	**7**	0	0	0	6
Congo	12	0	0	0	**0**	0	0	8
Thailand	12	0	0	0	0	**1**	0	8
Indonesia	12	0	0	0	0	0	**5**	8
C. colombiana	*21*	*8*	*7*	*6*	*8*	*8*	*8*	**0**

measurements of each structure and these are presented in this study as; (minimum-) stdv minus the mean – stdv plus the mean (-maximum).

RESULTS

Isolates

Twenty-five isolates obtained from CMW that had been isolated from *Eucalyptus* trees were included in this study (Table 1). Fifteen of these originated from natural or artificially induced wounds on trees in three countries, South Africa, Thailand, and Indonesia. In addition, ten of the isolates were from trees that are believed to have been killed by the fungus. The latter isolates were from Brazil, Congo, Uganda, and Uruguay.

PCR and sequencing reactions

Results were obtained for three separate datasets. The first provided a broad phylogenetic placement (i.e. Latin American or North American, Asian, and African clade) of the *C. fimbriata s. lat.* isolates from *Eucalyptus*. A more focussed analysis determined whether these isolates could be linked to any of the previously described species in the *C. fimbriata s. lat.* complex that were obtained from *Eucalyptus*. Thereafter, the isolates from *Eucalyptus* apparently representing undescribed species were considered in combined as well as single gene trees generated from the sequence data for these isolates. This was to determine whether they could be grouped based on geographical origin.

Combined gene tree for all described species in the *Ceratocystis fimbriata s. lat.* complex

Amplicons for the three gene regions were on average 500 bp for the ITS and βt-1 gene regions and 800 bp for the TEF1-α region (Table 1). The PHT for the data set including all described species in the *C. fimbriata s. lat.* complex, had a low value (P=0.01), but could be combined (Cunningham 1997).

Of the 1 989 characters in this dataset, 1 102 were constant, 45 were parsimony uninformative while 842 were parsimony informative. One hundred and forty two most parsimonious trees were obtained, of which one was selected for presentation (Fig. 1). The tree topology was as follows: Tree length (TL) = 2054 steps, Consistency Index (CI) = 0.7, Retention Index (RI) = 0.9 and Rescaled Consistency (RC) = 0.6. Phylogenetic analyses revealed a clade specific for the isolates from *Eucalyptus* (Fig. 1). Isolates in this large clade had high bootstrap (88 %) and Bayesian (88 %) support and included some substructure (Fig. 1). The substructure in the large clade for the isolates from *Eucalyptus* was not strongly supported and these isolates were treated as reflecting a single group of genetically related, but not identical isolates. The closest phylogenetic relative of the isolates in the *Eucalyptus* clade was *C. colombiana* (van Wyk *et al.* 2010a).

The models obtained using MrModeltest2 were the HKY+I+G model for both the ITS and the TEF1-α genes and the GTR+G model for the βt-1 gene region. Including these models in the Bayesian analyses resulted in a burnin of 7000. These 7000 trees were discarded from the final analyses. The posterior probabilities obtained with the Bayesian analyses supported the bootstrap values obtained in PAUP (Fig. 1).

Combined and separate gene trees for undescribed *Ceratocystis fimbriata s. lat.* isolates from *Eucalyptus*

In the dataset for the combined gene regions, there were 1 765 characters of which 1 680 were constant, 31 were parsimony uninformative while 54 were parsimony informative. Twenty-four most parsimonious trees were obtained, one of which was selected for presentation (Fig. 2). The tree topology was as follows: TL = 107 steps, CI = 0.8, RI = 0.9 and RC = 0.7. One well-supported clade (100 % bootstrap, 100 % Bayesian) was observed with high variation. Three clades that were supported within this large clade were also observed (Fig. 2). The models obtained for this dataset were the HKY model for the ITS gene, the F81 model for the βt-1 gene region and the HKY+I model for the TEF1-α gene region. A burn-in of 1000 was obtained and these 1000 trees were discarded from the final analyses. The posterior probabilities obtained with the Bayesian analyses supported the bootstrap values obtained with PAUP (Fig. 2).

Three well-supported clades were observed; the first included Asian (Indonesia and Thailand) and South American (Brazil and Uruguay) isolates; the second clade included African (Republic of Congo and South Africa) isolates while the third clade included African (Uganda) and Asian (Thailand) isolates. The previously described species, *C. colombiana*, grouped apart from these three clades (Fig. 2).

Where the data were treated separately, the trees for the ITS, βT and TEF1-α gene regions had a different topology when compared with those for the combined gene regions

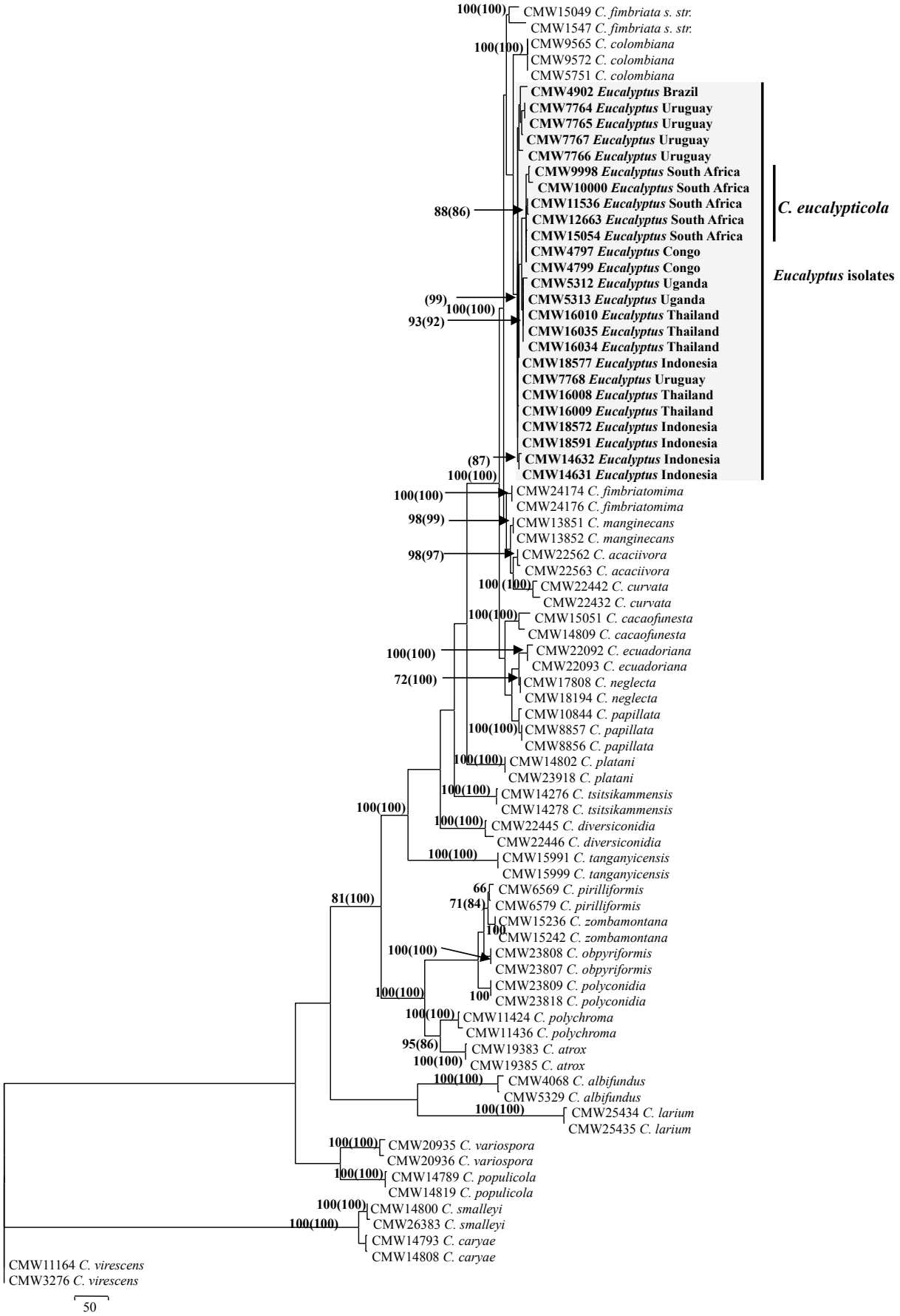


Fig. 1. Phylogenetic tree based on the combined sequences of the ITS, βt and TEF1-α gene regions for isolates from *Eucalyptus* including those provided the name *C. eucalypticola* and other described species in the *C. fimbriata s. lat.* complex. *Ceratocystis virescens* represents the outgroup taxon. Bootstrap values are indicated at the branch nodes and Bayesian values in brackets.

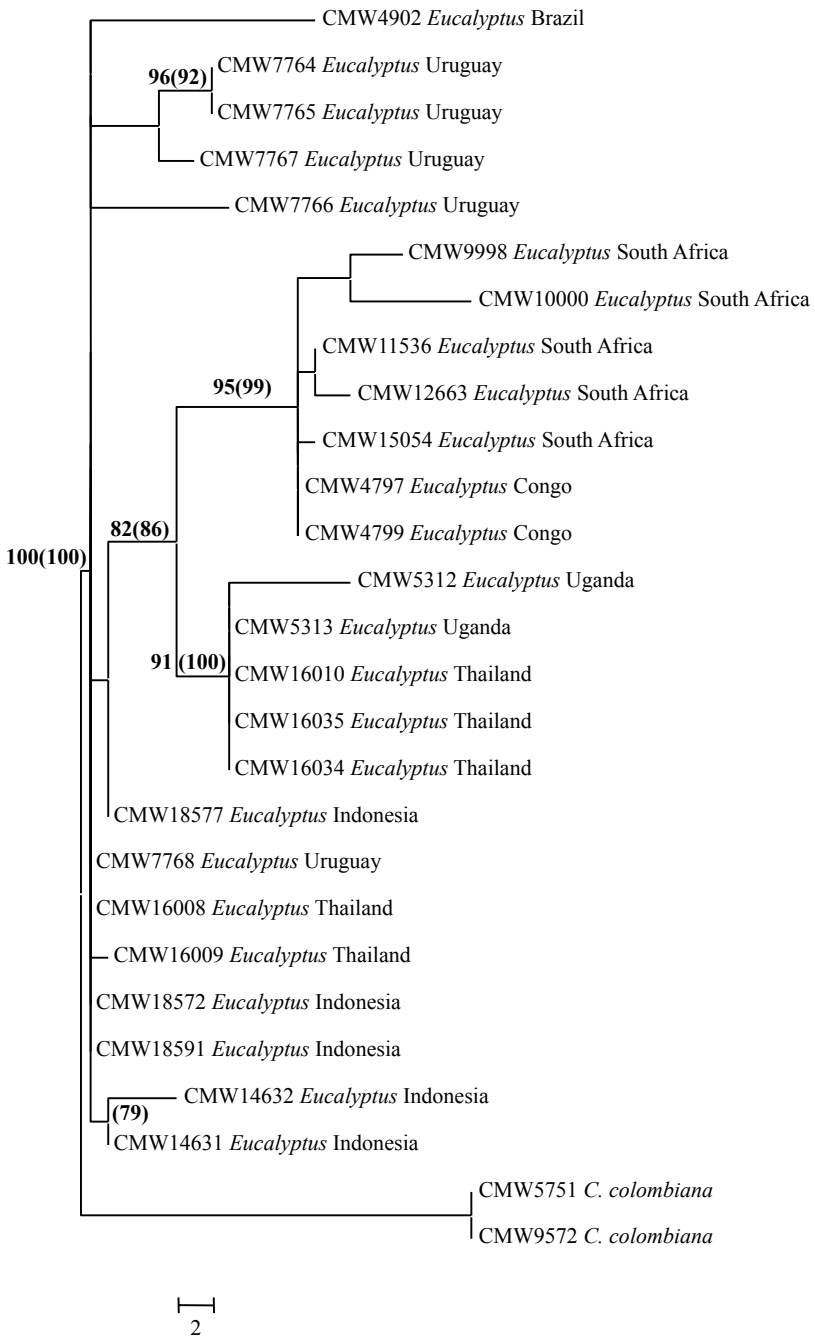


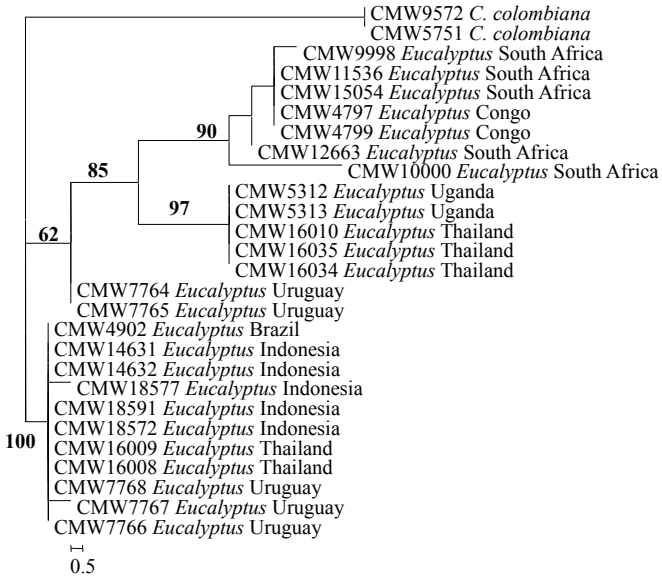
Fig. 2. A phylogenetic tree for the combined sequences of the ITS, βt and EF1-α gene regions, including only the undescribed *C. fimbriata s. lat.* isolates with *Eucalyptus* as their host. The closely related species, *C. colombiana*, is included as outgroup. Bootstrap support is indicated at the branch nodes while Bayesian support is indicated in brackets.

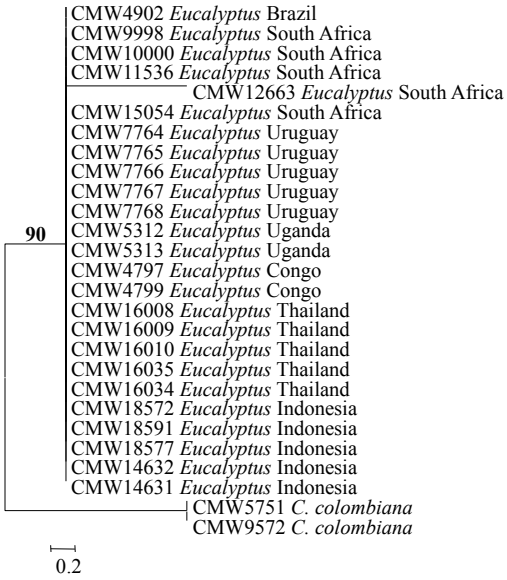
(Fig. 3). For the ITS gene tree, the same three clades emerged as in the combined dataset and included those for Asian and South American isolates, the African isolates and the African together with Asian isolates. However, only the African and Asian clade had strong support (97 %), the other two clades, African (63 %) and the Asian/ South American (55 %) clades had weak support (Fig. 3). In the case of the βt-1 gene tree, there was no support and all branches collapsed (Fig. 3). For the TEF1-α gene tree, there were two small clades encompassing the South African isolates that had high and medium support (85 % and 65 % respectively), while the rest of the isolates grouped in a single clade with strong (85 %) support (Fig. 3).

Where data for the *C. fimbriata s. lat.* isolates were analysed in MEGA, the results showed that in the ITS gene region, the *C. fimbriata s. lat.* isolates obtained from *Eucalyptus* were separated from *C. colombiana* by an average of 23 nucleotide differences (Table 2). Where isolates from different countries were compared, there was also variation in the ITS with a maximum of 13 bp and average of 5 bp differences (Table 2).

Where isolates of *C. fimbriata s. lat.* from *Eucalyptus* were compared with *C. colombiana* in the βt-1 gene region, there were only 3bp differences between them (Table 2). Within the clade representing the *C. fimbriata s. lat.* group from *Eucalyptus*, there was only one base pair difference observed in the South African group and no differences between isolates from different countries (Table 2).

For the TEF1-α gene region, there were 21bp differences between the isolate from Brazil and *C. colombiana* and an average of 8 bp differences between *C. colombiana* and the other isolates from *Eucalyptus*. Only the single isolate from





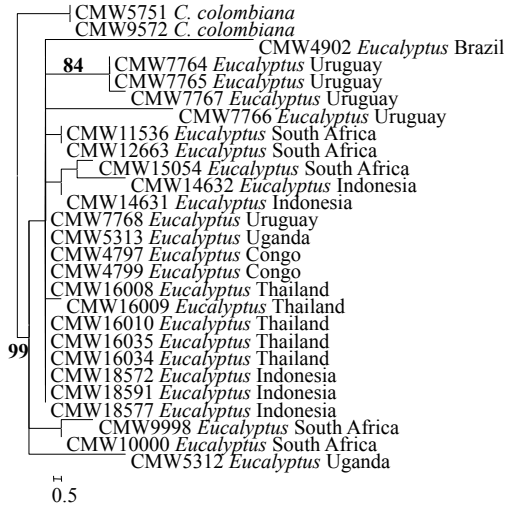


Fig. 3. Three phylograms each representing a single gene region (ITS, βt and TEF-1α, top to bottom) for the undescribed isolates from *Eucalyptus* representing *C. fimbriata s. lat.* showing low variation in the three separate gene regions as well as no support for the sub-clades observed in the combined gene trees. No outgroup was assigned to this dataset.

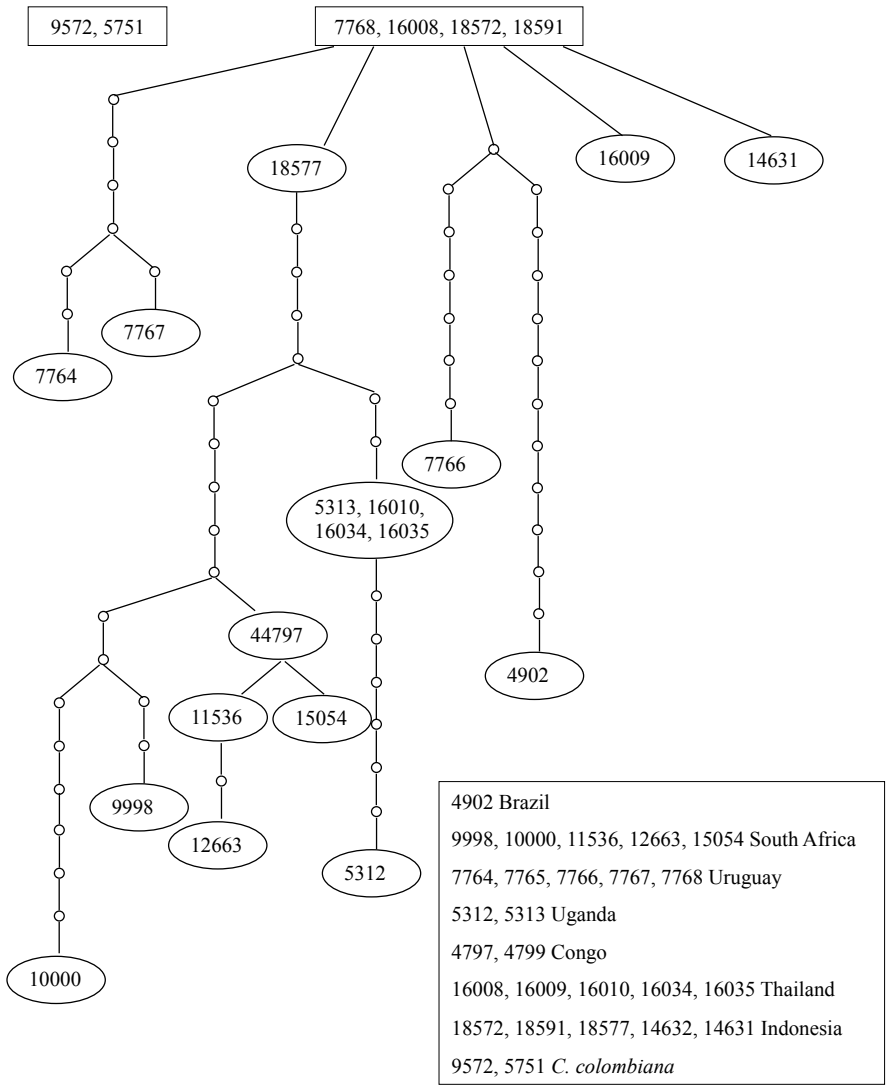


Fig. 4. Allele networks obtained from the three combined gene regions (ITS, βt and TEF1-α for all isolates from *Eucalyptus* as well as *C. colombiana.* The species *C. colombiana* is represented as highly different to the *Eucalyptus* isolates due to the fact that it formed a separate allele tree. The *C. fimbriata s.lat.* isolates from *Eucalyptus* all formed one allele tree with high variation observed within the tree.

Brazil differed from the other isolates while no differences were observed between the isolates from the other countries. The allele networks drawn from the combined gene regions (ITS, βt-1 and TEF1-α) for the *C. fimbriata s. lat.* obtained from *Eucalyptus* revealed a single tree with high variation (Fig. 4). There was no obvious geographic structure with regards to the origin of the eucalypt isolates. The previously described species, *C. colombiana*, formed a separate allele tree (Fig. 4).

Culture characteristics and morphology

All isolates from *Eucalyptus* had a similar greenish olivaceous (33'''f) (Rayner 1970) colony colour. The cultures had a banana odour similar to that of many *Ceratocystis* species.

The cultures all grew optimally at 30 ºC. No clear morphological differences could be observed between isolates from different countries (Table 3).

Isolate CMW 11536 from *Eucalyptus* in South Africa was chosen to represent the global collection of isolates obtained from *Eucalyptus*. Three additional isolates (CMW 9998, CMW 10000 and CMW 15054), also from South Africa, were chosen as additional specimens for description. Cultures of these isolates were grown on 2 % MEA, dried down and have been deposited with the National Collection of Fungi (PREM), Pretoria, South Africa. Living cultures are maintained in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI) at the University of Pretoria, South Africa and the Centraalbureau voor Schimmelcultures (CBS) in Utrecht, The Netherlands.

Where growth in culture was characterised based on the average colony diameter (from the five inoculated plates) for the four selected *Eucalyptus* isolates from South Africa, after 7 d, limited growth was observed at 4 °C (8 mm), 10 °C (7 mm), 15 °C (19 mm) and 35 °C (10 mm). Intermediate growth was observed after 7 d at 20 °C (34 mm) and 25 °C (35 mm), while the optimum temperature for growth in culture was 30 °C at which isolates reached an average of 39 mm diam after 7 d.

TAXONOMY

Isolates of the *Ceratocystis* from *Eucalyptus,* originating from many different countries, were phylogenetically distinct from all other *Ceratocystis* species residing in the *C. fimbriata s. la*t. clade. They also formed distinct phylogenetic groups based on geographic origin and might be found to represent distinct taxa in the future. For the present, those isolates from South Africa, which also had a morphology different to all described species from *Eucalyptus* (Table 4) are described as representing a novel taxon.

Table 3. Morphological comparison of two representative isolates from Indonesia, South Africa, Thailand, and Uruguay. Ten measurements were taken of each structure and the (minimum-) average minus standard deviation – average plus standard deviation and (-maximum) given below.

Characteristic / Country	Indonesia	South Africa	Thailand	Uruguay
Ascomatal bases				
Shape	Globose	Globose	Globose	Globose
Length	(125–)162–199(–200)	(120–)142–190(–202)	(188–)190–197(–200)	(144–)170–197(–200)
Width	(143–)173–193(–200)	(132–)143–193(–216)	(154–)177–199(–212)	(141–)164–184(–197)
Ascomatal necks				
Length	(390–)400–450(–470)	(372–)392–460(–486)	(354–)370–400(–424)	(354–)368–386(–409)
Width (bases)	(24–)25–35(–40)	(24–)25–35(–42)	(24–)25–35(–39)	(23–)26–32(–38)
Width (apices)	(15–)16–18(–20)	(15–)16–20(–22)	(16–)17–19(–20)	(15–)16–22(–25)
Ostiolar hyphae				
Shape	Divergent	Divergent	Divergent	Divergent
Length	(36–)43–53(–63)	(39–)40–52(–62)	(33–)35–39(–41)	(38–)41–51(–53)
Ascospores				
Length	3–5	3–5	3–4	3–4
Width (excluding sheath)	4–6	4–6	4–6	4–6
Width (including sheath)	5–8	5–7(–8)	5–7	6–7
Primary phialides				
Length	(69–)70–100(–134)	(73–)76–114(–131)	(67–)76–96(–100)	(73–)75–83(–88)
Width (bases)	4–6	4–6	4–6	2–4
Width (broadest point)	4–6	4–6	6–8	4–5
Width (apices)	3–5	3–5	3–5	3–4
Secondary phialides				
Length	(60–)70–100(–143)	(64–)69–109(–143)	(63–)68–77(–99)	(69–)72–96(–109)
Width (bases)	3–6	3–6	5–6	3–6
Width (apices)	5–7	5–7	4–8	6–8
Primary conidia				
Length	(13–)19–20(–24)	(15–)18–24(–25)	(10–)13–17(–18)	(10–)11–15(–18)
Width	4–5	4–5	3–4	2–3
Secondary conidia				
Length	6–8	6–8	6–8	(7–)9–11
Width	5–8	5–7	5–8	6–8
Chlamydospores				
Shape	Globose/Subglobose	Globose/Subglobose	Globose/Subglobose	Globose/Subglobose
Length	10–15	10–13	12–15	(6–)7–11(–13)
Width	8–13	8–10	10–13	(5–)7–11(–12)

Ceratocystis eucalypticola M. van Wyk & M.J. Wingf., **sp. nov.**
MycoBank MB512397
(Fig. 5)
Etymology: The name refers to *Eucalyptus* on which the fungus occurs.

All species of *Ceratocystis* from *Eucalyptus* are phylogenetically distinct. Colonies of *C. eucalypticola* are typically green colonies, relatively slow growing, and have a fruity banana odour.

Type: **South Africa**: *Kwa-Zulu Natal*: KwaMbonambi, isolated from artificially wounded *Eucalyptus*, 15 Dec. 2002, *M. van Wyk & J. Roux* (PREM 60168 – holotype; cultures ex-holotype CMW 11536 = CBS 124016)

Description: Ascomatal bases dark brown to black, globose, un-ornamented (105–)140–186(–222) µm wide, (118–)146–184(–216) µm high. *Ascomatal necks* dark brown to black at bases becoming lighter towards the apices, (274–)376–464(–499) µm long, apices (14–)16–20(–22) µm wide, bases (19–)25–33(–42) µm wide. *Ostiolar hyphae* divergent, (39–)45–59(–66) µm long. *Ascospores* hyaline, hat-shaped in side view, invested in sheath, 3–5 µm long, 4–6 µm wide

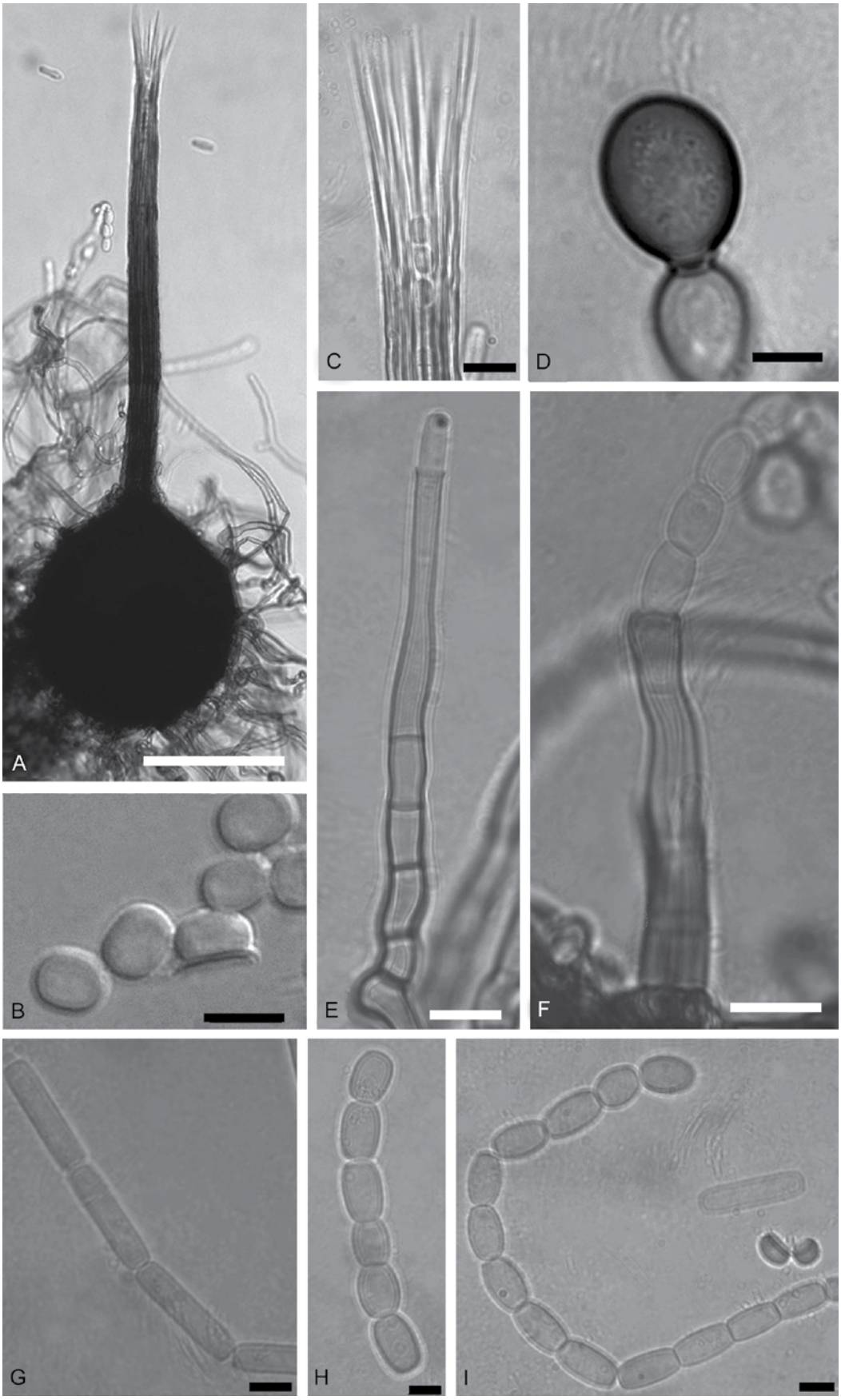


Fig. 5. Morphological characteristics of *Ceratocystis eucalypticola.* **a.** Ascomata with globose base. **b.** Hat-shaped (in side view) and cucullate (in top view) ascospores. **c.** Divergent ostiolar hyphae **d.** Dark, globose to sub-globose chlamydospore. **e.** Primary conidiophore, flask-shaped phialide, producing cylindrical conidia. **f.** Tubular shaped secondary conidiophore, producing a chain of barrel-shaped conidia. **g.** Chain of cylindrical conidia. **h.** Chain of barrel-shaped conidia. **i.** A chain of barrel-shaped conidia, two hat-shaped ascospores and a cylindrical conidium. Bars: **a.** = 100 µm, **b, f–i** = 5 µm, **c–e** = 10 µm.

Table 4. Morphological comparison of previously described species in the *C. fimbriata s. lat.* species complex obtained from *Eucalyptus* trees compared to *C. eucalypticola*.

Character / Species	*C. atrox*	*C. eucalypticola*	*C. fimbriatomima*	*C. neglecta*	*C. colombiana*	*C. pirilliformis*
Ascomatal bases						
Shape	Globose	Globose	Globose	Globose	Globose	Obpyriform
Length	(120–)140–180 (–222)	(105–)140–186 (–222)	(142–)173–215 (–234)	(173–)202–244 (–281)	(140–)177–237 (–294)	145–216(–279)
Width	(120–)150–178 (–200)	(118–)146–184 (–216)	(145–)178–225 (–255)	(153–)178–228 (–250)	(140–)177–237 (–294)	115–186(–206)
Ascomatal necks						
Length	(270–)310–400 (–460)	(274–)376–464 (–499)	(446–)660–890 (–1070)	(691–)745–840 (–889)	(375–)448–560 (–676)	372–683(–778)
Width (bases)	(21–)26–34(–40)	(19–)25–33(–42)	(28–)32–42(–47)	(27–)31–39(–46)	(24–)27–35(–43)	18–33(–40)
Width (apices)	(13–)14–16(–19)	(14–)16–20(–22)	(16–)18–24(–28)	(14–)16–20(–22)	(12–)14–18(–19)	12–21(–25)
Ostiolar hyphae						
Shape	Divergent	Divergent	Divergent	Divergent	Divergent	Convergent
Length	(18–)20–26(–28)	(39–)45–59(–66)	(40–)49–61(–68)	(35–)41–49(–54)	(28–)38–46(–52)	N/A
Ascospores						
Length	3–4	3–5	2–4	3–6	3–4	4–6
Width (excluding sheath)	3–4	4–6	4–6	4–7	(3–)4–6(–7)	3–5
Width (including sheath)	4–6	5–7(–)8	5–7	5–8	6–8(–11)	3–5
Primary phialides						
Length	(78–)87–151(–218)	(58–)77–113(–131)	(49–)60–94(–122)	(75–)80–114(–152)	(58–)65–83(–106)	62–147(–216)
Width (bases)	5–7(–13)	(3–)4–6(–7)	4–7	(4–)5–7(–8)	4–6(–8)	N/A
Width (broadest point)	4–7	4–6(–7)	5–9	5–9	(3–)6–8(–9)	N/A
Width (apices)	4–9	3–5	3–5	(3–)4–6(–7)	3–5(–6)	N/A
Secondary phialides						
Length	(39–)43–57(–66)	(43–)60–100(–143)	Absent	(38–)48–76(–89)	(42–)49–71(–85)	N/A
Width (bases)	5–7(–9)	(3–)4–6(–7)	Absent	(3–)5–7(–8)	(4–)5–7	N/A
Width (apices)	4–6)–7)	(4–)5–7(–8)	Absent	(3–)5–7(–8)	(5–)6–8	N/A
Primary conidia						
Length	(9–)11–15(–17)	(14–)16–22(–25)	(14–)20–28(–31)	(11–)15–27(–30)	(12–)16–24(–29)	12–25(–33)
Width	3–5	3–5	3–5	(3–)5–6	4–6	2–5
Secondary conidia						
Length	(7–)8–12(–14)	(6–)7–9(–12)	Absent	(6–)10–11	9–14	4–6
Width	(5–)6–8(–9)	4–6(–7)	Absent	(4–)5–7(–9)	6–8(–11)	3–5
Chlamydospores						
Shape	Absent	Globose/ Subglobose	Subglobose	Globose	Globose	Oval
Length	Absent	(10–)11–13(–15)	(6–)10–14(–15)	(8–)10–12(–13)	11–14	8–12(–13)
Width	Absent	8–10(–11)	(6–)7–11(–12)	(9–)10–14(–16)	11–15(–17)	5–8(–10)
Reference	Van Wyk *et. al.* 2007	This study	Van Wyk *et. al.* 2008	Rodas *et. al.* 2008	Van Wyk *et. al.* 2010a	Barnes *et. al.* 2003

without sheath, 5–7(–8) µm wide including sheath. *Anamorph* thielaviopsis-like, conidiophores of two types: *Primary conidiophores* phialidic, flask-shaped, (58–)77–113(–131) µm long, (3–)4–6 µm wide at the bases, 4–6(–7) µm wide at broadest points and 3–5 µm wide at apices. *Secondary conidiophores* flaring or wide mouthed, (43–)60–100(–143) µm long, (3–)4–6(–7) µm wide at bases and (4–)5–7(–8) µm wide at apices. *Primary conidia* cylindrical in shape (14–)16–22(–25) µm long, 3–5 µm wide. *Secondary conidia*, barrel-shaped, abundant, (6–)7–9(–12) µm long, 4–6(–7) µm wide. *Chlamydospores*, scarce, hair brown (17””i), globose to sub-globose (10–)11–13(–15) µm long, 8–10(–11) µm wide.

Habitat: Wounded and diseased *Eucalyptus*.

Known distribution: South Africa.

Other material examined: **South Africa**: Mpumalanga, Sabie, isolated from artificially wounded *Eucalyptus* trees, 14 July 2002, *M. van Wyk & J. Roux* (PREM 60169; living cultures CMW 9998 = CBS 124017); *loc. cit.*, isolated from artificially wounded *Eucalyptus* trees, 14 July 2002, *M. van Wyk & J. Roux* (PREM 60170; living cultures CMW 10000 = CBS 124019).

DISCUSSION

Isolates of *Ceratocystis fimbriata s. lat.* collected from *Eucalyptus* in Brazil, Indonesia, Republic of Congo, South Africa, Thailand, Uganda, and Uruguay were shown to be phylogenetically related. These included isolates taken from wounds on trees and also those that were associated with trees dying as result of infection by the fungus. Although all isolates from *Eucalyptus* resided in a single large clade, there was a high degree of diversity among them. It is thus possible that they represent a number of different cryptic species that cannot be resolved. For the present, those isolates from South Africa are provided with the name *C. eucalypticola* here. Future studies should seek to include additional isolates from *Eucalyptus* as well as to include sequences for gene regions not considered in this study, and that might discriminate more clearly between species in the *C. fimbriata s. lat.* complex. Currently, the group is unified based on a specific host and relatively strong phylogenetic similarity. In this respect, it also provides the foundation for further studies including a suite of isolates that would be difficult to obtain.

The species of *Ceratocystis* most closely related to *C. eucalypticola* is *C. colombiana*. *Ceratocystis colombiana* is a pathogen of coffee trees (Marin *et al.* 2003) as well as numerous other hosts including indigenous crops in Colombia. Although the two species are phylogenetically related, they are ecologically distinct and are not likely to be confused.

Ceratocystis eucalypticola is one of a number of species in the *C. fimbriata s. lat.* complex to be described from *Eucalyptus* trees. Other species from this host include; *C. atrox* (van Wyk *et al.* 2007) and *C. corymbiicola* (Kamgan Nkuekam *et al.* 2012) from Australia, *C. pirilliformis* (Barnes *et al.* 2003b) from Australia and South Africa, *C. neglecta* (Rodas *et al.* 2007) from Colombia, *C. fimbriatomima* (van Wyk *et al.* 2008) from Venezuela, and *C. zombamontana* (Heath *et al.* 2009) from Malawi. All of these species from *Eucalyptus* can be distinguished from each other based on phylogenetic inference and they have some morphological features that can be used to recognise them.

Morphologically, the specimens of *C. eucalypticola* cited here resemble species in the *C. fimbriata s. lat.* complex. The fungus has the typical green colony colour, is relatively slow growing, and has a fruity banana odour. *Ceratocystis eucalypticola* can be distinguished from other species in the *C. fimbriata s. lat.* complex in that they occur on *Eucalyptus* and based on differences in size of some diagnostic characters for this group of fungi.

Ceratocystis eucalypticola includes isolates only from wounds on trees in South Africa in the absence of disease, but is very closely related to isolates that originated from dying trees and that have been shown to be pathogenic (Laia *et al.* 1999; Roux *et al.* 2000, 2001, 2004). The species is also closely related to isolates that were collected from wounds on trees in countries other than South Africa where a *Ceratocystis* disease on *Eucalyptus* has not been seen. *Eucalyptus* death associated with *C. eucalypticola* has never been found in South Africa although trees dying of unknown causes are thought to have died due to infection by this fungus, which can be difficult to isolate. The fungus collected from wounds on trees has also been shown to be pathogenic in greenhouse inoculation trials (Roux *et al.* 2004, van Wyk *et al.* 2010b).

Isolates of *C. eucalypticola* from South Africa represent a clonal population (van Wyk *et al.* 2006b) and it was most likely introduced into the country. It is thus intriguing that *Eucalyptus* death associated with this fungus has not been seen. This might be due to planting stock susceptible to *C. eucalypticola* not having occurred in the country, or that conditions for infection were not suitable. Alternatively, it is possible that trees dying of unexplained causes might have been killed by *C. eucalypticola*, even though the fungus was not isolated from them. This is a question that is currently being pursued, particularly linked to unexplained *Eucalyptus* death in South Africa and where *Ceratocystis* cultures emerge from isolations.

ACKNOWLEDGEMENTS

We thank the National Research Foundation (NRF), members of the Tree Protection Co-operative Programme (TPCP), the THRIP initiative of the Department of Trade and Industry and the Department of Science and Technology (DST)/NRF Centre of Excellence in Tree Health Biotechnology (CTHB) for funding.

REFERENCES

Barnes I, Gaur A, Burgess T, Roux J, Wingfield BD, Wingfield MJ (2001) Microsatellite markers reflect intra-specific relationships between isolates of the vascular wilt pathogen, *Ceratocystis fimbriata*. *Molecular Plant Pathology* **2**: 319–325. DOI. org/10.1046/j.1464-6722.2001.00080.x

Barnes I, Roux J, Wingfield BD, O'Neil M, Wingfield MJ (2003a) *Ceratocystis fimbriata* infecting *Eucalyptus grandis* in Uruguay. *Australasian Plant Pathology* **32**: 361–355. DOI.org/10.1071/AP03032

Barnes I, Roux J, Wingfield MJ, Old KM, Dudzinski M (2003b) *Ceratocystis pirilliformis,* a new species from *Eucalyptus nitens* in Australia. *Mycologia* **95**: 865–871. DOI org/10.2307/3762015

Clement M, Posada D, Crandall K (2000) TCS: a computer program to estimate gene genealogies. *Molecular Ecology* **9**: 1657–1660. DOI.org/10.1046/j.1365-294x.2000.01020.x

Cunningham CW (1997) Can three incongruence tests predict when data should be combined? *Molecular Biology and Evolution* **14**: 733-740.

Engelbrecht CJB, Harrington TC (2005) Intersterility, morphology and taxonomy of *Ceratocystis fimbriata* on sweet potato, cacao and sycamore. *Mycologia* **97**: 57–69. DOI.org/10.3852/

mycologia.97.1.57

Feirreira EM, Harrington TC, Thorpe DJ, Alfenas AC (2010) Genetic diversity and interfertility among highly differentiated populations of *Ceratocystis fimbriata* in Brazil. *Plant Pathology* **59**: 721–735. DOI.org/10.1111/j.1365-3059.2010.02275.x

Glass NL, Donaldson GC (1995) Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous Ascomycetes. *Applied and Environmental Microbiology* **61**: 1323–1330.

Heath RN, Wingfield BD, Wingfield MJ, Meke G, Mbaga A, Roux J (2009) *Ceratocystis* species on *Acacia mearnsii* and *Eucalyptus* spp. in eastern and southern Africa including six new species. *Fungal Diversity* **34**: 41–67.

Jacobs K, Bergdahl DR, Wingfield MJ, Halik S, Seifert KA, Bright DE, Wingfield BD (2004) *Leptographium wingfieldii* introduced into North America and found associated with exotic *Tomicus piniperda* and native bark beetles. *Mycological Research* **108**: 411–418. DOI.org/10.1017/S0953756204009748

Johnson JA, Harrington TC, Engelbrecht CJB (2005) Phylogeny and taxonomy of the North American clade of the *Ceratocystis fimbriata* complex. *Mycologia* **97**: 1067–1092. DOI.org/10.3852/mycologia.97.5.1067

Kamgan Nkuekam G, Wingfield MJ, Mohammed C, Carnegie AJ, Pegg GS, Roux J (2012) *Ceratocystis* species, including two new species associated with nitidulid beetles on *Eucalyptus* in Australia. *Antonie van Leeuwenhoek* **101**: 217–241. DOI 10.1007/s10482-011-9625-7.

Katoh K, Misawa K, Kuma K, Miyata T (2002) MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research* **30**: 3059–3066. DOI.org/10.1093/nar/gkf436

Kile GA (1993) Plant diseases caused by species of *Ceratocystis sensu stricto* and *Chalara*. In: *Ceratocystis and Ophiostoma: taxonomy, ecology and pathogenicity* (MJ Wingfield, KA Seifert, JA Webber, eds): 173–183. St Paul, MN: American Phytopathological Society Press.

Laia ML, Alfenas AC, Harrington TC (1999) Isolation, detection in soil, and inoculation of *Ceratocystis fimbriata*, causal agent of wilting, dieback and canker in *Eucalyptus*. In: *Proceedings of the 12th Biennial Conference of the Australasian Plant Pathology Society, 27–30 September* (L Morin, ed): 77. Canberra: Australasian Plant Pathology Society.

Marin M, Castro B, Gaitan A, Preisig O, Wingfield BD, Wingfield MJ (2003) Relationships of *Ceratocystis fimbriata* isolates from Colombian coffee-growing regions based on molecular data and pathogenicity. *Journal of Phytopathology* **151**: 395–400. DOI org/10.1046/j.1439-0434.2003.00738.x

Moller WJ, DeVay JE (1968) Carrot as a species-selective isolation medium for *Ceratocystis fimbriata*. *Phytopathology* **58**: 123–124.

Nylander JAA (2004) *MrModeltest v2.* [Program distributed by the author.] Uppsala: Evolutionary Biology Centre, Uppsala University.

Old KM, Wingfield MJ, Yuan ZQ (2003) *A manual of diseases of Eucalypts in South-East Asia.* Jakarta: Centre for International Forestry Research.

Rayner RW (1970) *A Mycological Colour Chart*. Kew: Commonwealth Mycological Institute.

Rodas C, Roux J, van Wyk M, Wingfield BD, Wingfield MJ (2007) *Ceratocystis neglecta* sp. nov., infecting *Eucalyptus* trees in Colombia. *Fungal Diversity* **28**: 73–84.

Ronquist F, Huelsenbeck JP (2003) *MrBayes 3:* Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**: 1572–1574. DOI.org/10.1093/bioinformatics/btg180

Roux J, Coutinho TA, Munjuni Byabashaija D, Wingfield MJ (2001) Diseases of plantation *Eucalyptus* in Uganda. *South African Journal of Science* **97**: 16–18.

Roux J, van Wyk M, Hatting H, Wingfield MJ (2004) *Ceratocystis* species infecting stem wounds on *Eucalyptus grandis* in South Africa. *Plant Pathology* **53**: 414–421. DOI.org/10.1111/j.0032-0862.2004.01014.x

Roux J, Wingfield MJ (2009) *Ceratocystis* species: emerging pathogens of non-native plantation *Eucalyptus* and *Acacia* species. *Southern Forests* **71**: 115–120. DOI org/10.2989/SF.2009.71.2.5.820

Roux J, Wingfield MJ, Wingfield BD, Bouillett JP, Alfenas AC (2000) A serious new disease of *Eucalyptus* caused by *Ceratocystis fimbriata* in Central Africa. *Forest Pathology* **30**: 175–184. DOI. org/10.1046/j.1439-0329.2000.00202.x

Swofford DL (2002) *PAUP*: phylogenetic analysis using parsimony (*and other methods)*. Version 4. Sunderland, MA:Sinauer Associates.

Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Molecular Biology and Evolution* **24**: 1596–1599. DOI. org/10.1093/molbev/msm092

van Wyk M, Pegg G, Lawson S, Wingfield MJ (2007) *Ceratocystis atrox* sp. nov. associated with *Phoracanthta acanthocera* infestations on *Eucalyptus* in Australia. *Australian Journal of Plant Pathology* **36**: 407–414. DOI.org/10.1071/AP07042

van Wyk M, Roux J, Barnes I, Wingfield BD, Wingfield MJ (2006a) Molecular phylogeny of the *Ceratocystis moniliformis* complex and description of *C. tribilliformis* sp. nov. *Fungal Diversity* **21**: 181–201.

van Wyk M, Van der Merwe NA, Roux J, Wingfield BD, Kamgan GN, Wingfield MJ (2006b) Population biology of *Ceratocystis fimbriata* from *Eucalyptus* trees in South Africa. *South African Journal of Science* **102**: 259–263.

van Wyk M, Wingfield BD, Marin M, Wingfield MJ (2010a) Two new *Ceratocystis* species infecting coffee, cacao, citrus and native trees in Colombia. *Fungal Diversity* **40**: 103–117. DOI. org/10.1007/s13225-009-0005-9

van Wyk M, Wingfield BD, Wingfield MJ (2010b) Comparison of procedures to evaluate the virulence of *Ceratocystis fimbriata sensu lato* isolates from *Eucalyptus* in South Africa. *Southern Forests* **72(2)**: 57–62. DOI.org/10.2989/20702620.2010.507011

van Wyk M, Wingfield BD, Mohali S, Wingfield MJ (2008) *Ceratocystis fimbriatomima,* a new species in the *C. fimbriata sensu lato* complex isolated from *Eucalyptus* trees in Venezuela. *Fungal Diversity* **34**: 173–183.

White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: *PCR Protocols: a sequencing guide to methods and applications* (MA Innis, DH Gelfand, JJ Sninsky, TJ White, eds): 315–322. San Diego: Academic Press.

Wingfield MJ, Slippers B, Hurley BP, Coutinho TA, Wingfield BD, Roux J (2008) Eucalypt pests and diseases: growing threats to plantation productivity. *Southern Forests* **70**: 139–144.DOI. org/10.2989/SOUTH.FOR.2008.70.2.9.537

doi:10.5598/imafungus.2012.03.01.07

IMA FUNGUS · VOLUME 3 · NO 1: 59–79

ARTICLE

Aspergillus section *Versicolores*: nine new species and multilocus DNA sequence based phylogeny

Zeljko Jurjevic[1], Stephen W. Peterson[2], and Bruce W. Horn[3]

[1]EMSL Analytical, Inc., 200 Route 130 North, Cinnaminson, NJ 08077

[2]Bacterial Foodborne Pathogens and Mycology Research Unit, National Center for Agricultural Utilization Research, Agricultural Research Service, U.S. Department of Agriculture, 1815 North University Street, Peoria, Illinois 61604 USA; corresponding author email: Stephen.peterson@ars.usda.gov

[3]National Peanut Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, P.O. Box 509, Dawson, GA 39842-0509

Abstract: β-tubulin, calmodulin, internal transcribed spacer and partial lsu-rDNA, RNA polymerase 2, DNA replication licensing factor *Mcm7*, and pre-rRNA processing protein *Tsr1* were amplified and sequenced from numerous isolates belonging to *Aspergillus* sect. *versicolor*. The isolates were analyzed phylogenetically using the concordance model to establish species boundaries. *Aspergillus austroafricanus, A. creber, A. cvjetkovicii, A. fructus, A. jensenii, A. puulaauensis, A. subversicolor, A. tennesseensis* and *A. venenatus* are described as new species and *A. amoenus, A. protuberus, A. sydowii, A. tabacinus* and *A. versicolor* are accepted as distinct species on the basis of molecular and phenotypic differences. PCR primer pairs used to detect *A. versicolor* in sick building syndrome studies have a positive reaction for all of the newly described species except *A. subversicolor*.

Key words:
Aspergillus amoenus
Aspergillus austroafricanus
Aspergillus creber
Aspergillus cvjetkovicii
Aspergillus fructus
Aspergillus jensenii
Aspergillus protuberus
Aspergillus puulaauensis
Aspergillus subversicolor
Aspergillus sydowii
Aspergillus tabacinus
Aspergillus tennesseensis
Aspergillus venenatus
Aspergillus versicolor
concordance analysis
phylogeny
systematics

Article info: Submitted: 17 January 2012; Accepted: 7 June 2012; Published: 21 June 2012.

INTRODUCTION

Aspergillus section *Versicolores* was originally erected as the *Aspergillus versicolor* group by Thom & Church (1926) and was subsequently revised by Thom & Raper (1945) to contain four species. Raper & Fennell (1965) revised the genus *Aspergillus* and accepted 18 species in the *A. versicolor* group. Gams *et al.* (1985) formalized the sectional taxonomy of Raper & Fennell's (1965) groups. Using scanning electron microscopy (SEM), Kozakiewicz (1989) examined conidial surface ornamentation of most species of the section and removed seven species from section *Versicolores*. Klich (1993) revised the section based on morphological and other characteristics and accepted the seven species previously removed by Kozakiewicz (1989) from section *Versicolores*. Peterson (2008) accepted four phylogenetically distinct species in the section based on multilocus DNA sequence analysis, placing the other 14 species in different clades of *Aspergillus*.

Aspergillus versicolor is the most widely reported and studied species in section *Versicolores*. It has been isolated from soil (Domsch *et al.* 1980), indoor environments (Samson *et al.* 2001, Shelton *et al*. 2002, Engelhart *et al*. 2002, Amend *et al.* 2010, Anderson *et al.* 2011), various foods and feeds (Pitt & Hocking 2009) and hypersaline water (Kis-Papo *et al*. 2003, Mbata 2008), and is associated with many health issues of humans and animals (Jussila 2003, Perri *et al*. 2005, Baddley *et al*. 2009, Edmondson *et al*. 2009, Pitt & Hocking 2009, Moreno & Arenas 2010). It is a producer of the mycotoxin sterigmatocystin that is a precursor of aflatoxin B_1 (Mills & Abramson 1986, Tuomi *et al*. 2000, Nielsen 2003, Veršilovskis & Saeger 2010).

Environmental isolates of section *Versicolores* species exhibit great variation in macro-phenotypic ones but few differences in micro-phenotypic characters (Domsch *et al.* 1980, Klich 2002, Raper & Fennell 1965, Thom & Church 1926, Vesonder & Horn 1985), leading us to conduct a DNA-based phylogenetic study. to determine the limits of

variation within species, we amplified and sequenced DNA from 6 loci and used concordance analysis to identify species boundaries (Dettman *et al*. 2003) within section *Versicolores*. The species described and accepted are monophyletic.

MATERIALS AND METHODS

Fungal isolates

The provenance of fungal isolates examined in this study is detailed in Table 1 and these cultures are available from the Agricultural Research Service Culture Collection (NRRL), Peoria, Illinois (http://nrrl.ncaur.usda.gov).

Culture methods

Cultures were grown on Czapek yeast extract agar (CYA) at 5 °C, 25 °C, and 37 °C and on malt extract agar (MEA), CY20S, M40Y and M60Y, all at 25 °C for 10 d in darkness (Pitt 1980, Klich 2002). M40Y contained 2 % malt extract, 0.5 % yeast extract and 40 % sucrose; M60Y contained 2 % malt extract, 0.5 % yeast extract and 60 % sucrose. Colony diameters and appearance were recorded and photographs were made from 10-d culture plates incubated at 25 °C. Color names are from Ridgway (1912) and are referred to with plate number, e.g. R45.

Microscopy

Microscopic examination was performed by teasing apart a small amount of mycelium in a drop of 0.1 % Triton X-100 and examining the preparation under bright field or DIF illumination. Additional microscopic samples were made by gently pressing a *ca* 20 × 5 mm piece of transparent tape onto a colony, rinsing the tape with one or two drops of 70 % ethanol and mounting the tape in lactic acid with fuchsin dye. A Leica DM 2500 microscope with bright field, phase contrast and DIF contrast optics was used to view the slides. The Spot camera with spot imaging software was mounted on the microscope and used for photomicrography. A Nikon digital SLR camera with D70 lens was used for colony photography. Photographs were resized and fitted into plates with Microsoft PowerPoint 2003 or Adobe Photoshop.

DNA methods

Conidia from agar slant cultures were used to inoculate 125-mL Erlenmeyer flasks containing 25 mL of malt extract broth. Cultures were grown on a rotary platform (200 rpm) for 2–3 d at 25 °C. Biomass was collected by vacuum filtration, and then frozen and freeze-dried in microfuge tubes. Dry mycelium was ground to a powder, rehydrated with CTAB buffer and extracted with chloroform; the phases were separated by centrifugation and DNA was precipitated from the aqueous phase with an equal volume of isopropanol. Total nucleic acids were collected by centrifugation, the pellet was rinsed with 70 % ethanol, and the nucleic acids were dissolved in 100 µL sterile deionized water.

DNA was diluted *ca* 1:100 with sterile deionized water for use in amplifications. β-tubulin (*BT2*), calmodulin (*CF*), ITS and partial lsu-rDNA (*ID*), RNA polymerase 2 (*RPB2*), DNA replication licensing factor (*Mcm7*), and pre-rRNA processing protein (*Tsr1*) were amplified with primers used by Peterson *et al*. (2010). Standard buffer and conditions were used with a thermal profile of 95 °C for 2 min followed by 35 cycles of 96 °C for 30 sec; 51 °C for 60 sec; 72 °C for 60 sec; and a final extension phase of 72 °C for 5 min. Occasionally, multiple amplification bands were obtained and a higher annealing temperature was used to obtain single amplification bands. DNA sequencing was performed on both template strands using dye terminator technology (v3.1) and an ABI 3730 sequencer, both from Applied Biosystems (http://www.appliedbiosystems.com/). Raw sequences (bi-directional) were corrected using Sequencher (http://www.genecodes.com/). Corrected sequences were aligned for phylogenetic analysis using CLUSTALW (Thompson *et al.* 1994). Sequences were deposited in GenBank as accessions JN853798–JN854131, EF652176, EF652178, EF652185–EF652187, EF652196, EF652203, EF652209–EF652211, EF652214–EF652216, EF652226, EF652264, EF652266, EF652273–EF652275, EF652284, EF652291, EF652297–EF652299, EF652302–EF652304, EF652314, EF652352, EF652354, EF652361–EF652363, EF652372, EF652379, EF652385–EF652387, EF652390–EF652392, EF652402, EF652440, EF652442, EF652449–EF652451, EF652460, EF652467, EF652473–EF652475, EF652478–EF652480, EF652490 and JQ301889–JQ301896.

Parsimony analysis was conducted using PAUP* 4.0b10 (Swofford 2003). For single-locus data sets, the criterion was parsimony, addition order was random (5000 replications), branch swapping was NNI (nearest neighbor interchange) and max trees was set at 5000. The set of trees generated was used as the starting point for parsimony analysis with addition order “as is” and TBR branch swapping. Bootstrap analysis was conducted with “as is” addition order and TBR branch swapping for 1000 replications.

Bayesian posterior probabilities were calculated using MrBayes 3.12 (Huelsenbeck & Ronquist 2001, Ronquist & Huelsenbeck 2003). The *Mcm7, Tsr1* and *RPB2* data sets included only protein-coding sequences and each data set was partitioned into codon positions 1, 2, and 3. The *BT2* and *CF* loci included protein-coding and intron regions and the data were partitioned into intron and exon data. A GTR (general time-reversible) model was used with a proportion of invariant sites and a gamma-shaped distribution of rates across the sites. Markov chain Monte Carlo (MCMC) analysis was conducted for up to 5 × 10^6 generations until the chains converged.

Concordance analysis was based on the exclusionary principle of Baum & Shaw (1995) and the genealogical concordance phylogenetic species recognition concepts of Taylor *et al.* (2000). Clades were recognized as independent evolutionary lineages if 1) the clade was present in the majority of single-locus genealogies (majority rule consensus) or 2) if a clade was strongly supported by both parsimony and Bayesian analysis in at least one locus, and was not contradicted by another strongly supported locus (Dettman *et al.* 2003). Strong support was assessed as >70 % bootstrap and >0.95 posterior probability (Dettman *et al.* 2003).
The primers used for identification of *A. versicolor* in a PCR amplification (Dean *et al*. 2005) were tested using the primer sequences and amplification thermal profile recommended, but in a uniplex rather than multiplex amplification system (Dean *et al*. 2005).

Table 1. Provenance of fungal isolates used.

NRRL number	Provenance
Aspergillus amoenus MycoBank MB250654	
226	USA: isol. ex mammary gland, 1913.
236	Germany: Munster, isol. ex a *Berberis* sp. fruit, 1930, *M. Roberg*.
658	UK: isol. ex brined meat, 1929, *G. A. Ledingham*.
4838	Equivalent to NRRL 236, received from Centraalbureau voor Schimmelcultures, 1962, ex-type.
35600	USA: Hawaii, Kapuka Pauula, isol. ex the basidiomata of *Gandoderma australe*, 2005, *D.T. Wicklow*.
A-23228	India: Karnataka, isol. ex coffee berry, 1978, *B. Muthappa*.
Aspergillus asperescens Stolk MycoBank MB292835	
4770	Ex-type, out-group species.
Aspergillus austroafricanus **sp. nov.,** MycoBank MB800597	
233	South Africa: Capetown, unknown, 1922, sent by *V. A. Putterill*, ex-type.
Aspergillus creber **sp. nov.,** MycoBank MB800598	
231	South Africa: Capetown, unknown, 1922, sent by *V. A. Putterill*.
6544	Atlantic Ocean: isol. ex a floating tar ball, 1979, *A. Wellman*.
25627	Japan: Ibaraki, isol. ex tea field soil, 1996, *T. Goto*.
58583	USA: Pennsylvania, isol. ex indoor air sampler, 2008, *Z. Jurjevic*.
58584	USA: California, isol. ex indoor air sample, 2008, *Z. Jurjevic*.
58587	USA: California, isol. ex indoor air sample, 2008, *Z. Jurjevic*.
58592	USA: California, isol. ex indoor air sample, 2008, *Z. Jurjevic*, ex-type.
58597	USA: New Jersey, isol. ex indoor air sample, 2008, *Z. Jurjevic*.
58601	USA: New Jersey: isolated from indoor air sample, 2009, *Z. Jurjevic*.
58606	USA: Pennsylvania, isol. ex indoor air sample, 2009, *Z. Jurjevic*.
58607	USA: Pennsylvania, isol. ex indoor air sample, 2009, *Z. Jurjevic*.
58612	USA: New Jersey, isol. ex indoor air sample, 2009, *Z. Jurjevic*.
58670	USA: New Jersey, isol. ex indoor air sample, 2009, *Z. Jurjevic*.
58672	USA: Georgia, isol. ex indoor air sample, 2009, *Z. Jurjevic*.
58673	USA: Georgia, isol. ex indoor air sample, 2009, *Z. Jurjevic*.
58675	USA: Ohio, isol. ex indoor air sample, 2009, *Z. Jurjevic*.
Aspergillus cvjetkovicii **sp. nov.,** MycoBank MB800599	
227	USA: New Jersey, isol. ex soil, 1915, *G.W. Wilson*, ex-type.
230	China: isol. ex soy sauce, 1917, *Round*.
4642	Unknown: sent to NRRL, 1969, *D. I. Fennell* as WB4642.
58593	USA: California, isol. ex indoor air sample, 2008, *Z. Jurjevic*.
Aspergillus fructus **sp. nov.,** MycoBank MB800600	
239	USA: California, isol. ex date fruit, 1939, *Bliss*, ex-type.
241	Unknown: isol. ex pomegranate fruit, 1916, *L. McCulloch*.
Aspergillus jensenii **sp. nov.,** MycoBank MB800601	
225	UK: unknown, 1913, sent to C. Thom by *Dade*.
235	UK: London, isol. ex paraffin, 1930, *H. Raistrick*.
240	USA: New York, Ithaca, isol. ex the rhizosphere of pepper plants, 1911, *C. N. Jensen,* sent to C. Thom by Whetzel as type strain of *A. globosus*.
58582	USA: Montana, isol. ex indoor air sample, 2008, *Z. Jurjevic*.
58600	USA: Montana, isol. ex indoor air sample, 2008, *Z. Jurjevic*, ex-type.
58671	USA: Pennsylvania, isol. ex indoor air sample, 2009, *Z. Jurjevic*.
58674	USA: Ohio, isol. ex indoor air sample, 2009, *Z. Jurjevic*.
Aspergillus multicolor Sappa MycoBank MB292849	
4775	Ex-type, out-group species.

Table 1. (Continued).

NRRL number	Provenance
Aspergillus protuberus MycoBank MB326650	
661	UK: isol. ex brined meat, 1929, *G. A. Ledingham*.
3505	Yugoslavia, isol. ex rubber coated electrical cables, ca 1968, ex-type.
58613	USA: New Jersey, isol. ex indoor air sample, 2009, *Z. Jurjevic*.
58747	USA: New Jersey, isol. ex indoor air sample, 2009, *Z. Jurjevic*.
58748	USA: New Jersey, isol. ex indoor air sample, 2009, *Z. Jurjevic*.
58990	USA: Connecticut, isol. ex indoor air sample, 2009, *Z. Jurjevic*.
58991	USA: Connecticut, isol. ex indoor air sample, 2009, *Z. Jurjevic*.
Aspergillus puulaauensis **sp. nov.,** MycoBank MB800602	
35641	USA: Hawaii, Pu'u la'au Highway 200, isol. ex dead hardwood branch, 2003, *D. T. Wicklow*, ex-type.
58602	USA: West Virginia, isol. ex indoor air sample, 2009, *Z. Jurjevic*.
62124	USA: Hawaii, mesic mountain forest, isol. ex basidiomata of *Inonotus* sp., 2003, *D. T. Wicklow*.
62516	Canada: Alberta, isol. ex air sample in bee house, *ca* 1990, *S. P. Abbot,* equivalent to UAMH 7651.
Aspergillus subversicolor **sp. nov.,** MycoBank MB800603	
58999	India: Karnataka, isol. ex coffee berry, 1970, *B. Muthappa*, ex-type.
Aspergillus sydowii MycoBank MB279636	
250	Unknown: prior to 1930, sent to C. Thom by *M. Swift*.
254	USA: Georgia, Waycross, clinical isolate, 1940, *M. M. Harris*.
4768	USA: California, isol. ex soil, 1969.
62450	Thailand: isol. ex dead plant stem, 1977, *E. G. Simmons*.
Aspergillus tabacinus MycoBank MB539544	
659	UK: isol. ex brined meat, 1929, *G. A. Ledingham*.
4791	Unknown: isol. ex tobacco, 1934, *Y. Nakazawa*, ex-type.
5031	Unknown: type isolate of *A. versicolor* var. *magnus* Sasaki, received from IFO, 1962.
62481	Nepal: Kathmandu, isol. ex maize, 1977.
Aspergillus tennesseensis **sp. nov.,** MycoBank MB800604	
229	Unknown: sent to C. Thom, 1917, by *R. Thaxter*.
234	USA: Maryland, Beltsville, isol. ex chestnut seed, 1927, *C. Thom*.
13150	USA: Tennessee, isol. ex toxic dairy feed, 1984, *B. W. Horn*, ex-type.
13152	USA: Tennessee, isol. ex toxic dairy feed, 1984, *B. W. Horn*.
Aspergillus venenatus **sp. nov.,** MycoBank MB800605	
13147	USA: Tennessee: isolated from toxic dairy feed, 1984, *B. W. Horn*, ex-type.
13148	USA: Tennessee, isol. ex toxic dairy feed, 1984, *B. W. Horn*.
13149	USA: Tennessee, isol. ex toxic dairy feed, 1984, *B. W. Horn*.
62457	USA: Missouri, isol. ex corn, 1989, *D. T. Wicklow*.
Aspergillus versicolor MycoBank MB172159	
238	USA: isol. ex unrecorded substrate, 1935, *V. K. Charles*, ex-type.
5219	South Africa: Pretoria, received 1970, from *J. P. van der Walt*.
13144	USA: Tennessee, isol. ex toxic dairy feed, 1984, *B. W. Horn*.
13145	USA: Tennessee, isol. ex toxic dairy feed, 1984, *B. W. Horn*.
13146	USA: Tennessee, isol. ex toxic dairy feed, 1984, *B. W. Horn*.
Aspergillus species, undescribed	
530	East Indies: isol. ex natural rubber, 1938, *Shumard*.
13151	USA: Tennessee: isol. ex toxic dairy feed, 1984, *B. W. Horn*.

RESULTS

Phylogenic analysis of sequence data

Sixteen independent evolutionary lineages were detected using both criteria for concordance (Dettman *et al.* 2003). The accepted species (Peterson 2008) *A. versicolor, A. tabacinus, A. amoenus, A. protuberus* and *A. sydowii* each were identified as independent lineages (Fig. 1). Four

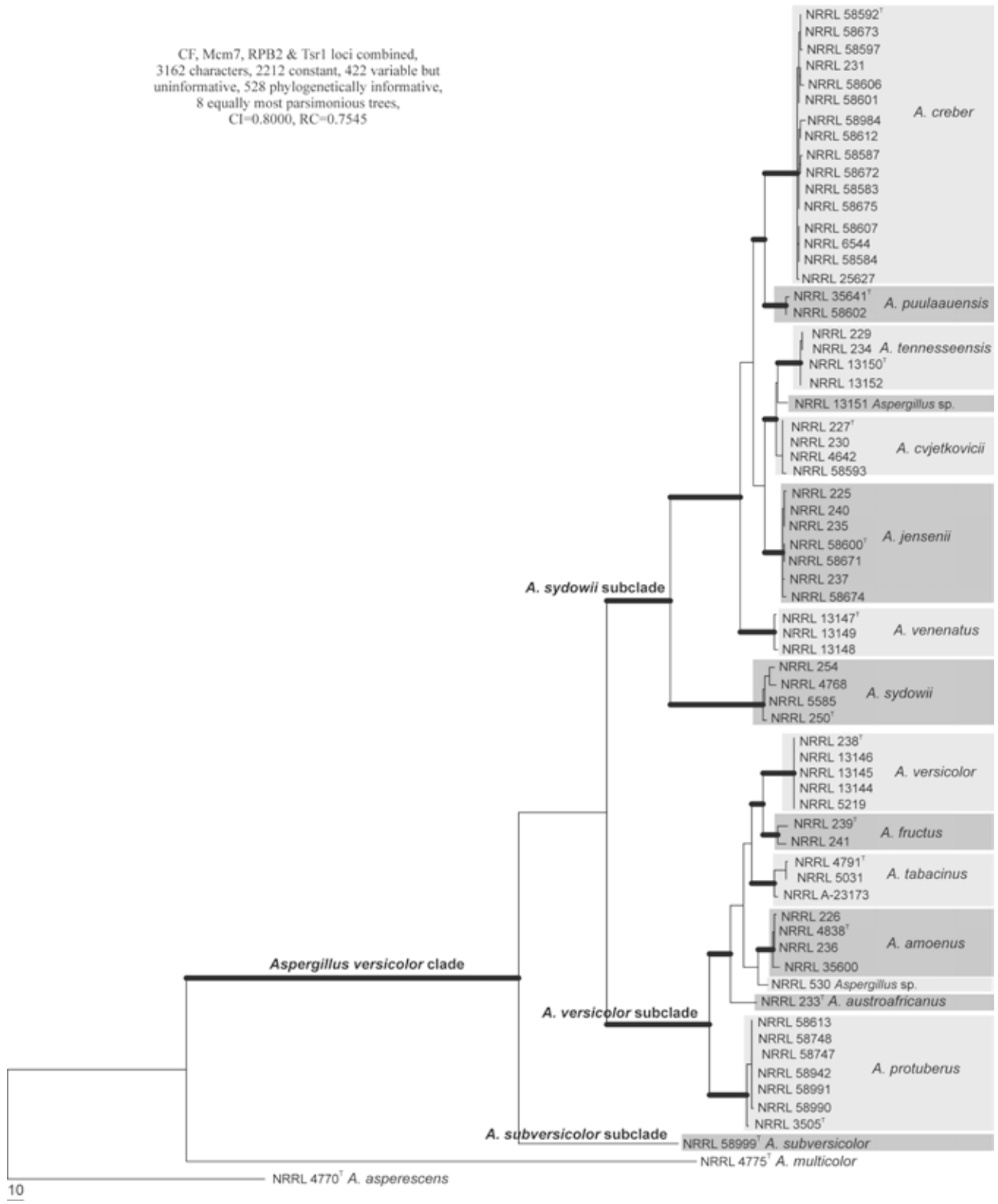


Fig. 1. Phylogenetic tree calculated from DNA sequence data from four concatenated loci. The section *Versicolores* contains three subclades, the *A. versicolor* subclade, the *A. sydowii* subclade and the *A. subversicolor* subclade. Thick branches indicate >90 % bootstrap and >0.90 Bayesian posterior probability for the node. Isolate NRRL 13151 is similar in colony appearance to *A. tennesseensis* but may represent a distinct species. Isolate NRRL 530 is similar in colony appearance to *A. amoenus* but also may represent a distinct species.

lineages contained a single isolate. Two of these single-isolate lineages, *A. subversicolor* and *A. austroafricanus*, were sufficiently distinct phenotypically from other species in the section and are described as new. The other two single-isolate lineages (NRRL 13151 and NRRL 530) were phenotypically difficult to distinguish from their siblings, and species descriptions were not accorded them.

The section *Versicolores* clade contained three subclades (Fig. 1): the *A. sydowii* subclade containing *A. sydowii, A. creber, A. venenatus, A. tennesseensis, A.*

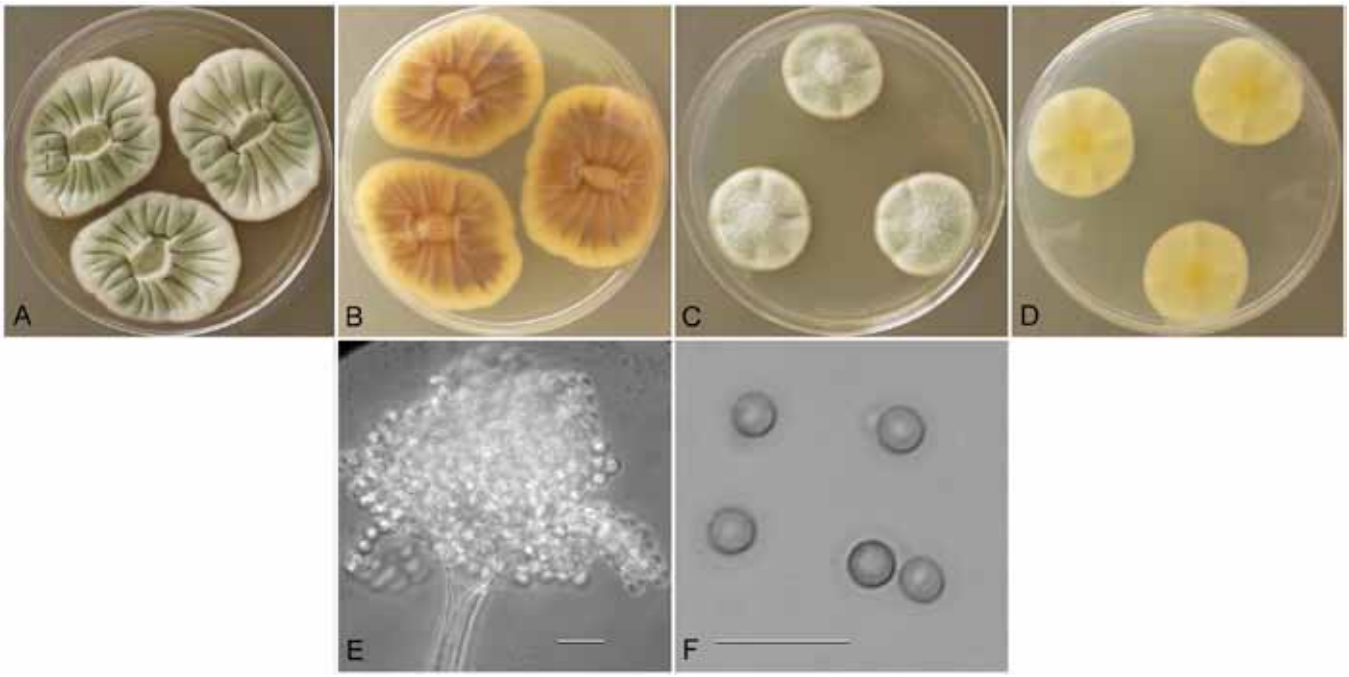


Fig. 2. *Aspergillus amoenus* (NRRL 4838), culture plates are 9 cm diam, colonies grown at 25 °C for 10 d. a. CYA colonies. b. CYA colony reverse. c. MEA colonies. d. MEA colony reverse. e. Smooth stipe, subglobose vesicle, and conidia, bar=10 µm. f. Globose, smooth-walled conidia, bar=10 µm.

cvjetkovicii, A. jensenii and *A. puulaauensis*; the *A. versicolor* subclade containing *A. versicolor, A. tabacinus, A. fructus, A. protuberus, A. amoenus* and *A. austroafricanus*; and the *A. subversicolor* subclade containing the single species *A. subversicolor*. Single-locus trees placed *A. sydowii* in the *A. sydowii* subclade, in the *A. versicolor* subclade or in a distinct clade containing only *A. sydowii* (Figs S1–S5, Supplementary Information, online only) with low confidence levels. The *Mcm7* locus from *A. sydowii* was not amplified despite numerous attempts and thus *A. sydowii* does not appear in Fig. S3 (Supplementary Information, online only). The combined data tree (Fig. 1) depicts *A. sydowii* as a member of the *A. sydowii* subclade with strong statistical support. In the combined data tree, each species' group of isolates resides on a branch with >90 % bootstrap proportion and >0.90 Bayesian posterior probability.

TAXONOMY

Previously described species

Aspergillus amoenus M. Roberg, *Hedwigia* **70**:138 (1931).
MycoBank MB250654
(Fig. 2a–f)

Type: **Germany**: Munster, isol. ex *Berberis* sp. fruit, 1930. *M. Roberg* (NRRL 4838—ex holotype culture).

Description: *Colonies* grown 10 d on CYA at 25 °C (Fig. 2a–b) attained 25–40 mm diam, radially sulcate, centrally raised or sunken 3–4 mm, one older isolate (NRRL 226) plane, sporulating moderately to well, conidial heads in grayish green shades near tea green (R47), clear to pale orange exudate present in some isolates, faint reddish soluble pigment present in some isolates, reverse mostly reddish brown hues, with some isolates uncolored. Colonies grown 10 d on MEA at 25 °C (Fig. 2c–d) attained 23–33 mm diam, low, velutinous, some isolates with shallow sulcations, colony center often with funicular hyphal aggregates, sporulation in blue-green to gray-green shades, no soluble pigment except NRRL 226 with pale brown pigment, no exudate, reverse colored light orange yellow to pale yellow red. Incubation for 7 d on CYA at 5 °C produced no growth or germination of conidia. Incubation for 7 d on CYA at 37 °C commonly produced growth up to 6 mm diam.

Stipes (Fig. 2e) smooth walled, hyaline to yellow with brownish shades, (35–)100–600(–1100) × (2.5–)4–7(–8) µm, *vesicles* pyriform to spatulate, (4–)7–17(–21) µm diam, *conidial heads* biseriate, *metulae* covering 1/3 to entire vesicle, 3–6(–8) × 2.5–4.0(–5.5) µm, *phialides* (5–)6–8(–11) × 2–3 µm, fragmentary heads resembling penicillate fructifications abundant, *conidia* (Fig. 2f) spherical to subspherical, occasionally ellipsoidal, 2.5–3.5(–5) µm, smooth walled, NRRL 35600 produced globose hülle cells 12–22 µm diam when grown on M40Y medium, other isolates did not.

Aspergillus protuberus Muntañola-Cvetković, *Mikrobiologija* **5**: 119 (1968).
MycoBank MB326650
(Fig. 3a–h)
Synonym: Aspergillus versicolor var. *protuberus* (Muntañola-Cvetković) Kozak., *Mycol. Pap.* **161**: 139 (1989).

Type: **Yugoslavia:** isol. ex rubber coated electrical cables, *ca* 1968 (NRRL 3505—ex holotype culture).

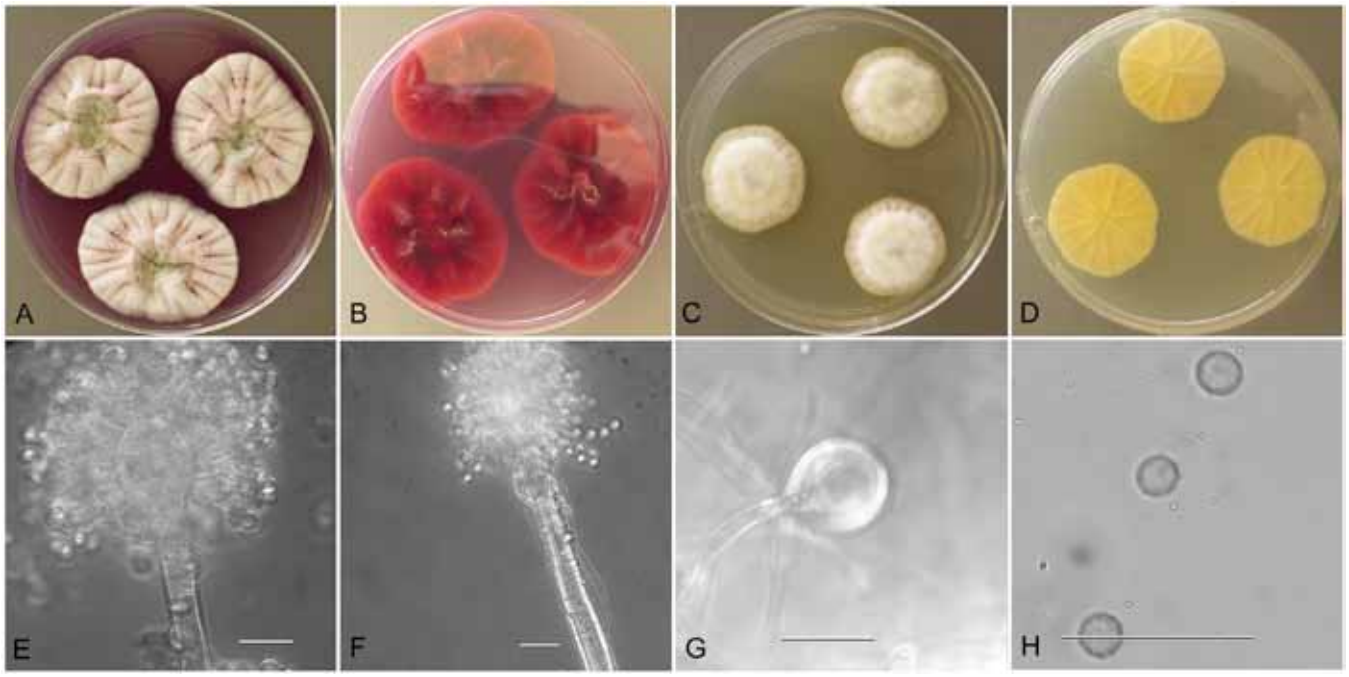


Fig. 3. *Aspergillus protuberus* (NRRL 3505), culture plates are 9 cm diam, colonies grown at 25 °C for 10 d. a. CYA colonies. b. CYA colony reverse. c. MEA colonies. d. MEA colony reverse. e. Stipe, subglobose to clavate vesicle, and conidia, bar=10 µm. f. Roughened surface of stipe, bar=10 µm. g. Globose hülle cell, bar=10 µm. h. Globose, finely roughened conidia, bar=10 µm.

Description: *Colonies* grown 10 d on CYA at 25 °C (Fig. 3a–b) attained 28–34 mm diam, radially and concentrically sulcate, wrinkled, centrally raised 2–4 mm, clumped aerial hyphae give a mealy appearance in some areas of some isolates, sporulation moderate with conidial heads often creamy white but sometimes patches of yellow-green conidia (celandine green R47) are present, scarlet red (R1) exudate moderately abundant, vinaceous-fawn (R40) to pale yellow soluble pigment present, reverse brownish red or orange cinnamon (R20), one isolate brazil red (R1). Colonies grown 10 d on MEA at 25 °C (Fig. 3c–d) attained 27–32 mm diam, floccose, mounded 4–5 mm centrally, radially sulcate, no exudate, no soluble pigment, reverse light pinkish yellow to pinkish yellow. Incubation for 7 d on CYA at 5 °C or 37 °C produced no growth or germination of conidia.

Stipes (Fig. 3e–f) smooth to tuberose, hyaline to yellow or occasionally with brownish shades, (120–)300–800(–1250) × 4–10 µm, occasionally terminating with two vesicles, *vesicles* pyriform to spatulate, rarely subspherical, (6–)10–24(–27) µm diam, *conidial heads* biseriate, *metulae* covering half to entire vesicle, (3–)4–7(–8) × 2.5–4.5(–5.5) µm, *phialides* (4–)5–8(–11) × 2–3(–3.5) µm, fragmentary heads resembling penicillate fructifications occasionally present, *conidia* (Fig. 3h) spherical to subspherical or occasionally ellipsoidal to pyriform, (2.0–)2.5–3.5(–5) µm, finely roughened wall, *hülle cells* (Fig. 3g) globose sometimes present.

Aspergillus sydowii (Bain. & Sart.) Thom & Church *Aspergilli*:147 (1926).
MycoBank MB279636
(Fig. 4a–g)
Basionym: *Sterigmatocystis sydowi* Bainer & Sartory, *Ann. Mycol*. **11**: 25 (1913).

Type: *Sine loc.*: sent to C. Thom, prior to 1930, *M. Swift* (NRRL 250—culture ex neotype).

Description: *Colonies* grown 10 d on CYA at 25 °C (Fig. 4a–b) attained 27–37 mm diam, velutinous, radially sulcate, sporulating well, conidial heads deep bluish gray-green (R42), exudate moderate to abundant, clear to yellowish to reddish brown, reddish-brown soluble pigment, reverse tawny olive (R39) to orange cinnamon (R29) on the periphery. Colonies grown 10 d on MEA at 25 °C (Fig. 4c–d) attained 37–48 mm diam, velutinous, some isolates with shallow sulcations, sporulating in dark grayish blue-green color, funicular hyphal aggregates often seen centrally, no exudate, no soluble pigment, reverse unpigmented to brownish pink in NRRL 4768. Incubation for 7 d on CYA at 5 °C produced no growth or germination of conidia. Incubation at 37 °C produced colonies 10–17 mm diam in 10 d.

Stipes (Fig. 4e) smooth, colorless, 100–500 µm × 4–7 µm, *vesicles* subglobose, 5–10 (–15) µm diam, *conidial heads* biseriate, *metulae* covering most of the vesicle, 6–7 × 2–3 µm, *phialides* 7–10 × 2.0–2.5 µm, fragmentary heads (Fig. 4f) resembling penicillate fructifications abundant, *conidia* (Fig. 4g) globose to subglobose, 2.5–3.0 (–5) µm, spinulose.

Aspergillus tabacinus Nakaz *et al*., *J. Agr. Chem. Soc. Japan* **10**: 177 (1934).
MycoBank MB539544
(Fig. 5a–f)
Synonym: *Aspergillus versicolor* var. *magnus* Sasaki, *J. Fac. Agric. Hokkaido Univ.* **49**: 144 (1950).

Type: *Sine loc.*: isol. ex tobacco, 1934, *Y. Nakazawa* (NRRL 4791—culture ex neotype).

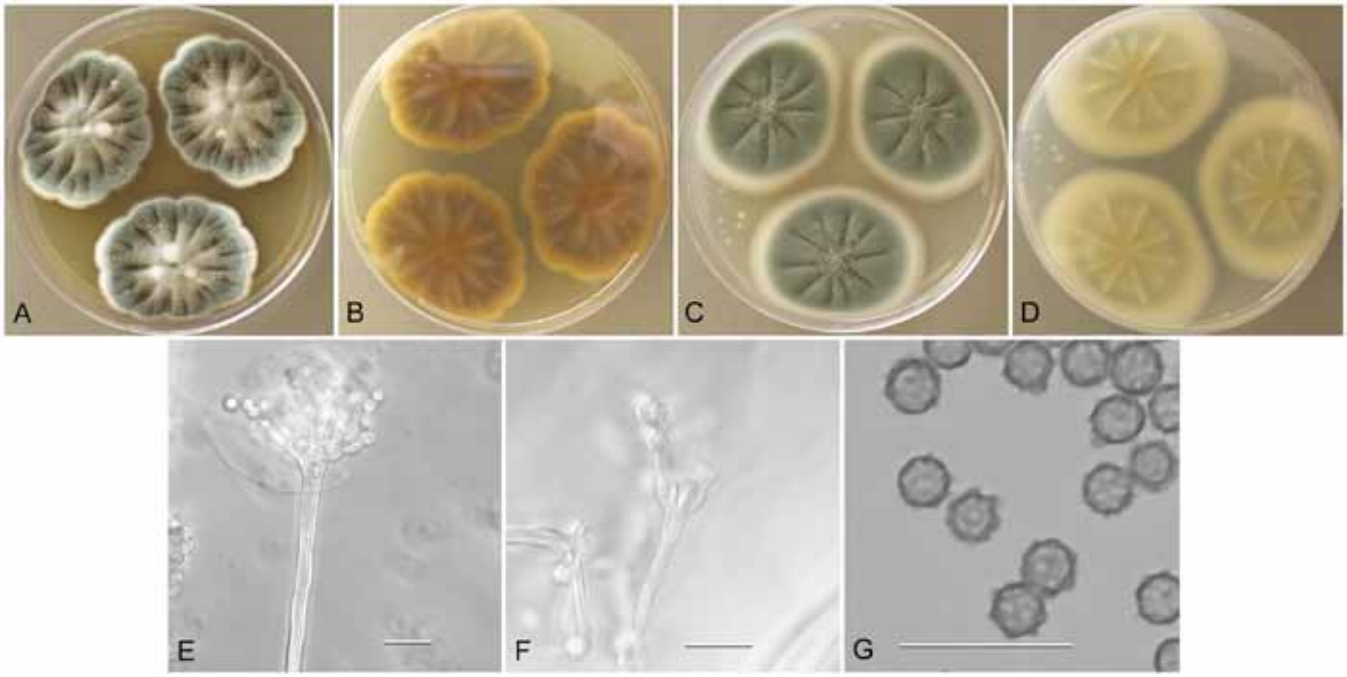


Fig. 4. *Aspergillus sydowi* (NRRL 250), culture plates are 9 cm diam, colonies grown at 25 °C for 10 d. a. CYA colonies. b. CYA colony reverse. c. MEA colonies. d. MEA colony reverse. e. Smooth stipe, subglobose vesicle, and conidia, bar=10 µm. f. Penicillate conidiophore from aerial hyphae, bar=10 µm. g. Subglobose, spinulose conidia, bar=10 µm.

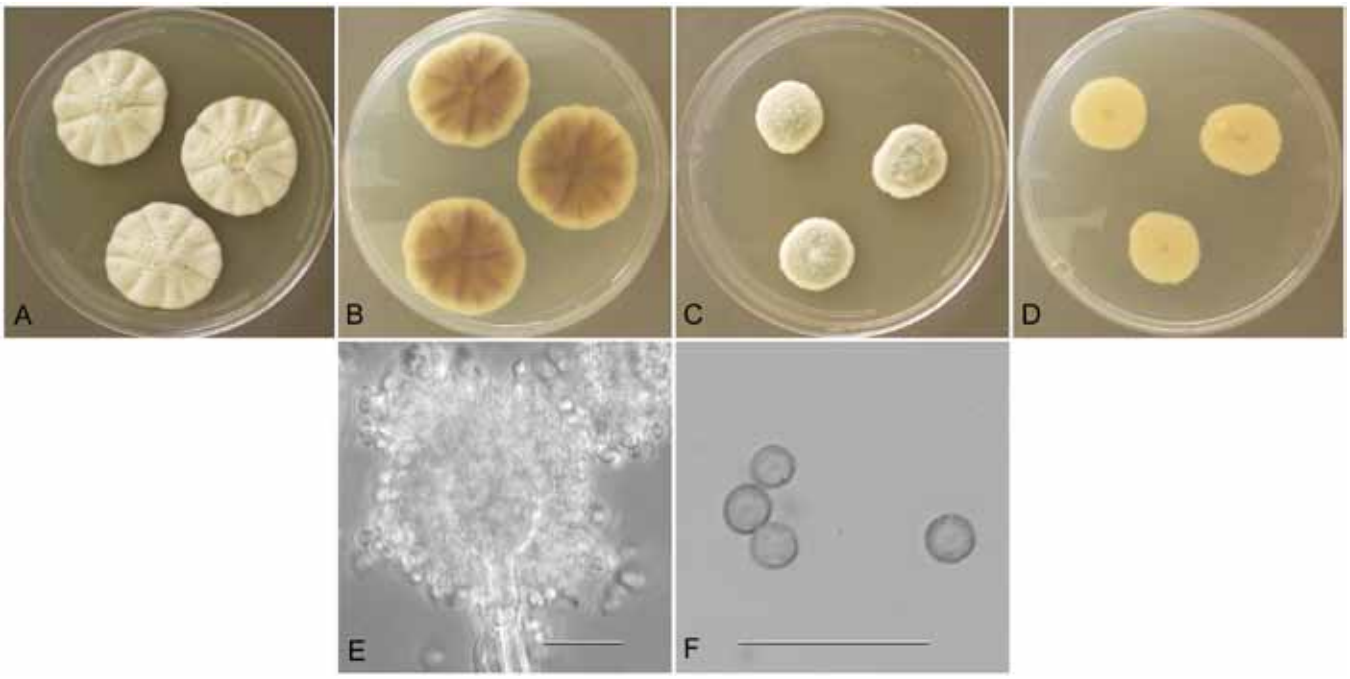


Fig. 5. *Aspergillus tabacinus* (NRRL 4791), culture plates are 9 cm diam, colonies grown at 25 °C for 10 d. a. CYA colonies. b. CYA colony reverse. c. MEA colonies. d. MEA colony reverse. e. Smooth stipe, with clavate vesicle, and conidia, bar=10 µm. f. Globose, smooth-walled conidia, bar=10 µm.

Description: *Colonies* grown 10 d on CYA at 25 °C (Fig. 5a–b) attained 30–32 mm diam, sulcate, centrally raised 2–3 mm, often sporulating heavily throughout but sometimes sporulation is delayed, conidial heads artemisia green (R47), sporulation from aerial branches pronounced, exudate clear when present, no soluble pigment, reverse uncolored in NRRL 5031, or brown in other isolates. Colonies grown 10 d on MEA at 25 °C (Fig. 5c–d) attained 17–30 mm diam, NRRL 4791 is velutinous and covered with funicular hyphal aggregates, NRRL 5031 and NRRL 62481 are floccose, sporulation in bluish-green shades, no exudate, no soluble pigment, reverse uncolored to cream or very pale yellow. Incubation for 7 d on CYA at 5 °C or 37 °C produced no growth or germination of conidia.

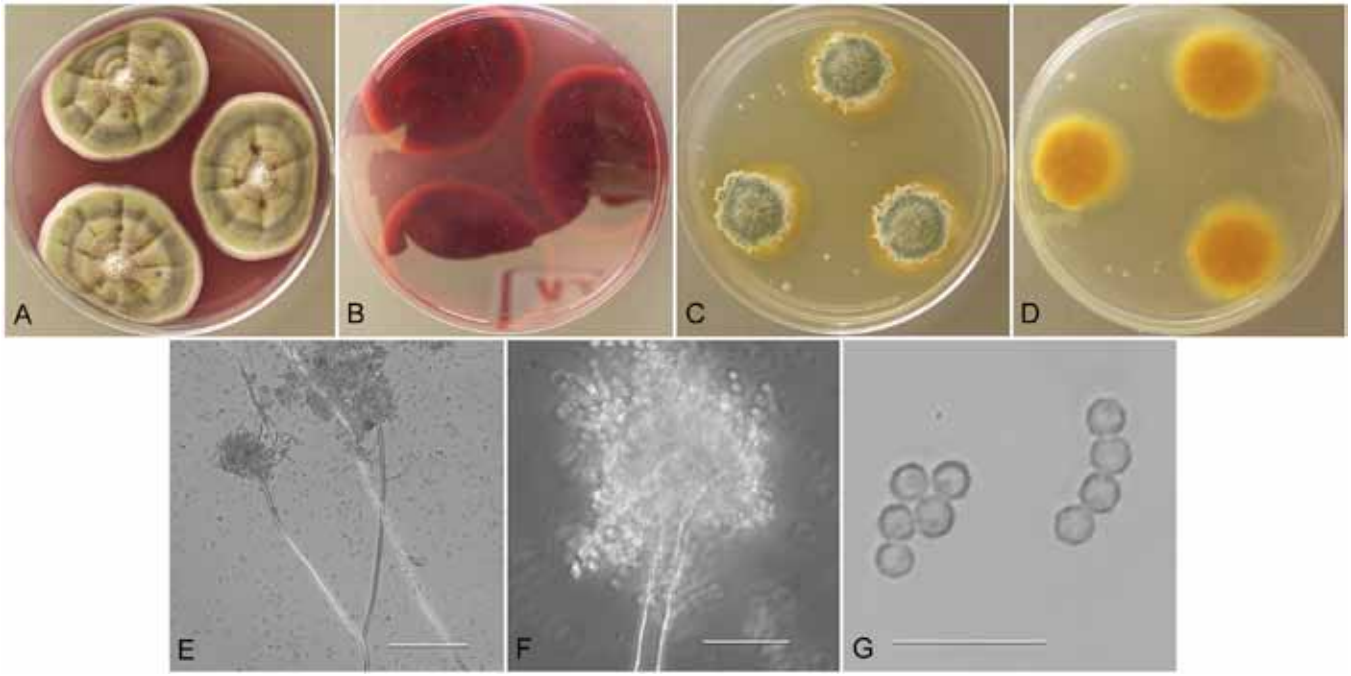


Fig. 6. *Aspergillus versicolor* (NRRL 238), culture plates are 9 cm diam, colonies grown at 25 °C for 10 d. a. CYA colonies. b. CYA colony reverse. c. MEA colonies. d. MEA colony reverse. e. Bifurcating stipe producing two conidiophores, bar=50 µm. f. Smooth stipe, subglobose vesicle, and conidia, bar=10 µm. g. Globose conidia with roughened walls, bar=10 µm.

Stipes smooth walled (Fig. 5e), septate, hyaline to yellow with brownish tint, (70–)300–700(–900) × 4–8(–9) µm, *vesicles* pyriform to spatulate, (5–)8–15(–22) µm diam, *conidial heads* biseriate, *metulae* covering half to entire vesicle, 3–8(–9)um × 2.5–4.5(–5.5) µm, *phialides* 5–8(–11) × 2–3(–3.5) µm, fragmentary heads resembling penicillate fructifications abundant, *conidia* (Fig. 5f) spherical to subspherical, occasionally ellipsoidal, (2.5–)3–4(–7) µm, smooth walled.

Aspergillus versicolor (Vuill.) Tirab., *Annali Bot*. **7**: 9 (1908).
MycoBank MB172159
(Fig. 6a–g)
Basionym: *Sterigmatocystis versicolor* Vuill., *in* Mirsky, *Thèse de médicine* (Nancy) **27**:15 (1903).

Type: *Sine loc.:* 1935, *V. K. Charles* (NRRL 238—culture ex neotype).

Description: *Colonies* grown 10 d on CYA at 25 °C (Fig. 6a–b) attained 28–36 mm diam, sulcate, centrally raised 4–5 mm, sporulating well, conidial heads pale grayish green near tea green (R47), central area mealy from aggregated aerial hyphae, exudate present in mostly clear to pale pink shades (brownish red in one isolate), faint to very obvious pinkish soluble pigment, reverse vinaceous or brown or scarlet (NRRL 238). Colonies grown 10 d on MEA at 25 °C (Fig. 6c–d) attained 21–31 mm diam, low, with funicular hyphal aggregates, sometimes dominating colony appearance, sporulating in pale to dark bluish green to gray green color, no exudate seen, soluble pigment yellow in some isolates, not present in others, reverse pale yellow, yellow orange or orange. Incubation for 7 d on CYA at 5 °C produced no growth or germination of conidia. Incubation for 7 d on CYA at 37 °C produced growth up to 8 mm diam.

Stipes (Fig. 6e–f) smooth, occasionally lightly tuberose, hyaline to yellow with brownish shades, (45–)200–750(–1050) × (4–)5–8(–12) µm, *vesicles* pyriform to spatulate, (6–)9–17(–20) µm in diam, *conidial heads* biseriate, *metulae* covering half to entire vesicle, 3–6(–9) × 2.5–4.5 µm, *phialides* (4–)5–7(–11) × 2–3 µm, fragmentary heads resembling penicillate fructifications occasionally present, *conidia* (Fig. 6g) spherical to subspherical, occasionally ellipsoidal, (2–)2.5–3.5(–6.5) µm, finely roughened wall, *hülle cells* globose, produced by NRRL 5219 when grown on M40Y medium, but not other isolates.

Observations: The ex-neotype culture NRRL 238 (isolated in 1935) is quite different in appearance, particularly in production of dark red soluble pigment and scarlet colony reverse on CYA, from the more recent isolates that were placed in the ARS Culture Collection between 1970 and 1984. The more recent isolates (NRRL 5219, NRRL 13144, NRRL 13145 and NRRL 13146) are quite similar in appearance and are the primary basis of the phenotypic description. Although there is phenotypic distinction, all five isolates are *A. versicolor* based on DNA sequence analysis.

New species

Aspergillus austroafricanus Jurjevic, S. W. Peterson & B. W. Horn, **sp. nov.**
MycoBank MB800597
(Fig. 7a–f)
Etymology: Isolated from soil in South Africa.

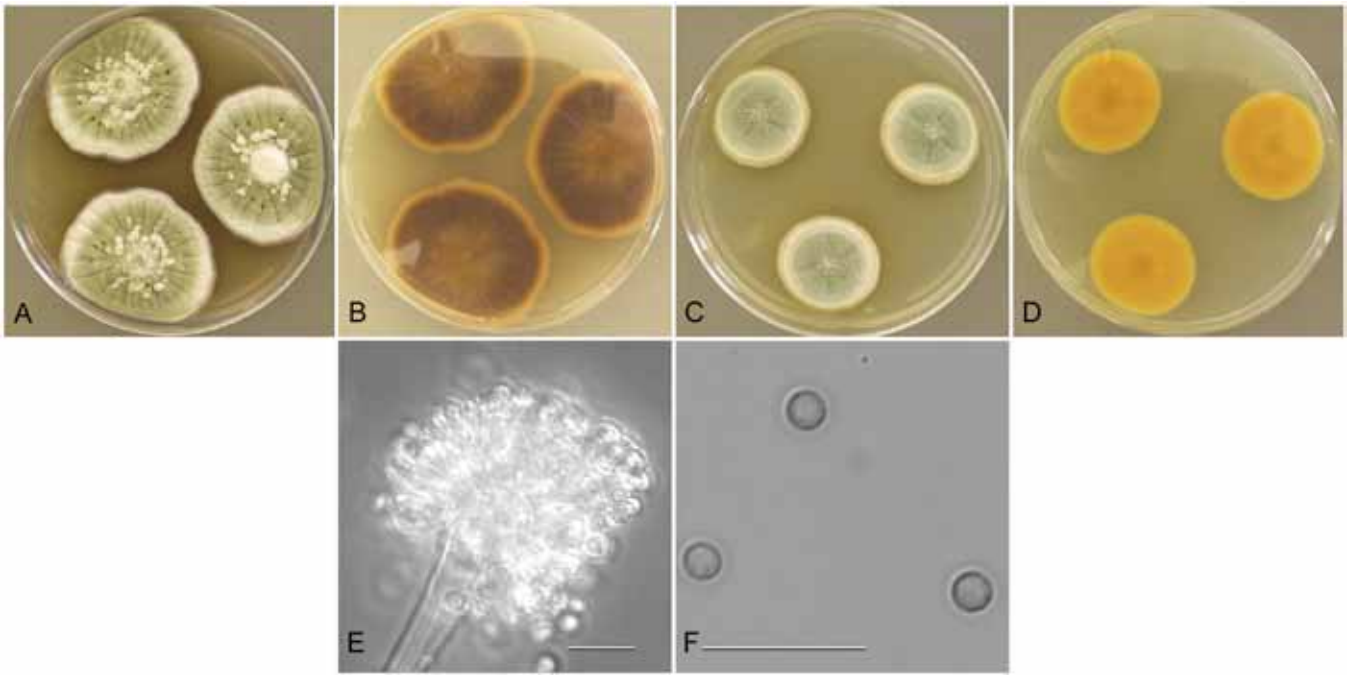


Fig. 7. *Aspergillus austroafricanus* (NRRL 233), culture plates are 9 cm diam, colonies grown at 25 °C for 10 d. a. CYA colonies. b. CYA colony reverse. c. MEA colonies. d. MEA colony reverse. e. Smooth stipe, subglobose vesicle and conidia, bar=10 µm. f. Globose, smooth-walled conidia, bar=10 µm.

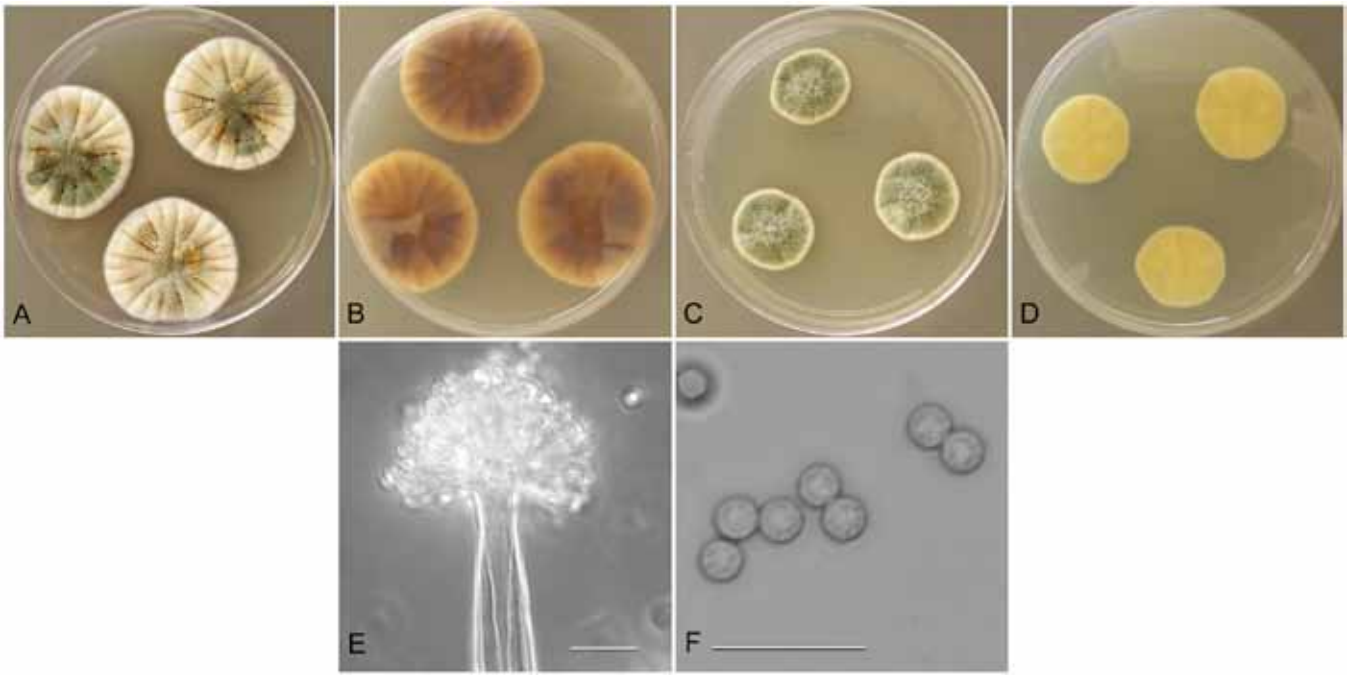


Fig. 8. *Aspergillus creber* (NRRL 58583), culture plates are 9 cm diam, colonies grown at 25 °C for 10 d. a. CYA colonies. b. CYA colony reverse. c. MEA colonies. d. MEA colony reverse. e. Smooth stipe, subglobose vesicle, and conidia, bar=10 µm. f. Globose, finely roughened conidia, bar=10 µm.

Type: **South Africa**: Capetown, sent to C. Thom, 1922, *V. A. Putterill* (BPI 880914 – holotype [from dried colonies of NRRL 233 grown 7 d at 25 °C on CYA and MEA]).

Diagnosis: Conidia smooth-walled, no growth at 37 °C, produces reddish brown soluble pigment when grown on CYA.

Description: *Colonies* grown 10 d on CYA at 25 °C (Fig. 7a–b) attained 23–24 mm diam, mounded, shallowly sulcate, overgrowth by clumped hyphae making surface appear mealy, sporulating well, conidial heads near sage green (R47), sparse clear exudate, soluble pigment reddish brown, reverse dull brown. Colonies grown 10 d on MEA at 25 °C (Fig. 7c–d) attained 27 mm diam, velutinous, sporulation pale blue green, central hyphal tufts, no exudate, no soluble pigment, reverse yellowish orange. Incubation for 7 d on CYA at 5 °C or 37 °C produced no growth or germination of conidia.

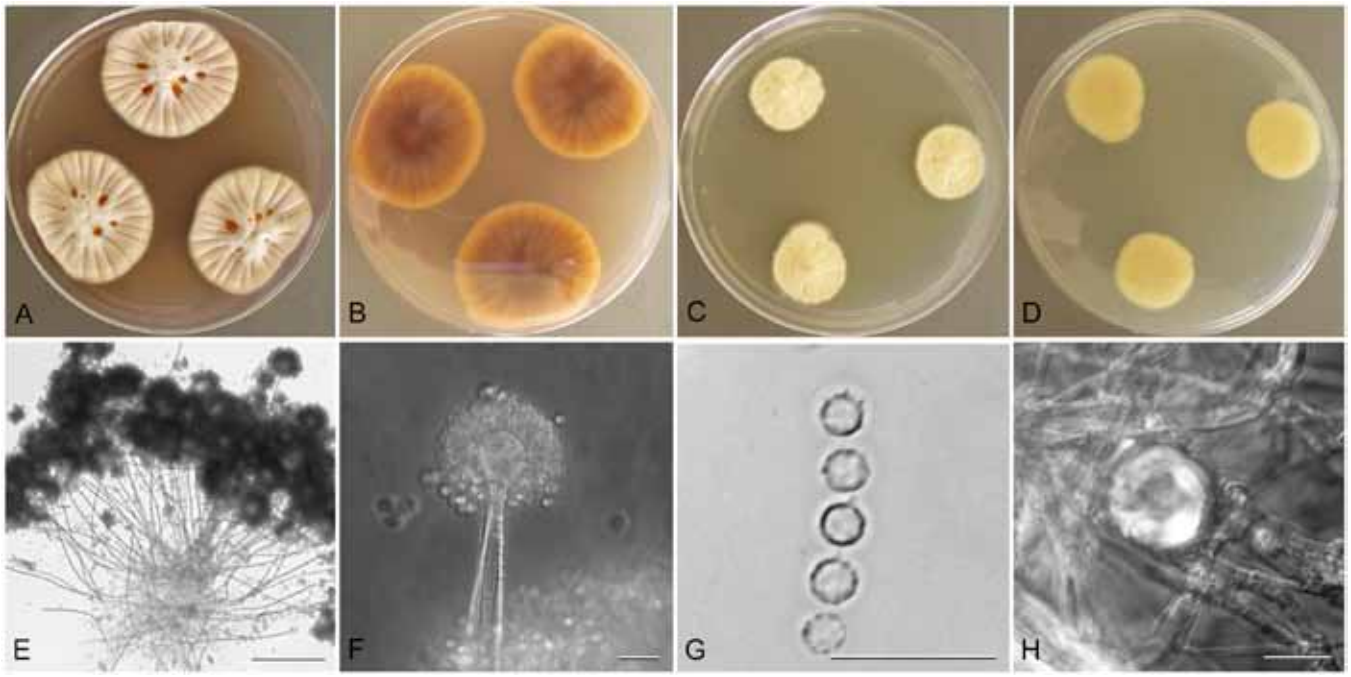


Fig. 9. *Aspergillus cvjetkovicii* (NRRL 4642), culture plates are 9 cm diam, colonies grown at 25 °C for 10 d. a. CYA colonies. b. CYA colony reverse. c. MEA colonies. d. MEA colony reverse. e. Numerous conidiophores arising from the basal colony, bar=50 µm. f. Stipe, subglobose vesicle, and conidia, bar=10 µm. g. Globose, spinulose conidia, bar=10 µm. h. Globose hülle cell, bar=10 µm.

Stipes (Fig. 7e) smooth walled, hyaline to yellowish, (40–)100–350(–500) µm × 3–5(–6) µm, *vesicles* pyriform to spatulate, (4–)6–12(–15) µm diam, *conidial heads* biseriate, *metulae* covering 1/3 to entire vesicle, 3–7(–9)um × 2.5–4.5 µm, *phialides* (4–)5–7(–9) × (2–)2.5–3(–4) µm, fragmentary heads resembling penicillate fructifications occasionally present, *conidia* (Fig. 7f) spherical to subspherical, 2.5–3.5 (–4.5) µm, smooth walled.

Aspergillus creber Jurjevic, S. W. Peterson & B. W. Horn, **sp. nov.**
MycoBank MB800598
(Fig. 8a–f)
Etymology: From the Latin word *creber* meaning numerous or frequent.

Type: **USA**: *California*: isol. ex air sample, Nov. 2008, *Z. Jurjevic* (BPI 800912 – holotype; [from dried colonies of NRRL 58592 grown 7 d at 25 °C on CYA and MEA]).

Diagnosis: Produces rough-walled conidia, no growth at 37 °C, no soluble pigments formed on CYA or MEA, conidial color pea green or sage green on CYA and MEA.

Description: *Colonies* grown 10 d on CYA at 25 °C (Fig. 8a–b) attained 18–26 mm diam, radially sulcate, raised 3–5 mm centrally, peripheral areas white or yellow, central area sporulating well, conidial heads pea green to artemisia green (R47), exudate when present yellowish to reddish, no soluble pigment, reverse clay colored to cinnamon or reddish brown (R29). Colonies grown 10 d on MEA at 25 °C (Fig. 8c–d) attained 18–22 mm diam, low to 1–2 mm mounded, often overgrown centrally with hyphae aggregated into funicles, sporulation in yellow-green shades (pea green to sage green R47), with *ca* 1 mm white border, one isolate (NRRL 231) with vivid brown soluble pigment, other isolates no soluble pigment, no exudate, reverse pale yellow orange or olive drab or orange brown. Incubation for 7 d on CYA at 5 °C or 37 °C produced no growth or germination of conidia.

Stipes (Fig. 8e) smooth walled, (10–)70–450(–650) x (3–)4–7(–8) µm, *vesicles* pyriform to spatulate and occasionally subglobose, (4–)7–17(–25) µm diam, *conidial heads* biseriate, *metulae* (3–)4–6(–8) x 2.5–4.5(–5) µm, *phialides* (4–)5–8(–10) x 2–3(–4) µm, *conidia* (Fig. 8f) spherical to subspherical, occasionally ellipsoidal to pyriform, (2.5–)3–4(–9) µm, finely roughened wall.

Aspergillus cvjetkovicii Jurjevic, S. W. Peterson & B. W. Horn, **sp. nov.**
MycoBank MB800599
(Fig. 9a–h)
Etymology: Named in honor of Bogdan Cvjetković (University of Zagreb); pronunciation \`chet-kO-``vi-chi\.

Type: **USA**: *New Jersey:* isol. ex soil, 1915, *W. Wilson* (BPI 880909 – holotype [from dried colonies of NRRL 227 grown 7 d at 25 °C on CYA and MEA]).

Diagnosis: Produces spinulose conidia, no growth at 37 °C, colonies producing red exudate and red soluble pigment on CYA.

Description: *Colonies* grown 10 d on CYA at 25 °C (Fig. 9a–b) attained 24–29 mm diam, radially sulcate, either centrally sunken or raised (2–3 mm), sporulating well, conidial heads white to cream in most isolates, pea green (R47) in NRRL 58593, exudate generally abundant, reddish brown to orange cinnamon, reddish brown soluble pigment, reverse yellowish

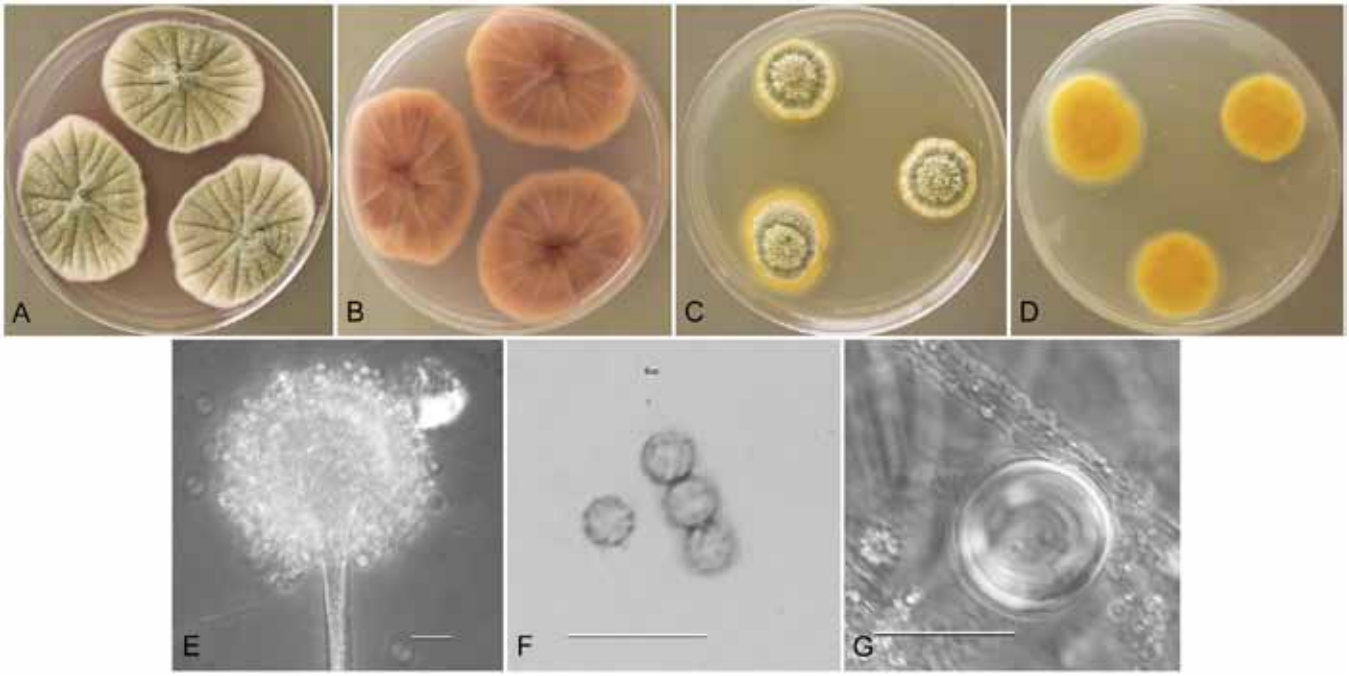


Fig. 10. *Aspergillus fructus* (NRRL 239), culture plates are 9 cm diam, colonies grown at 25 °C for 10 d. a. CYA colonies. b. CYA colony reverse. c. MEA colonies. d. MEA colony reverse. e. Smooth stipe, spathulate vesicle, and conidia, bar=10 µm. f. Globose, finely roughened conidia, bar=10 µm. g. Globose hülle cell, bar=10 µm.

red shades near orange cinnamon (R29) or tawny olive (R39). Colonies grown 10 d on MEA at 25 °C (Fig. 9c–d) attained 17–36 mm diam, low, slightly sulcate, sporulating throughout in creamy yellow shades, NRRL 58593 conidia are yellowish green, NRRL 227 and NRRL 230 produce brown soluble pigment while NRRL 4642 and 58593 do not produce soluble pigment, reverse brownish orange or pale creamy yellow. Incubation for 7 d on CYA at 5 °C or 37 °C produced no growth or germination of conidia.

Stipes (Fig. 9e–f) smooth walled, hyaline to yellow, (40–)200–700(–850) × (3–)4–7(–8) µm, *vesicles* pyriform to spatulate, rarely subspherical, (5–)9–18(–23) µm diam, *conidial heads* biseriate, *metulae* covering half to entire vesicle, 3–6(–8) × 2.5–4.5 µm, *phialides* 5–8(–10) × 2–3(–4) µm, occasionally solitary phialides present up to 32 µm long, fragmentary heads resembling penicillate fructifications occasionally present, *conidia* (Fig. 9g) spherical to subspherical, occasionally ellipsoidal, (2–)2.5–3.5(–5) µm, spinulose, *hülle cells* (Fig. 9h) globose, sometimes present.

Aspergillus fructus Jurjevic, S. W. Peterson & B. W. Horn, **sp. nov.**
MycoBank MB800600
(Fig. 10a–g)
Etymology: From fruit.

Type: **USA**: *California*: isol. ex date fruit, 1939, *Bliss* (BPI 880915 – holotype [from dried colonies of NRRL 239 grown 7 d at 25 °C on CYA and MEA]).

Diagnosis: Resembling *A. versicolor* growth at 37 °C, but forming shorter conidiophores 150–400 µm versus 200–750 µm conidiophores in *A. versicolor.*

Description: *Colonies* grown 10 d on CYA at 25 °C (Fig. 10a–b) attained 29–39 mm diam, sulcate, centrally raised 4–5 mm, funicular clumps of aerial hyphae abundant, sporulating well, conidial heads celandine green (R47), exudate clear to yellow, moderately abundant, soluble pigment clear, orange red in NRRL 239, reverse uncolored or mahogany red to orange-rufous (R2). Colonies grown 10 d on MEA at 25 °C (Fig. 10c–d) attained 22–32 mm diam, slightly sulcate, centrally covered by hyphal tufts, sporulation in yellow-green hues near artemisia green (R47), no exudate, no soluble pigment, reverse uncolored or drab orange. NRRL 241 was floccose on MEA. Incubation for 7 d on CYA at 5 °C produced no growth or germination of conidia. Incubation for 7 d on CYA at 37 °C produced growth up to 4 mm diam.

Stipes (Fig. 10e) smooth walled, hyaline to yellow, (50–)150–400(–500) × 4–7 µm, *vesicles* pyriform to spatulate, (6–)9–17(–21) µm diam, *conidial heads* biseriate, *metulae* covering half to entire vesicle, (2–)3–7(–9) × 2.5–4.5(–7) µm, *phialides* (5–)6–8(–11) × 2–3(–4) µm, fragmentary heads resembling penicillate fructifications abundant, *conidia* (Fig. 10f) spherical to subspherical, occasionally ellipsoidal, (2–)2.5–3.5(–4.5) µm, finely roughened wall, *hülle cells* (Fig. 10g) globose, sometimes present.

Aspergillus jensenii Jurjevic, S. W. Peterson & B. W. Horn, **sp. nov.**
MycoBank MB800601
(Fig. 11a–g)
Etymology: Named in honor of C. N. Jensen who first reported this species as *Aspergillus globosus* Jensen, a later homonym of *A. globosus* Link.

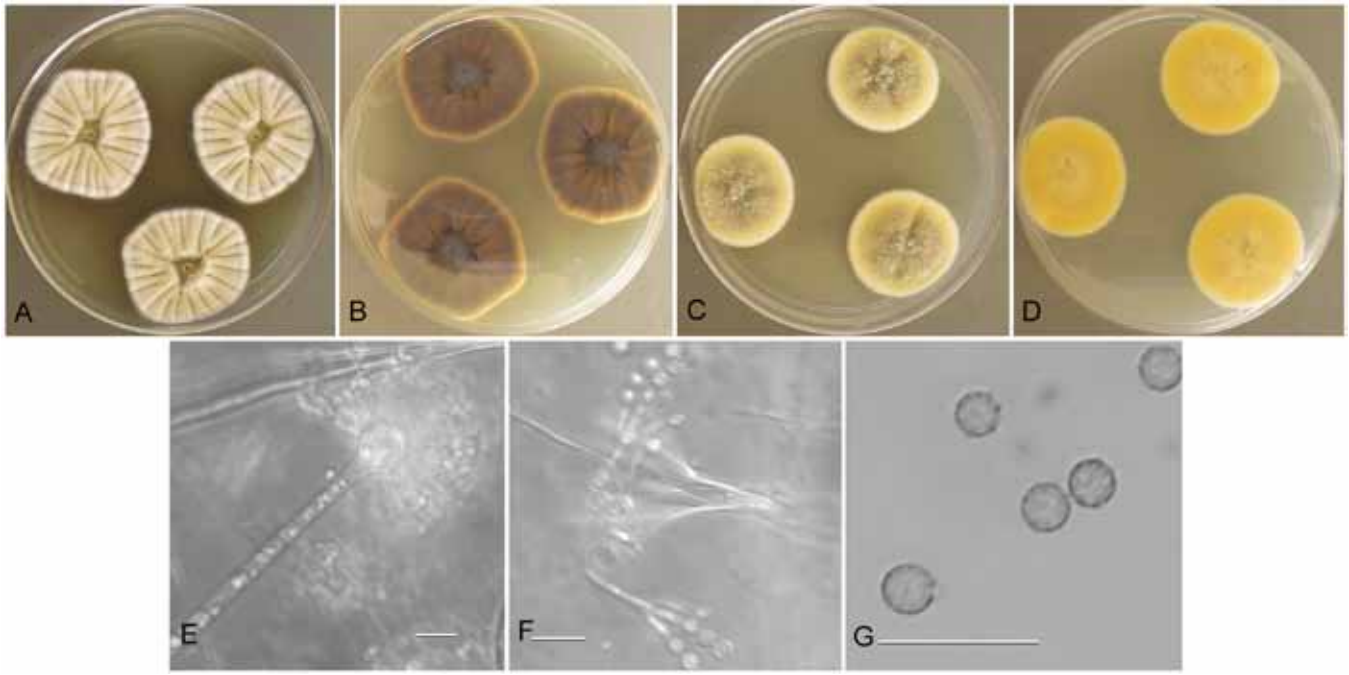


Fig. 11. *Aspergillus jensenii* (NRRL 58671), culture plates are 9 cm diam, colonies grown at 25 °C for 10 d. a. CYA colonies. b. CYA colony reverse. c. MEA colonies. d. MEA colony reverse. e. Smooth stipe, subglobose vesicle, metulae and phialides, bar=10 µm. f. Penicillate conidiogenous cells from aerial hyphae, bar=10 µm. g. Globose, finely roughened conidia, bar=10 µm.

Type: **USA**: *Montana*: isol. ex air sample, Oct. 2008, *Z. Jurjevic* (BPI 880910 – holotype [from dried colonies of NRRL 58600 grown 7 d at 25 °C on CYA and MEA]).

Synonym: Aspergillus globosus Jensen, Cornell University Agricultural Experiment Station Bulletin 315: 482 (1912); non Link 1809.

Diagnosis: Conidial walls roughened, no growth at 37 °C, conidial color near celandine, tawny olive to dark umber colony reverse on CYA.

Description: *Colonies* grown 10 d on CYA at 25 °C (Fig. 11a–b) attained 20–27 mm diam, radially sulcate, centrally raised or sunken, with clumped hyphal aggregates common in some isolates, sporulating moderately well, conidial heads celandine (R47) centrally and often white peripherally, exudate when present reddish brown or yellow brown, soluble pigment faint or intense yellow brown, in one case reddish brown, reverse tawny olive (R39) to dark brown near dark umber (R3). Colonies grown 10 d on MEA at 25 °C (Fig. 11c–d) attained 17–30 mm diam, low, plane, most isolates have funicular tufts of aerial hyphae centrally, sporulating well in yellowish blue-green shades, no exudate seen, soluble pigment either light brown or reddish brown, brownish orange in one isolate, reverse pale yellow or orange or brownish red. Incubation for 7 d on CYA at 5 °C or 37 °C produced no growth or germination of conidia.

Stipes (Fig. 11e) smooth walled, hyaline to yellow, occasionally with brownish shades, (45–)200–700(–1000) × (–3)4–7(–8) µm, *vesicles* pyriform to spatulate, rarely subspherical, (5–)7–16(–22) µm diam, *conidial heads* biseriate, *metulae* covering 1/3 to entire vesicle, 3–8 × 2.5–4(–5) µm, *phialides* (4–)5–8(–11) × 2–3 µm, rarely solitary phialides present up to 32 µm long and up to 4.5 µm diam, fragmentary heads resembling penicillate fructifications (Fig. 11f) commonly present, *conidia* (Fig. 11g) spherical to subspherical, occasionally ellipsoidal to pyriform, (2.5–)3–4.5(–7) µm, finely roughened wall, globose hülle cells 15–20 µm diam produced by NRRL 58582 but not other isolates.

Aspergillus puulaauensis Jurjevic, S. W. Peterson & B. W. Horn, **sp. nov.**
MycoBank MB800602
(Fig. 12a–h)
Etymology. Isolated near the Pu'u la'au Highway on Hawaii; pronunciation \pU-U-la-U-en-sis\

Type: **USA**: *Hawaii*: isol. ex dead hardwood branch, 2003, *D.T. Wicklow* (BPI 880911 – holotype [from dried colonies of NRRL 35641 grown 7 d at 25 °C on CYA and MEA]).

Diagnosis: Isolates produce abundant hülle cells when grown on M40Y agar, no growth at 37 °C,.

Description: *Colonies* grown 10 d on CYA at 25 °C (Fig. 12a–b) attained 22–25 mm diam, sulcate, centrally raised 5–6 mm with funicular hyphal clumps, sporulation light, conidial heads artemisia green (R47), exudate when present clear or reddish, soluble pigment when present brown, reverse yellowish to clay color (R39) or cinnamon (R29). Colonies grown 10 d on MEA at 25 °C (Fig. 12c–d) attained 21–25 mm diam, sulcate or plane, low, velutinous, deep green (artemisia to lily green R47), no exudate seen, no soluble pigment, reverse pale yellow near chamois or pale orange. Incubation for 7 d on CYA at 5 °C and 37 °C produced no growth or germination of conidia.

Stipes (Fig. 12e) smooth walled, hyaline to yellow, (35–)100–500(–700) × (3–)4–7 µm, *vesicles* pyriform to spatulate,

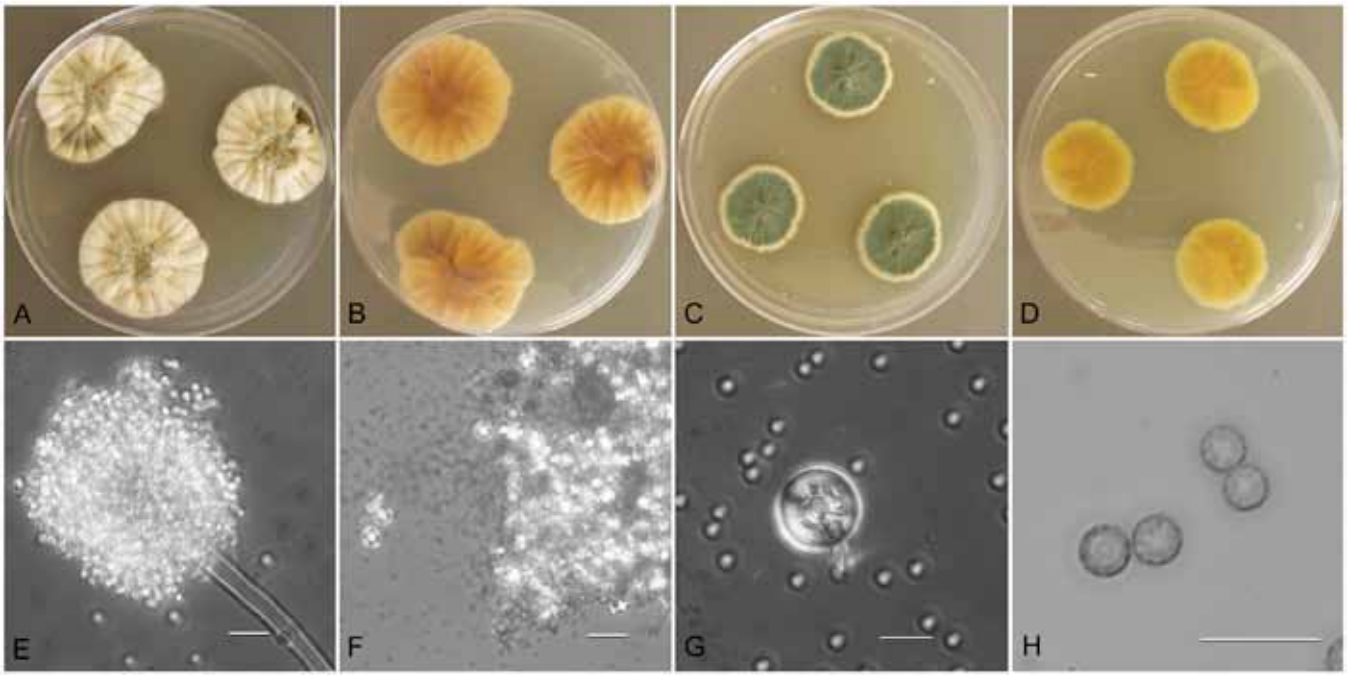


Fig. 12. *Aspergillus puulaauensis* (NRRL 35641), culture plates are 9 cm diam, colonies grown at 25 °C for 10 d. a. CYA colonies. b. CYA colony reverse. c. MEA colonies. d. MEA colony reverse. e. Smooth stipe, vesicle, and conidia, bar=10 µm. f. Mass of hülle cells, bar=50 µm. g. Hülle cell, bar=10 µm. h. Globose conidia with finely roughened walls, bar=10 µm.

occasionally subspherical, (5–)8–18(–21) µm diam, *conidial heads* biseriate, *metulae* covering half to entire vesicle, (3–)4–7(–9) × 2.5–4 µm, *phialides* 5–7(–10) × 2–3 µm, fragmentary heads resembling penicillate fructifications occasionally present, *conidia* (Fig. 12h) spherical to ellipsoidal, (2.5–)3–4(–5.5) µm, finely roughened wall, *hülle cells* (Fig. 12f–g) spherical 11–19 µm diam seen in all isolates when grown on M40Y medium.

Aspergillus subversicolor Jurjevic, S. W. Peterson & B. W. Horn, **sp. nov.**
MycoBank MB800603
(Fig. 13a–f)
Etymology: Beneath or at the foot of *Aspergillus versicolor*.

Type: **India**: *Karnataka*: isol. ex green coffee berries, 1970, *B. Muthappa* (BPI 880918 – holotype [from dried colonies of NRRL 58999 grown 7 d at 25 °C on CYA and MEA]).

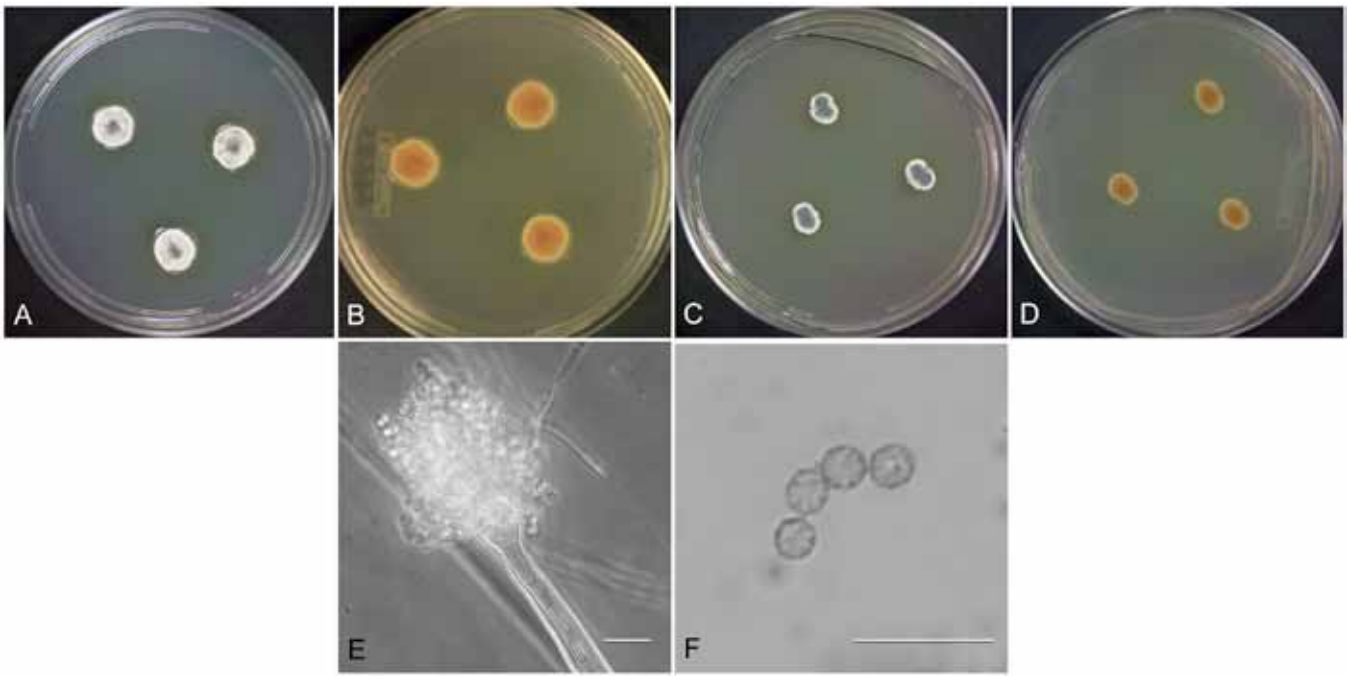


Fig. 13. *Aspergillus subversicolor* (NRRL 58999), culture plates are 9 cm diam, colonies grown at 25 °C for 10 d. a. CYA colonies. b. CYA colony reverse. c. MEA colonies. d. MEA colony reverse. e. Smooth stipe, subglobose vesicle, and conidia, bar=10 µm. f. Subglobose, finely roughened conidia, bar=10 µm.

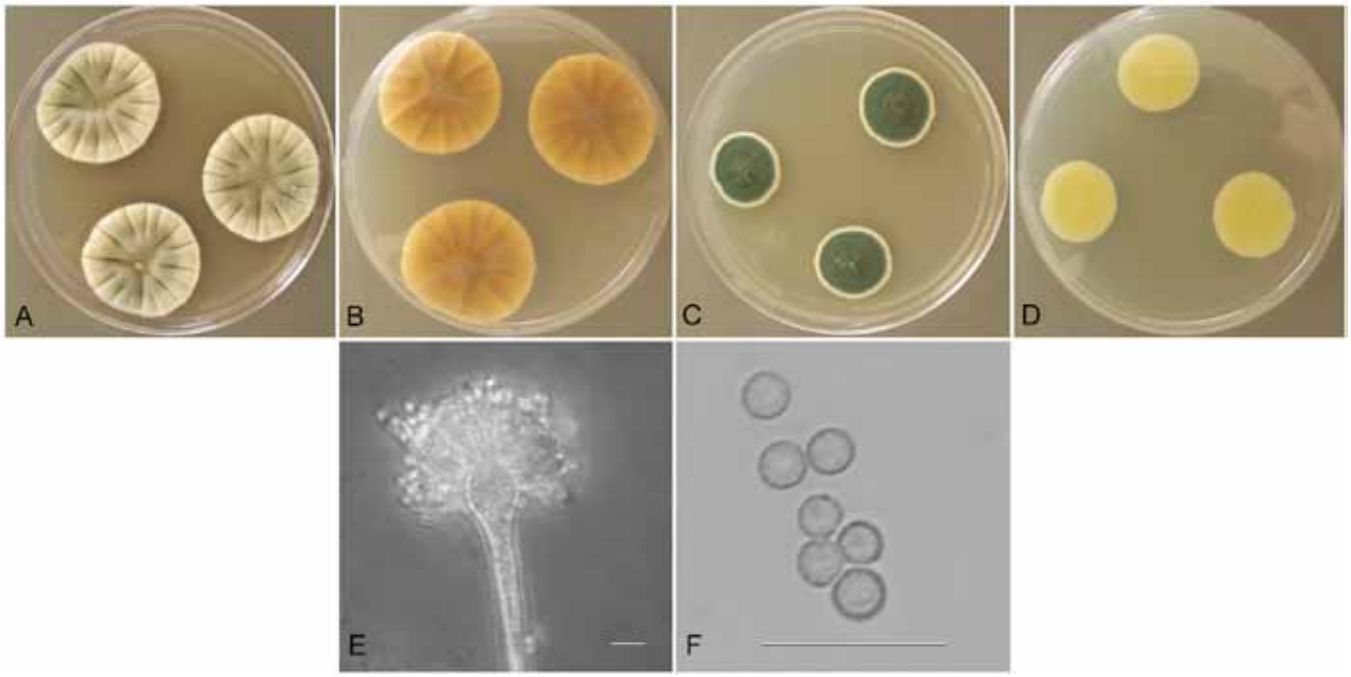


Fig. 14. *Aspergillus tennesseensis* (NRRL 13150), culture plates are 9 cm diam, colonies grown at 25 °C for 10 d. a. CYA colonies. b. CYA colony reverse. c. MEA colonies. d. MEA colony reverse. e. Smooth stipe, pyriform vesicle, and conidia, bar=10 µm. f. Globose, finely roughened conidia, bar=10 µm.

Diagnosis: Conidia rough-walled, no growth at 37 °C, growing slowly on all media, producing yellow soluble pigment on CYA but no exudate.

Description: *Colonies* grown 10 d on CYA at 25 °C (Fig. 13a–b) attained 18–20 mm diam, sulcate, raised 5–6 mm centrally, wrinkled, sporulating sparsely, conidial heads artemisia green (R47), no exudate, soluble pigment faint yellow, reverse tawny (R15) to ochraceous orange. Colonies grown 10 d on MEA at 25 °C (Fig. 13c–d) attained 12–14 mm diam, low, plane, velutinous, sporulating in bluish green color (artemisia R47), no exudate, no soluble pigment, reverse brownish orange. Incubation for 7 d on CYA at 5 °C or 37 °C produced no growth or germination of conidia.

Stipes (Fig. 13e) smooth walled, hyaline to slightly brownish, (60–) 250–450 (–550) × 4–7(–10) µm, *vesicles* pyriform to subglobose (6–)10–17(–22) µm diam, *conidial heads* biseriate, *metulae* covering half to entire or rarely 1/3 of vesicle, (3–)4–7(–9) × (2–)2.5–4 µm, bearing 2–3 ampuliform *phialides,* 5–8(–10) × 2–3 µm, fragmentary heads resembling penicillate fructifications occasionally present, *conidia* (Fig. 13f) spherical to subspherical, occasionally ellipsoidal to pyriform, (2.5–)3–4(–7) µm, finely roughened wall.

Aspergillus tennesseensis Jurjevic, S. W. Peterson & B. W. Horn, **sp. nov.**
MycoBank MB800604
(Fig. 14a–f)
Etymology: Isolated in Tennessee.

Type: **USA**: *Tennessee*: isol. ex toxic dairy feed, 1984, *B.W. Horn* (BPI 880917 – holotype [from dried colonies of NRRL 13150 grown 7 d at 25 °C on CYA and MEA]).

Diagnosis: Producing rough-walled conidia, no growth at 37 °C, conidial color slate green when grown on MEA.

Description: *Colonies* grown 10 d on CYA at 25 °C (Fig. 14a–b) attained 22–30 mm diam, composed of a loose hyphal mat, radially sulcate, centrally raised or sunken, overgrown by clumps of aerial hyphae in some isolates, sporulating well centrally, pea green to artemisia green (R47), scant clear exudate usually present, soluble pigment absent, reverse in brownish orange shades near honey yellow or chamois (R30). Colonies grown 10 d on MEA at 25 °C (Fig. 14c–d) attained 20–46 mm diam, low, plane, velutinous, sporulating in dark green color near slate green (R47), no exudate, no soluble pigment, reverse uncolored, pale lemon yellow, or pale brown. Incubation for 7 d on CYA at 5 °C or 37 °C produced no growth or germination of conidia.

Stipes (Fig. 14e) smooth walled, hyaline to yellowish with brownish shades, (35–)100–300(–400) × 4–7 µm, *vesicles* pyriform, (7–)10–16(–18) µm diam, *conidial heads* biseriate, *metulae* covering half to entire vesicle, 4–6(–8) × 2.5–4 µm, *phialides* 5–8(–11) × 2–3 µm, fragmentary heads resembling penicillate fructifications occasionally present, *conidia* (Fig. 14f) spherical to subspherical, occasionally ellipsoidal to pyriform, (2.5–)3–4(–8) µm, finely roughened wall.

Aspergillus venenatus Jurjevic, S. W. Peterson & B. W. Horn, **sp. nov.**
MycoBank MB800605
(Fig. 15a–h)
Etymology: Producing toxins.

Type: **USA**: *Tennessee*: isol. ex toxic dairy feed, 1984, *B.W. Horn* (BPI 880916 – holotype [from dried colonies of NRRL 13147 grown 7 d at 25 °C on CYA and MEA]).

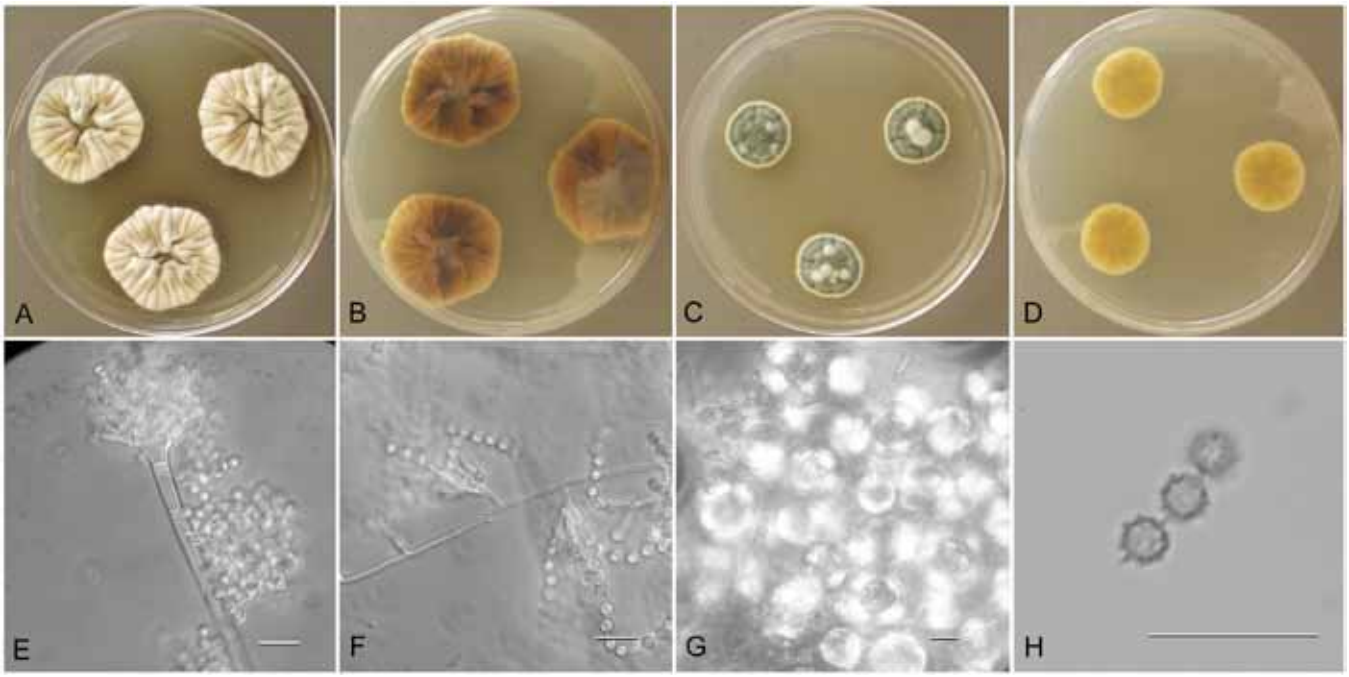


Fig. 15. *Aspergillus venenatus* (NRRL 13147), culture plates are 9 cm diam, colonies grown at 25 °C for 10 d. a. CYA colonies. b. CYA colony reverse. c. MEA colonies. d. MEA colony reverse. e. Smooth stipe, spathulate vesicle, and conidia, bar=10 µm. f. Penicillate conidiogenous cells on aerial hyphae, bar=10 µm. g. Globose hülle cells, bar=10 µm. h. Globose, spinulose conidia, bar=10 µm.

Diagnosis: Producing spinulose conidia, no growth at 37 °C, producing no exudate or soluble pigments on CYA or MEA.

Description: *Colonies* grown 10 d on CYA at 25 °C (Fig. 15a–b) attained 22–31 mm diam, radially sulcate, sporulating centrally in artemisia green (R47) to deep bluish gray-green (R42) in one isolate, no exudate, no soluble pigment, reverse deep olive buff to tawny or brown (R15). Colonies grown 10 d on MEA at 25 °C (Fig. 15c–d) attained 17–24 mm diam, lightly sulcate, low, central tufted funicular aggregates of aerial hyphae, sporulating well in deep green color near slate green (R47), no exudate, no soluble pigment, reverse pale lemon yellow, chamois, or light olive drab. Incubation for 7 d on CYA at 5 °C or 37 °C produced no growth or germination of conidia.

Stipes (Fig. 15e) smooth walled, hyaline to yellow with brownish shades, (20–)100–400(–500) × 4–7 µm, *vesicles* pyriform to spatulate, (6–)9–17(–21) µm diam, *conidial heads* biseriate, *metulae* covering half to entire vesicle, (3–)4–7(–9) × 2.5–4(–5) µm, *phialides* (5–)6–8(–11) × 2–3(3.5) µm, fragmentary heads resembling penicillate fructifications (Fig. 15f) commonly present, *hülle cells* (Fig. 15g) spherical, present in some isolates, *conidia* (Fig. 15h) spherical to subspherical, occasionally ellipsoidal to pyriform, 3–4(–6) µm diam, spinulose.

Phenotypic species recognition.

Growth rates of species on different media are presented in Table 2.

Phenotypic recognition of species in section *Versicolores* is based on smooth, roughened or spinulose conidia, conidial color, exudate and soluble pigment colors on CYA and MEA, growth rates and ability to grow at 37 °C, and on the uniform presence of hülle cells in one species.

Aspergillus cvjetkovicii, A. sydowii and *A. venenatus* isolates produce spinulose conidia. *A. sydowii* isolates grow at 37 °C, while *A. cvjetkovicii* and *A. venenatus* isolates do not. *A. cvjetkovicii* isolates produce reddish exudate and soluble pigment on CYA, while *A. venenatus* isolates produce no exudate or soluble pigment.

Aspergillus amoenus, A. austroafricanus and *A. tabacinus* produce smooth-walled conidia. Of these only *A. amoenus* isolates grow at 37 °C. *A. tabacinus* isolates produce no soluble pigment and *A. austroafricanus* produces reddish brown soluble pigment when grown on CYA.

The remaining eight species produce conidia with noticeably roughened walls, but the ornamentation is not pronounced enough to be considered spinulose. Two of the eight species, *A. versicolor* and *A. fructus*, have roughened conidial walls and grow at 37 °C. These two species are very similar but have somewhat distinct stipe lengths of 150–400 µm in *A. fructus* versus 200–750 µm in *A. versicolor*. We examined only two *A. fructus* isolates and five *A. versicolor* isolates and while separation of these species using phenotype on standard media appears possible, until more isolates are seen, it is recommended that strains be identified from gene sequences such as beta tubulin or calmodulin. Genealogical concordance species recognition clearly distinguishes these sibling species (Fig. 1).

Species with roughened conidia that do not grow at 37 °C are *A. protuberus, A. creber, A. jensenii, A. puulaauensis, A. subversicolor* and *A. tennesseensis*. *Aspergillus protuberus* isolates on CYA produce a red exudate (near scarlet R1) and a vinaceous or yellow soluble pigment, and MEA cultures are floccose. *A. jensenii* isolates produce brown CYA colony reverse colors from tawny olive to dark umber, and conidial color is near celandine green (R47). All *A. puulaauensis* isolates produce spherical hülle cells when

Table 2. Colony diameters (mm) of section *Versicolores* species on various media after 7d. Incubation at 25 °C except where noted.

Species	CYA	MEA	CY20	M40Y	M60Y	CYA at 37 °C
A. amoenus	20–29	11–15	11–20	14–22	15–24	6
A. austroafricanus	18–19	16–17	24–25	18–19	17–18	-
A. creber	17–23	11–15	15–22	18–23	19–24	-
A. cvjetkovicii	15–21	14–17	17–20	16–24	15–25	-
A. fructus	13–20	10–16	9–17	10–23	10–20	4
A. jensenii	16–20	9–13	15–20	21–26	22–28	-
A. protuberus	17–25	11–18	14–22	21–24	20–24	-
A. puulaauensis	18–21	11–12	17–19	19–22	18–21	-
A. subversicolor	13–14	6–7	10–11	15–16	16–18	-
A. sydowii	20–25	20–25	21–26	23–26	23–27	8
A. tabacinus	21–26	12–16	21–26	8–23	8–23	-
A. tennesseensis	20–22	12–14	17–19	19–22	19–22	-
A. venenatus	14–17	8–10	15–16	19–23	17–21	-
A. versicolor	20–26	10–18	19–22	21–26	22–25	8

Table 3. Predicted species identity based on ITS genotype and correlation of ITS genotypes and species in section *Versicolores*. ITS geneotypes were assigned arbitrary letter designations and species are determined by genealogical concordance.

ITS genotype	Predicted species
A	*A. amoenus, A. fructus, A. protuberus, A. tabacinus, A. versicolor*
B	*A. subversicolor*
C	*A. austroafricanus*
D	*A. cvjetkovicii, A. jensenii, A. tennesseensis, A. venenatus*
E	*A. sydowii*
F	*A. sydowii*
G	*A. amoenus*
H	*A. tabacinus*
I	*A. creber, A. versicolor*
J	*A. puulaauensis*
K	*A. creber*
L	*A. creber*
M	*A. jensenii*
N	*A. creber*

grown on M40Y medium and the species is distinguished by this consistent character. One isolate each of *A. versicolor* and *A. amoenus* (both grow at 37 °C) and one isolate of *A. jensenii* also produced hülle cells on M40Y. *A. subversicolor* isolates are relatively slow growing on MEA and M40Y (Table 2) and produce faint yellow soluble pigment on CYA. *A. tennesseensis*, when grown on MEA produce very dark green conidial areas (near slate green R47) not produced by other rough-spored species in the section. *Aspergillus creber* isolates produce no soluble pigment on either CYA or MEA, and conidial color on either medium is pea green to sage green (R47).

There is considerable variation in colony appearance within species and considerable overlap in colony appearance between species, making species separation within section *Versicolores* challenging. In addition, some of the isolates included in this study were propogated *in vitro* for several decades prior to preservation by lyophilization. Among those isolates, several appear to have mutated and consequently produce colonies that have a wet appearance when grown on CYA or produce only moist aerial aggregates of hyphae with little sporulation. Identification of these degenerate strains relies on DNA sequence analysis. DNA sequence analysis is the most reliable means for identifying species within this section.

ITS region genotypes from species in section *Versicolores* are presented in Table 3. Some genotypes are shared by two or more species. Genotype A is present in isolates of five different species and genotype D is present in four different species of the section. Isolates of some species, such as *A. creber* (genotypes I, K, L N), display two to four ITS genotypes within species.

DISCUSSION

Initial phenotypic examination of *Aspergillus* section *Versicolores* isolates was made using CYA cultures grown for 7 d at 25 °C (Klich & Pitt 1988). Those cultures did not provide sufficient data to reliably identify the species. Subsequently we tried culturing the isolates for 10 d at 25 °C on CYA to allow for further development of exudate, soluble pigment and conidial color. Raper & Fennell (1965) used incubation times of generally 10–14 d. We found that incubation for 10 d is necessary for characterizing isolates of section *Versicolores*.

Only four of the available genetic loci were used in preparing the combined data tree (Fig. 1). The ITS region was not included because it contained few informative nucleotides and because its veracity as a phylogenetic indicator is questionable (Galagan *et al.* 2005). The ITS data themselves however may be of interest for bar-coding studies (discussed later). The beta tubulin sequences from section *Versicolores* are of the "two intron" type and probably have a different evolutionary origin than the "three intron" type of beta tubulin found in the out-group species (Peterson 2008). Because of the suspected paralogy of this molecule, it was not included in the combined data tree. It was included as a possible target for DNA sequence-based identification of isolates.

Henig (1966) in his work on systematics required that all taxa be monophyletic. When working with phenotypic characters in section *Versicolores*, it was difficult to identify the informative characters that could satisfy Henig's requirement. Analysis of DNA sequences from unlinked loci using concordance (Taylor *et al*. 2000, Dettman *et al.* 2003) makes it possible to define monophyletic groups. Phylogenetic recognition of species occasionally makes it necessary to accept cryptic species (Perrone *et al*. 2011) because the phenotypic characters of the species overlap with their siblings to such an extent that the species cannot be reliably identified without molecular tools. For NRRL 530 and NRRL 13151 that form single isolate lineages, reliable characters to define the species have not been found, but with the identification of additional isolates it may be possible to phenotypically characterize and subsequently name these species. For *A. versicolor* and *A. fructus* the limited number of isolates and the observed intraspecific variation reduce confidence in the current phenotypic recognition of the species, but the phylogenetic data are unequivocal and so *A. fructus* was described as new.

Prior to this publication *A. versicolor* was a species with documented genetic and phenotypic variation that did not resolve into clearly recognizable species. Fourteen species are now known in section *Versicolores* and the ITS region variation is ca. 3 % as calculated from the data herein. By comparison ca. 4 % variation is found in the *Petromyces* clade (*Aspergillus* sect. *Flavi*) between *P. flavus* and *P. nomius* and 14 species have been named (Varga *et al.* 2009). In the *Petromyces* clade, one species may possess a phenotype very similar to another species (Kurtzman *et al.* 1987, Peterson *et al.* 2001, Soares *et al.*, 2012). While the validity of some species in the *Petromyces* clade have been questioned (Varga *et al.* 2009), phylogenetic distinction has served to validate species (Peterson 2008, Varga *et al.* 2009) regardless of the phenotypic similarities or overlapping character states of the species. Peterson (2008) suggested that sect. *Versicolores* could easily be dropped from *Aspergillus* taxonomy. This much broader study of *A. versicolor sensu lato* isolates suggests that section *Versicolores* should be retained as a monophyletic and useful subgeneric designation.

Aspergillus versicolor is the most reported fungal species in section *Versicolores* from damp indoor environments (Jussila 2003, Rydjord *et al.* 2005) and its presence is used as an indicator of Sick Building Syndrome (SBS) (Schwab & Straus 2004). We amplified each newly described species using *A. versicolor*-specific primers (Dean *et al*. 2005; data not shown) and obtained a positive signal in all cases except for *A. subversicolor* and *A. sydowii*; therefore the primer set retains its usefulness. In *A. creber* and *A. jensenii* some isolates did not amplify even though the genotypes were identical with isolates that did amplify, suggesting degradation or incorrect quantitation of the genomic DNA.

Twenty-four sect. *Versicolores* isolates in this study were obtained by one of us (ZJ) from air samples in buildings, but none comprised *A. versicolor* sensu stricto (Table 1). Of the five *A. versicolor* isolates examined, three were isolated from a single lot of toxic cattle feed in the USA and the substrate for the other two isolates, one from the USA and the other from South Africa, was not recorded. The species is widespread geographically, but was not commonly encountered among the isolates used in this study. *Aspergillus creber* was the most frequently isolated species from indoor air samples in the USA (13 strains from six states), followed by *A. protuberus* (five strains from two states) and *A. jensenii* (four strains from three states). *Aspergillus versicolor sensu stricto* may not be common in buildings. Two other species, *A. cvjetkovicii* and *A. puulaauensis*, were each isolated once from indoor air. The other newly described species, *A. fructus, A. austroafricanus*, *A. subversicolor, A. tennesseensis* and *A. venenatus*, were isolated from plant material or had unknown sources (Table 1). Amend *et al.* (2010) reported that fungi isolated from indoor air sources (e.g., dust, carpet) are highly diverse in the temperate regions of the world and are much less diverse in tropical regions. Therefore, our strains from indoor air samples from the USA in addition to culture collection strains from many regions of the world may represent much of the diversity present in section *Versicolores*.

There is considerable interest in using ITS sequences for bar-coding identification of fungi, particularly for large-scale ecological studies (Begerow *et al.* 2010, Schoch *et al*. 2012). In section *Versicolores* species, one particular ITS genotype is present in isolates of five different species and another genotype is found in four different species (Table 3). Because ITS genotypes do not uniquely identify species in this section, use of multiple loci is the most reliable means of DNA sequence-based identification in section *Versicolores* (Peterson 2012).

Viable propagules of *A. versicolor* have been recovered from the highly saline Dead Sea (Kis-Papo *et al*. 2003), showing an ability to survive conditions of salinity or drying. The ARS Culture Collection contains a few putative *A. versicolor* isolates obtained from brined meats in the UK. Upon sequence and phenotypic analysis, these isolates were

identified as three species, *A. amoenus*, *A. tabacinus* and *A. protuberus,* all of which occur in the *A. versicolor* subclade (Fig. 1). Additionally, *A. creber* NRRL 6544, from the *A. sydowii* subclade, was isolated from a tar ball floating in the Atlantic Ocean. High tolerance to salinity may extend to other species in section *Versicolores*. *Aspergillus versicolor* has also been identified from dust collected in the International Space Station (Vesper *et al*. 2008). In addition to CYA and MEA we used high sugar content media (CY20S, M40Y and M60Y) containing 20, 40 or 60 % sucrose, respectively. All isolates grew well on all of the media (Table 2), with no noticable reduction in growth rates even on M60Y medium. Species from section *Versicolores* have a remarkably broad tolerance for a wide range of water activity of their substrates.

Aspergillus versicolor isolates produce the aflatoxin precursor sterigmatocystin, a compound that is mutagenic and tumorigenic (Veršilovskis & Saeger 2010). Animal feed infested with three morphotypes of *A. versicolor*, all of which produce sterigmatocystin, have been implicated in dairy animal toxicosis, but it is unknown whether sterigmatocystin caused the toxicosis (Vesonder & Horn 1985). Those three morphotypes are now identified as *A. versicolor, A. tennesseensis* and *A. venenatus*, and as these species occur in the two main subclades of section *Versicolores* (Fig. 1), sterigmatocystin production may be present in additional species. The distribution of section *Versicolores* species in agricultural commodities and their role in stigmatocystin toxicoses require additional study. In addition to sterigmatocystin, recent studies have revealed numerous metabolites with biological activities (Finefield *et al.* 2011, Lee *et al.* 2011) from *A. versicolor sensu lato* Jaio *et al.* (2007) discovered novel nucleotide analogs from *A. puulaauensis* which was reported under the name *A. versicolor*.

Aspergillus versicolor has been implicated as the causitive agent of disseminated aspergillosis in dogs (Zhang *et al.* 2012), has probably caused aspergillosis in transplant recipients (Baddley *et al*. 2009), and has been isolated from the infected eye of a patient suffering from HIV (Perri *et al.* 2005). We included two section *Versicolores* clinical isolates in our study. NRRL 254 was identified as *A. sydowii* and NRRL 226, originally identified as *A. versicolor*, is here identified as *A. amoenus*. Because of different sensitivities of fungal species to fungal antibiotics, a more detailed study of *A. versicolor* clinical isolates might be of value to guide appropriate therapeutic regimens (Pfaller *et al.* 2011).

ACKNOWLEDGEMENTS

We thank Amy E. McGovern for valuable technical support. Patricia Eckel kindly advised us on Latin usage. David L. Hawksworth advised us on some nomenclatural issues. The authors thank Donald T. Wicklow (USDA, Peoria, IL) and Lynne Sigler (University of Alberta, Edmonton, Alberta) for the gift of Hawaiian and Canadian isolates. Mention of a trade name, proprietary product, or specific equipment does not constitute a guarantee or warranty by the United States Department of Agriculture and does not imply its approval to the exclusion of other products that may be suitable. USDA is an equal opportunity provider and employer.

REFERENCES

Amend AS, Seifert KA, Samson R, Bruns TD (2010) Indoor fungal composition is geographically patterned and more diverse in temperate zones than in the tropics. *Proceedings of the National Academy of Sciences*, USA **107**: 13748–13753.

Anderson B, Frisvad JC, Sondergaard I, Rasmussen IB, Larsen LS (2011) Associations between fungal species and water-damaged building materials. *Applied and Environmental Microbiology* **77**: 4180–4188.

Baddley JW, Marr KA, Andes DR, Walsh TJ, Kauffman CA, Kontoyiannis DP, Ito JI, Balajee SA, Pappas PG, Moser SA (2009) Patterns of susceptibility of *Aspergillus* isolates recovered from patients enrolled in the transplant-associated infection surveillance network. *Journal of Clinical Microbiology* **47**: 3271–3275.

Baum DA, Shaw KL (1995) Genealogical perspectives on the species problem. In: *Experimental and Molecular Approaches to Plant Biosystematics* (PC Hoch & AG Stephenson, eds): 289–303. St Louis. Mo: Missouri Botanical Garden.

Begerow D, Nilsson H, Unterseher M, Maier W. (2010) Current state and perspectives of fungal DNA barcoding and rapid identification procedures. *Applied Microbiology and Biotechnology* **87**: 99–108.

Dean TR, Roop B, Betancourt D, Menetrez MY (2005) A simple multiplex polymerase chain reaction assay for the identification of four environmentally relevant fungal contaminants. *Journal of Microbiological Methods* **61**: 9–16.

Dettman JR Jacobson DJ, Turner E, Pringle A, Taylor JW (2003) Reproductive isolation and phylogenetic divergence in *Neurospora*: comparing methods of species recognition in a model eukaryote. *Evolution* **57**: 2721–2741.

Domsch KH, Gams W, Anderson TH (1980) *Compendium of Soil Fungi* Vol. 1. Academic Press, UK.

Edmondson DA, Barrios CS, Brasel TL, Straus DC, Kurup VP, Fink JN (2009) Immune response among patients exposed to molds. *International Journal of Molecular Sciences* **10**: 5471-5484.

Engelhart S, Loock A, Skutlarek D, Sagunski H, Lommel A, Farber H, Exner M (2002) Occurrence of toxigenic *Aspergillus versicolor* isolates and sterigmatocystin in carpet dust from damp indoor environments. *Applied and Environmental Microbiology* **68**: 3886–3890.

Finefield JM, Sherman DH, Tsukamoto S, Williams RM (2011) Studies on the biosynthesis of the notoamides: synthesis of an isotopomer of 6-hydroxydeoxybrevianamide E and biosynthetic incorporation into notoamide J. *Journal of Organic Chemistry* **76**: 5954–5958.

Galagan JE, Calvo SE, Cuomo C, Ma L-J, Wortman JR, Batzoglou S, Lee S-I, Batürkmen M, Spevak CC, Clutterbuck J, Kapitonov V, Jurka J, Scazzocchio C, Farman M, Butler J, Purcell S, Harris S, Braus GH, Draht O, Busch S, D'Enfert C, Bouchier C, Goldman GH, Bell-Pedersen D, Griffiths-Jones S, Doonan JH, Yu J, Vienken K, Pain A, Freitag M, Selker EU, Archer DB, Peñalva MA, Oakley BR, Momany M, Tanaka T, Kumagai T, Asai K, Machida M, Nierman WC, Denning DW, Caddick M, Hynes M, Paoletti M, Fischer R, Miller B, Dyer P, Sachs MS, Osmani SA, Birren BW (2005) Sequencing of *Aspergillus nidulans* and comparative analysis with *A. fumigatus* and *A. oryzae. Nature* **438**: 1105–1115.

Gams W, Christensen M, Onions AHS, Pitt JI, Samson RA (1985) Infrageneric taxa of *Aspergillus*. In: *Advances in* Penicillium *and*

Aspergillus *Systematics* (RA Samson & JI Pitt, eds): 55–64. New York: Plenum Press.

Hennig W (1966) *Phylogenetic Systematics*. [English translation.] Urbana, IL: University of Illinois Press.

Huelsenbeck JP, Ronquist F (2001) MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* **17**: 754–755.

Jiao P, Murdur SV, Gloer JB, Wicklow DT (2007) Kipukasins, nucleoside derivatives from *Aspergillus versicolor*. *Journal of Natural Products* **70**: 1308–1311.

Jussila J (2003) *Inflammatory Responses in Mice after Intratracheal Instillation of Microbes Isolated from Moldy Buildings.* Helsinki, Finland: National Public Health Institute.

Kis-Papo T, Kirzhner V, Wasser SP, Nevo, E (2003) Evolution of genomic diversity and sex at extreme environments: fungal life under hypersaline Dead Sea stress. *Proceedings of the National Academy of Sciences, USA* **100**: 14970–14975.

Klich MA (1993) Morphological studies of *Aspergillus* section *Versicolor* and related species. *Mycologia* **85**: 100–107.

Klich MA (2002) *Identification of Common Aspergillus Species.* Utrecht: Centraalbureau voor Schimmelcultures.

Klich MA, Pitt JI (1988) *A Laboratory Guide to Common Aspergillus Species and Their Teleomorphs*. North Ryde, NSW: CSIRO.

Kozakiewicz Z (1989) *Aspergillus species* on stored products. *Mycological Papers* **161**: 1–188.

Kurtzman CP, Horn BW, Hesseltine CW (1987) *Aspergillus nomius*, a new aflatoxin-producing species related to *Aspergillus flavus* and *Aspergillus tamarii. Antonie van Leeuwenhoek* **53**: 147–158.

Lee YM, Dang HT, Li J, Zhang P, Hong J, Lee CO, Jung JH (2011) A cytotoxic fellutamide analogue from the sponge-derived fungus *Aspergillus versicolor*. *Bulletin of the Korean Chemical Society* **32**: 3817–3820.

Mbata TI (2008) Isolation of fungi in hyper saline Dead Sea water. *Sudanese Journal of Public Health* **3**: 170–172.

Mills JT, Abramson D (1986) Production of sterigmatocystin by isolates of *Aspergillus versicolor* from western Canadian stored barley and rapeseed/canola. *Canadian Journal of Plant Pathology* **82**: 151–153.

Moreno G, Arenas R. (2010) Other fungi causing onychomycosis. *Clinics in Dermatology* **28**: 160–163.

Nielsen KF (2003) Mycotoxin production by indoor molds. *Fungal Genetics and Biology* **39**: 103–117.

Perri P, Campa C, Incorvaia C, Parmeggiani F, Lamberti G, Costagliola C, Sebastian A (2005) Endogenous *Aspergillus versicolor* endophthalmitis in an immuno-competent HIV-positive patient. *Mycopathologia* **160**: 259–261.

Perrone G, Stea G, Epifani F, Varga J, Frisvad JC, Samson RA (2011) *Aspergillus niger* contains the cryptic phylogenetic species *A. awamori. Fungal Biology* **115**: 1138–1150.

Peterson SW (2008) Phylogenetic analysis of *Aspergillus* species using DNA sequences from four loci. *Mycologia* **100**: 205–226.

Peterson SW (2012) *Aspergillus* and *Penicillium* identification using DNA sequences: barcode or MLST? *Applied Microbiology and Biotechnology* DOI 10.1007/s00253-012-4165-2.

Peterson SW, Ito Y, Horn BW, Goto T. (2001) *Aspergillus bombycis*, a new aflatoxigenic species and genetic variation in its sibling species, *A. nomius*. *Mycologia* **93**: 689–703.

Peterson SW, Jurjevic Z, Bills GF, Stchigel AM, Guarro J, Vega FE (2010) The genus *Hamigera*, six new species and multilocus DNA sequence based phylogeny. *Mycologia* **102**: 847–864.

Pfaller MA, Castanheira M, Diekema DJ, Messer SA, Jones RN (2011) Triazole and echinocandin MIC distributions with epidemiological cutoff values for differentiation of wild-type strains from non-wild-type strains of six uncommon species of *Candida*. *Journal of Clinical Microbiology* **49**: 3800–3804.

Pitt JI (1980) ["1979"] *The Genus Penicillium and Its Teleomorph States Eupenicillium and Talaromyces*. London: Academic Press.

Pitt JI, Hocking AD (2009) *Fungi and Food Spoilage*. 3rd edn. London: Springer Verlag.

Raper KB, Fennell DI (1965) *The Genus Aspergillus*. Baltimore, MD: Williams & Wilkins.

Ridgway R (1912) *Color Standards and Color Nomenclature*. Washington, DC: Ridgway.

Ronquist F, Huelsenbeck JP (2003) MrBayes3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**:1572–1574.

Rydjord B, Hetlandy G, Wikerz HG (2005) Immunoglobulin G antibodies against environmental moulds in a Norwegian healthy population shows a bimodal distribution for *Aspergillus versicolor*. *Scandinavian Journal of Immunology* **62**: 281–288.

Samson RA, Houbraken J, Summerbell RC, Flannigan B, Miller JD (2001) Common and important species of fungi and actinomycetes in indoor environments. In: *Microorganisms in Home and Indoor Work Environments* (B. Flannigan, RA Samson & JD Miller, eds): 287–292. London: Taylor and Francis.

Schoch CL, Seifert KA, Huhndorf S, Robert V, Souge JL, Levesque CA, Chen W, Fungal Barcoding Consortium (2012) Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for *Fungi*. *Proceedings of the National Academy of Sciences, USA* **109**: 6241–6246.

Schwab CJ, Straus DC (2004) The roles of *Penicillium* and *Aspergillus* in sick building syndrome. *Advances in Applied Microbiology* **55**: 215–238.

Shelton BG, Kirkland KH, Dana W (2002) Profiles of airborne fungi in buildings and outdoor environments in the United States. *Applied and Environmental Microbiology* **68**: 1743–1753.

Soares C, Rodrigues P, Peterson SW, Lima N, Venâncio A (2012) Three new species of *Aspergillus* section *Flavi* isolated from almonds and maize in Portugal. *Mycologia*: doi:10.3852/11-088.

Swofford DL (2003) *PAUP*: Phylogenetic Analysis Using Parsimony (*and Other Methods)*. Version 4. Sunderland, MA: Sinauer Associates.

Taylor JW, Jacobson DJ, Kroken S, Kasuga T, Geiser DM, Hibbett DS, Fisher MC (2000) Phylogenetic species recognition and species concepts in fungi. *Fungal Genetics and Biology* **31**: 21–32.

Thom C, Church MB (1926) *The Aspergilli*. Baltimore, MD: Williams & Wilkins.

Thom C, Raper KB (1945) *Manual of the Aspergilli*. Baltimore, MD: Williams & Wilkins.

Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTALW: improving the sensitivity of progressive multiple sequence alignments through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* **22**: 4673–4680.

Tuomi T, Reijula K, Johnsson T, Hemminki K, Hintikka EL, Llindroos O, Kalso S, Koukila-Kähkölä P, Mussalo-Rauhamaa H, Haahtela T (2000) Mycotoxins in crude building materials from water-damaged buildings. *Applied and Environmental Microbiology* **66**: 1899–1904.

Varga J, Frisvad JC, Samson RA. (2009) A reappraisal of fungi producing aflatoxins. *World Mycotoxin Journal* **2**: 263−277

Veršilovskis A, Saeger SD (2010) Sterigmatocystin: occurrence in foodstuffs and analytical methods – an overview. *Molecular Nutrition and Food Research* **54**: 136–147.

Vesonder RF, Horn BW (1985) Sterigmatocystin in dairy cattle feed contaminated with *Aspergillus versicolor*. *Applied and Environmental Microbiology* **49**: 234–235.

Vesper SJ, Wong W, Kuo CM, Pierson DL (2008) Mold species in dust from the International Space Station identified and quantified by mold-specific quantitative PCR. *Research in Microbiology* **159**: 32–435.

Zhang S, Corapi W, Quist E, Griffin S, Zhang M (2012) *Aspergillus versicolor,* a new causative agent of canine disseminated aspergillosis. *Journal of Clinical Microbiology* **50**: 187–191.

SUPPLEMENTARY INFORMATION

Supplemental Fig 1

BT2 locus, 643 characters: 534 are constant
45 are variable but parsimony-uninformative
64 are parsimony-informative; <100 mp trees,
CI=0., RC=0.

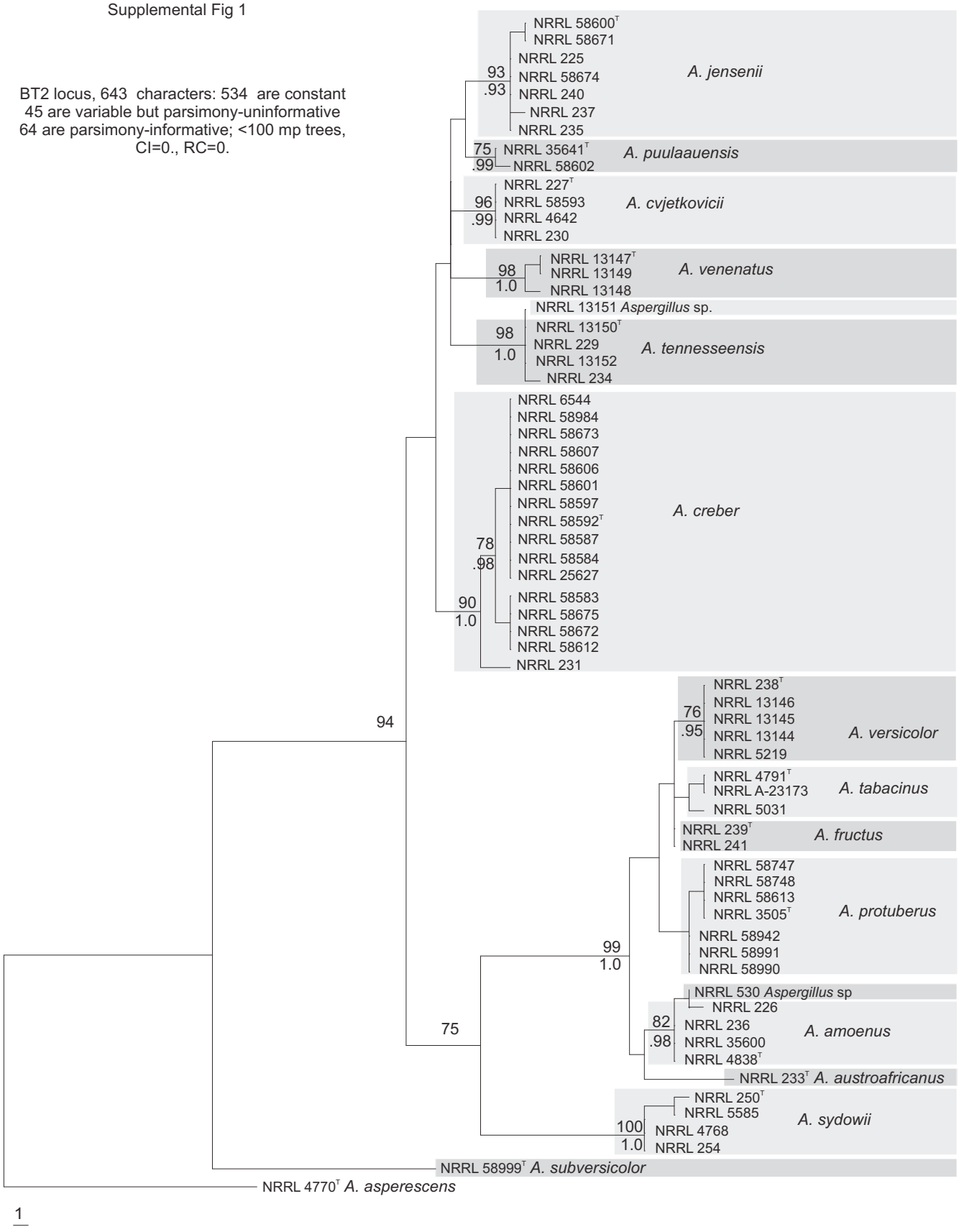


Fig. S1. Phylogenetic tree based on beta tubulin sequences; Bayesian posterior probabilities and bootstrap values based on 1000 replicates are placed on the internodes where values are significant.

SUPPLEMENTARY INFORMATION

Supplemental Fig. 2

Calmodulin locus, 694 characters: 510 are constant
86 are variable but parsimony-uninformative
98 are parsimony-informative; 18 mp trees
CI=0.8617, RC=0.8337

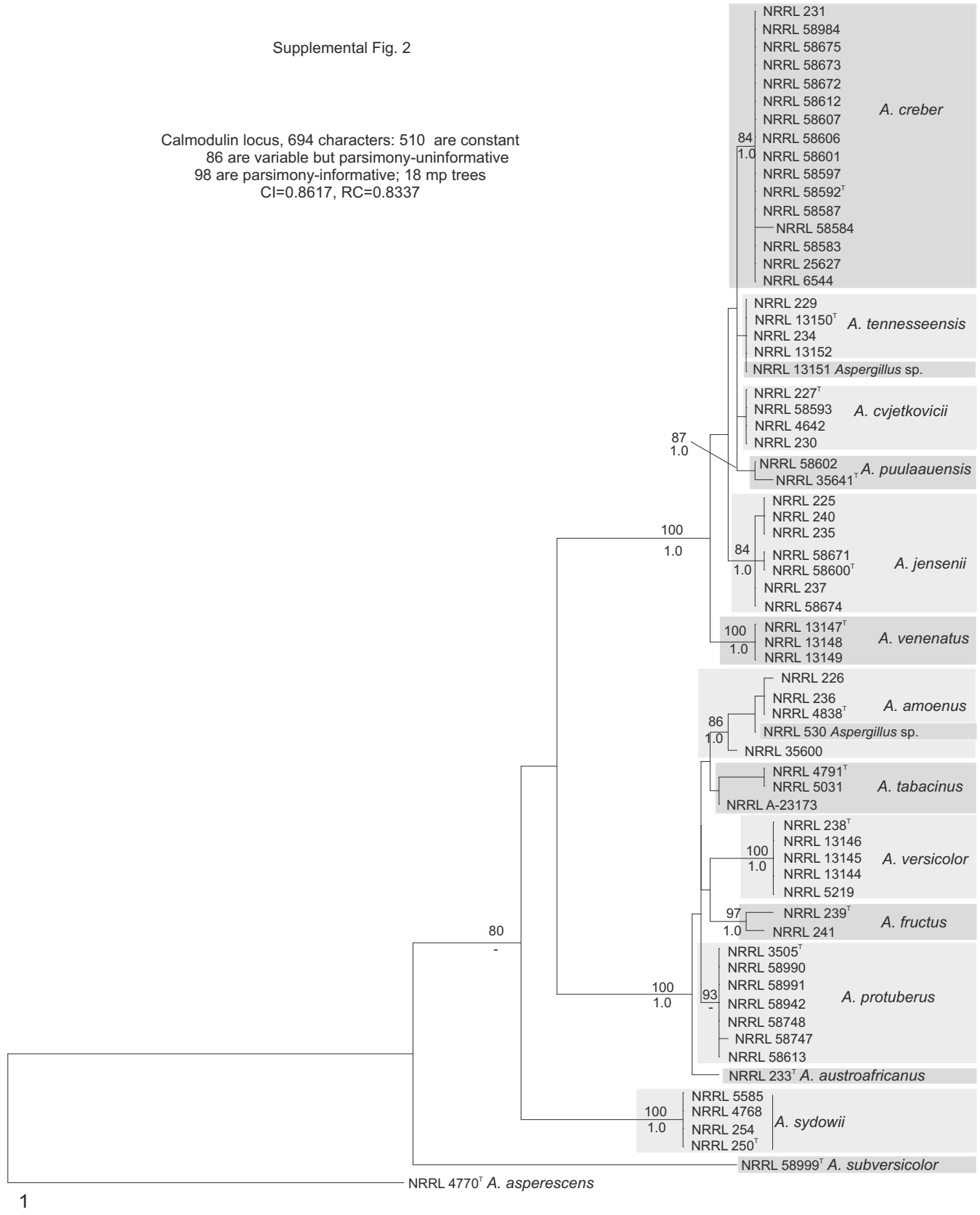


Fig. S2. Phylogenetic tree based on calmodulin sequences; Bayesian posterior probabilities and bootstrap values based on 1000 replicates are placed on the internodes where values are significant.

SUPPLEMENTARY INFORMATION

Supplemental Fig. 3

Mcm7 locus, 616 characters: 476 are constant, 72 are variable but parsimony-uninformative, 68 are parsimony-informative; 2 mp trees, CI=0.8324, RC=0.8074

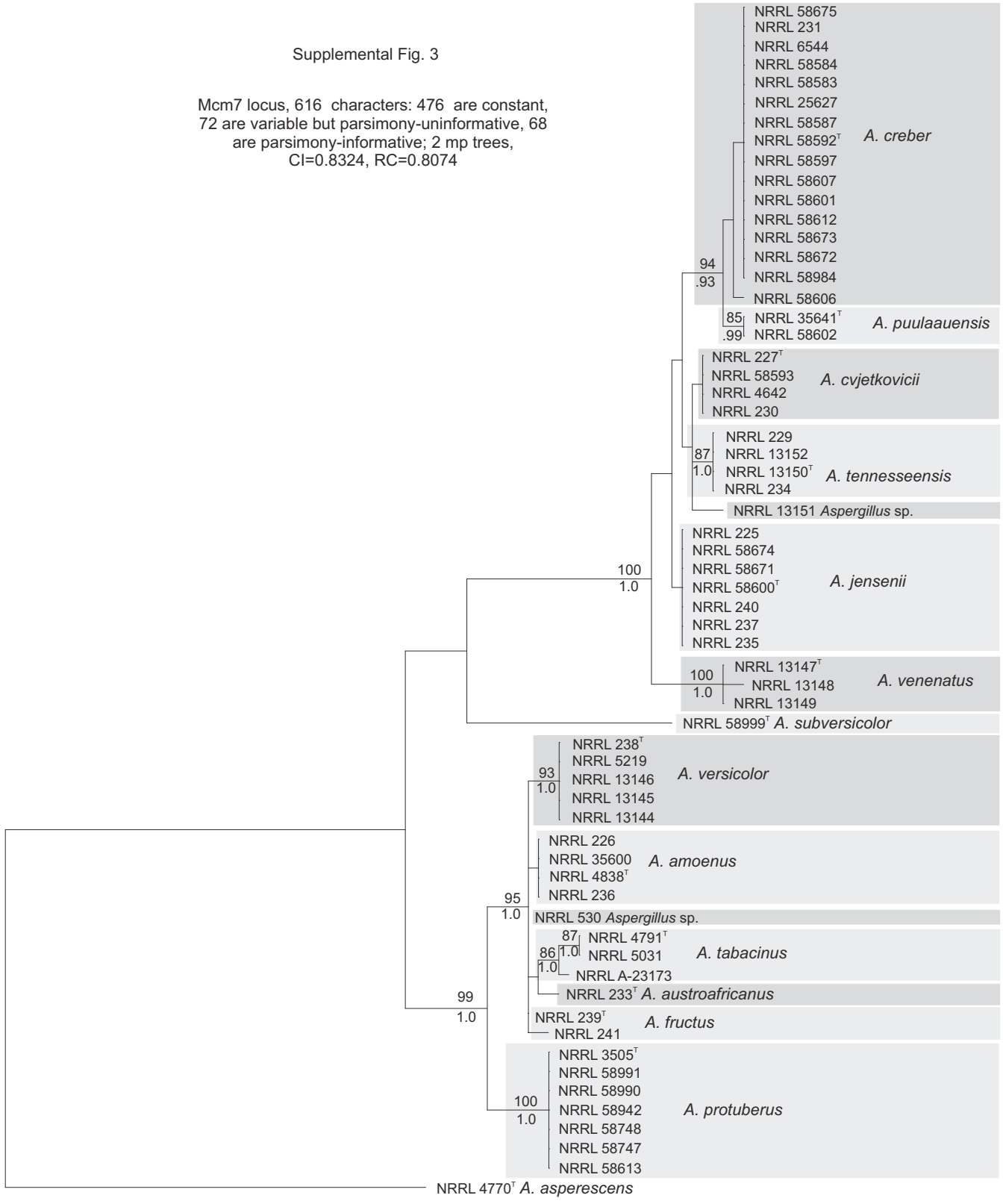


Fig. S3. Phylogenetic tree based on *Mcm7* locus sequences; Bayesian posterior probabilities and bootstrap values based on 1000 replicates are placed on the internodes where values are significant.

SUPPLEMENTARY INFORMATION

Supplemental Fig. 4

RPB2 locus, 1011 characters: 801 are constant, 88 are variable but parsimony-uninformative, 122 are parsimony-informative; 6 mp trees, CI=0.7935, RC=0.7585.

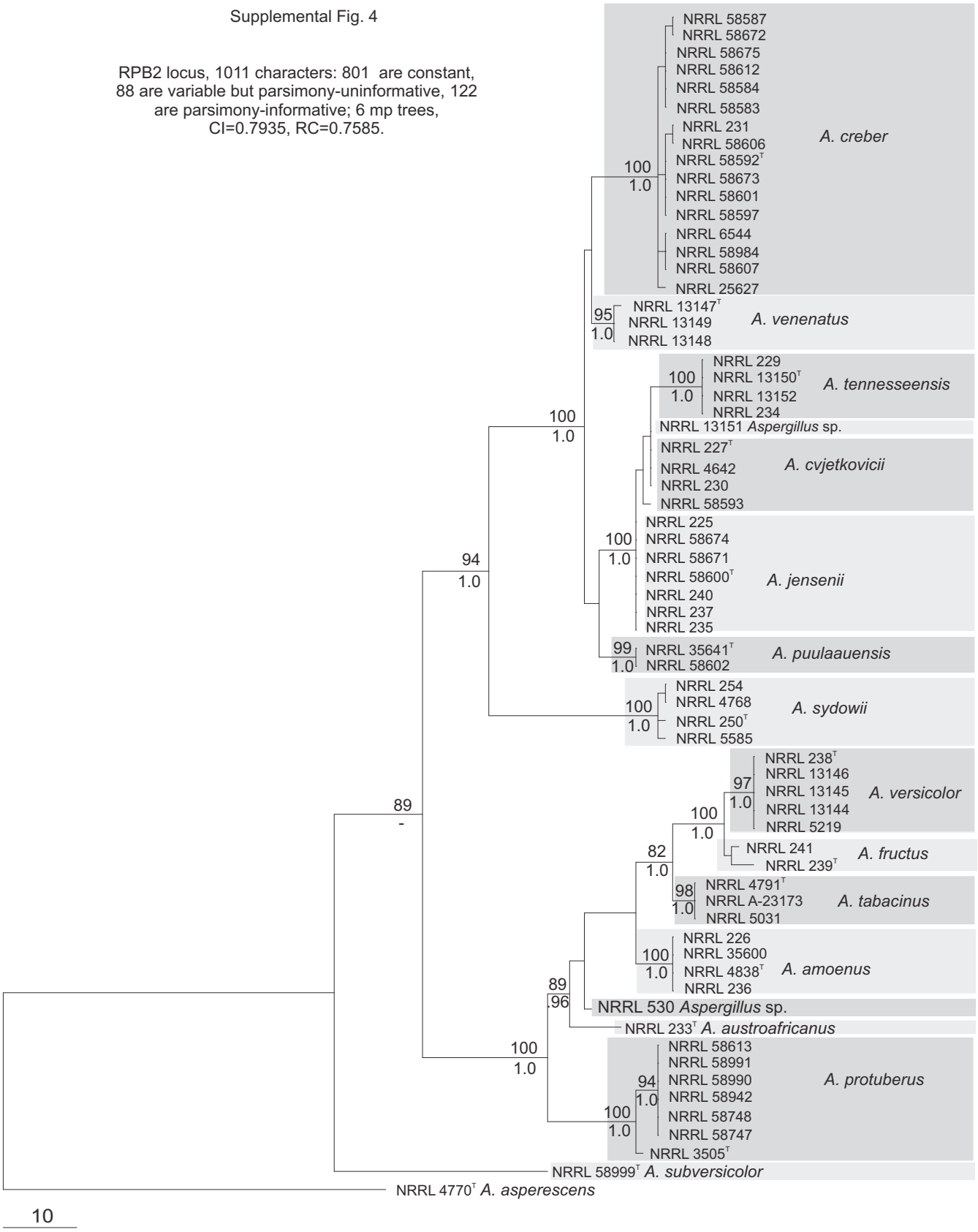


Fig. S4. Phylogenetic tree based on *RPB2* locus sequences; Bayesian posterior probabilities and bootstrap values based on 1000 replicates are placed on the internodes where values are significant.

SUPPLEMENTARY INFORMATION

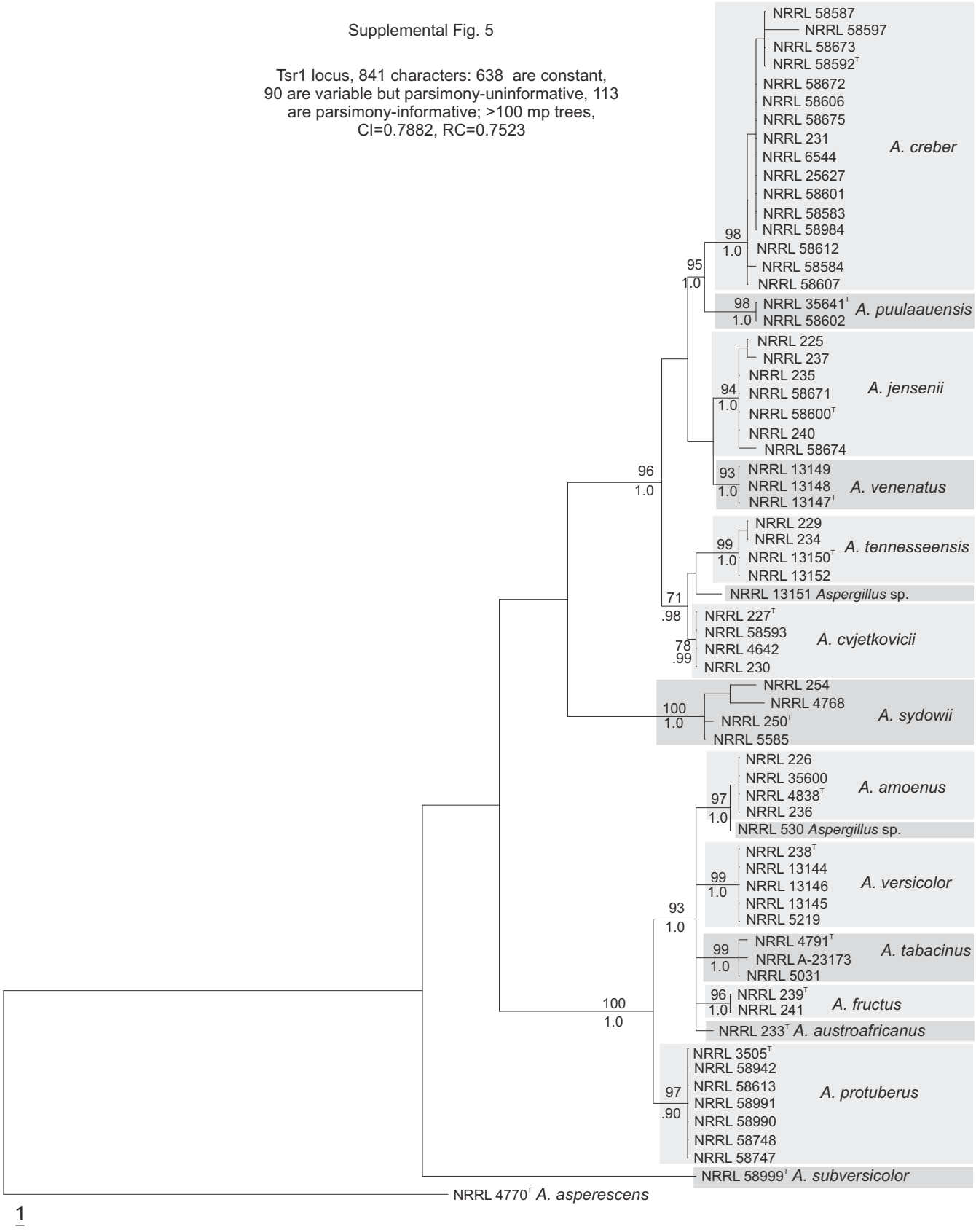


Fig. S5. Phylogenetic tree based on *Tsr1* locus sequences; Bayesian posterior probabilities and bootstrap values based on 1000 replicates are placed on the internodes where values are significant.

doi:10.5598/imafungus.2012.03.01.08

IMA FUNGUS · VOLUME 3 · NO 1: 81–86

ARTICLE

The impacts of the discontinuation of dual nomenclature of pleomorphic fungi: the trivial facts, problems, and strategies

Uwe Braun

Martin-Luther-Universität, Institut für Biologie, Bereich Geobotanik und Botanischer Garten, Herbarium, Neuwerk 21, 06099 Halle (Saale), Germany; corresponding author's e-mail: uwe.braun@botanik.uni-halle.de

Abstract: The symposium "One fungus = Which name" held in Amsterdam 12–13 April 2012, addressed the drastic changes in the naming of pleomorphic fungi adopted by the 18th International Botanical Congress in Melbourne in 2011. Possible solutions and ways to face resulting problems were suggested. The fundamental change is that under the new rules fungi in future will be treated nomenclaturally like plants and all other groups of organisms ruled by the ICN, i.e. with one correct name for each species. Numerous discussions and statements during the Symposium reflected widespread anxieties that these rules could negatively influence taxonomic work on pleomorphic fungi. However, they are groundless, being based on misunderstandings and confusion of nomenclature and taxonomy. With pleomorphic fungi, taxonomists will in future have to answer the question whether different morphs can represent one fungus (taxon), but this remains a taxonomic decision and has nothing to do with nomenclature. Furthermore, the ICN does not and cannot rule on how this decision is made. Thus it cannot provide rules based solely on methods involving morphology *in vivo* or *in vitro*, molecular analyses, physiological and biochemical data, inoculation experiments in pathogenic groups or any other methods or combinations of them. It is up to the taxonomist to select appropriate methods and to decide which data are sufficient to introduce new taxa. Some future problems and strategies around the application of anamorph- and teleomoph-typified taxon names (genera and species), are discussed here, using the recently monographed powdery mildews (*Erysiphales*) as an example.

Key words:
anamorph
Article 59
Erysiphales
fungi
International Code of Nomenclature for algae fungi, and plants
teleomorph

Article info: Submitted: 21 May 2012; Accepted: 1 June 2012; Published: 21 June 2012.

INTRODUCTION

During the KNAW-CBS Fungal Diversity Centre-organized symposium "One fungus = One name" held in Amsterdam in April 2011 ways to overcome dual nomenclature in pleomorphic fungi were discussed culminating in the "Amsterdam declaration" (Hawksworth *et al*. 2011) with recommendations on how to deal with such fungi in future. However, all aspects of this declaration did not receive general acceptance, and opposing arguments were also presented and published (Gams *et al.* 2011). A few months later, the sweeping decisions of the 18th International Botanical Congress in Melbourne, Australia, in July 2011 nullified the opposing viewpoints, discussions and proposals of the first Amsterdam Symposium, rendering the Amsterdam Declaration a 'fait accompli'.

Various proposals to emend the International Code of Botanical Nomenclature adopted by the Melbourne Congress caused worldwide surprise to most mycologists and can be considered revolutionary. The possibility to publish valid diagnoses or descriptions of new taxa in English besides Latin in future, the recognition of effective electronic publications of new taxa under certain, defined conditions, the mandatory requirement to deposit new fungal names in a recognized repository, the renaming of the *Code* (now the "International Code of Nomenclature for algae, fungi, and plants"), and some other changes have been accepted by the overwhelming majority of mycologists and are welcome. Detailed discussions and explanations of the Melbourne decisions have been published by Hawksworth (2011), Knapp *et al*. (2011), and Norvell (2011). However, the abolition of the special provisions of the previous Art. 59 of the ICN, allowing the separate naming of morphs of pleomorphic fungi, which was based on the most drastic 'floor' proposal concerning this Article made by Scott A. Redhead (the Secretary of a Committee appointed by the Vienna Congress in 2005 to address this matter) among two other less drastic ones (Norvell 2011), was unexpected and a shock to most mycologists. After the first shock, followed by deeper objective considerations of the consequences, advantages and disadvantages of the

new rules for fungi I came to the conclusion that these drastic changes are probably the best solution, since they provide a good prospect of more stability and flexibility in future and should prevent endless discussions and attempts to modify the old Art. 59. However, reactions and comments by numerous mycologists worldwide after the first symposium held in Amsterdam in 2011 ("One fungus = One name") and the Melbourne decisions, as well as various discussions during the second Amsterdam conference in 2012 ("One fungus = Which name?") revealed widespread anxieties that the new rules could negatively influence future taxonomic work with pleomorphic fungi. Viewed objectively, however, most of the discussed problems and obvious reservations are mainly based on a confusion of nomenclature and taxonomy, i.e. they have nothing to do with the changed rules and reflect a widespread misunderstanding concerning the function of the *Code*.

Various problems and open questions have already been addressed by Gams *et al*. (2012), and the present paper adds to the debate by addressing some further more minor points. Strategies to overcome problems and to prepare the mycological community for the enormous load of work caused by the new rules are also discussed using powdery mildews (*Erysiphales*) as an example. Comments, explanations and proposals summarized in this paper are based on a lecture given during the second Amsterdam symposium, discussions during this meeting, and other critical notes, enquiries and discussion between the first and second Amsterdam symposia.

GENERAL NOTES, PROBLEMS, AND STRATEGIES

Special problems at the generic level

At the generic level, the new rules provide obvious advantages and more freedom for the application of anamorph-typified genus names, which are now treated equally for priority purposes, so that they may now be used as holomorph names, i.e. for all morphs belonging to one fungus. Names of an anamorph-typified genus and a teleomorph-typified genus now compete nomenclaturally, if they belong to one taxon ("one fungus"). If in this case the anamorph genus represents the oldest valid and legitimate name, and it is the most widely used and preferred, (e.g. *Aspergillus*, *Cladosporium*, *Penicillium*), this name has priority over any younger meiosporic genus and can be applied and used immediately as the name for all morphs involved (holomorph). This applies, for instance, in the case of *Cladosporium* Link 1816, v. *Davidiella* Crous & U. Braun 2003. If anamorph-typified genera are younger but nevertheless preferred, proposals may be made in future to accept these genus names. If a teleomorph-typified genus name is younger, it may also be proposed as the name for all morphs. The procedures for such proposals, which can be submitted as Lists of entire fungal groups, are outlined in Art. 4.13 and Art. 56.3 of the new version of the *Code*. Hence, in future we have a high degree of flexibility in the application of competing names at generic rank.

However, problems in the application of genus names are usually connected with their typification and taxonomic implications. Anamorph as well as teleomorph genera are ruled by typification, i.e. by their type species. In cases where we indeed have "one fungus" that deserves "one name", decisions regarding synonymy can be made on the basis of molecular examinations (preferred), associated development of anamorphs and teleomorphs in culture or any other methods. This is not under the jurisdiction of the *Code*. As the application of all fungal names is ruled by their types, it is necessary to have convincing data for the type species of both, the anamorph-typified genus and the teleomorph-typified genus, showing that the taxa concerned are, indeed, congeneric. However, we have often only molecular or other indications that certain anamorph and teleomorph genera are probably congeneric merely based on data derived from non-type species. Fortunately in such cases, the synonymy of these generic names can also be proposed. This is then just a taxonomic decision leading to a proposal which in any case is allowed and is not under the jurisdiction of the *Code*. The *Code* only rules which name has to be adopted in this case of facultative synonymy. Any treatments and concepts of genera are possible, e.g. widening or reducing the circumscriptions, and in an extreme case reducing them to a monotypic genus only containing the type species, and these modifications are only nomenclaturally, not taxonomically, ruled by the *Code*.

Other problems, also discussed during the Amsterdam Symposium in April this year, concern the naming of often numerous phylogenetically unproven species previously assigned to a certain anamorph genus whose name, based on its type species, is now considered synonymous with (part of) a holomorph name. Allocations of species to certain genera are taxonomic decisions, not ruled by the *Code*, and can be done on the basis of any method, ranging from morphology to molecular sequence analysis. If an anamorph-typified generic name is reduced to synonymy with a teleomorph-typified generic name, based on molecular data referring to their two type species, it would be theoretically possible, but not in all cases advisable, to re-allocate all species names previously assigned to the anamorph genus to the teleomorph genus name that now has priority. The phylogenetically unproven species can be retained in the anamorph genus, which is then only a facultative (heterotypic) synonym.

According to the new Art. 59, names published prior to 1 January 2013 for the same taxon, but based on different morphs, are neither considered to be alternative names according to Art. 34.2 nor superfluous names according to Art. 52.1, i.e. they are legitimate if not illegitimate due to other reasons. Such synonyms are valid names, and valid names remain available for use. Therefore, such anamorph generic names may be retained and used for morphologically similar species with unproven phylogenetic affinity. Another case concerns the names of anamorph-typified genera having priority over competing names of teleomorph-typified genera or younger names being given priority following a proposal to use them in future for all morphs. In these cases, all species with unproven affinity may remain alongside type species with proven phylogenetic affinity and other phylogenetically proven species awaiting future clarification of their status and affinity. This is possible and may be advisable since any assignment of species to a genus is just a taxonomic decision, as explained above. The only alternative would be to re-allocate such unproven species to another genus, if

available, or even to introduce a new genus for them, which would result in numerous new genera and new combinations. That is not quite what was intended by the new rules. In such cases, the genera concerned remain paraphyletic or even polyphyletic for a certain time until the phylogenetic positions of all species assigned to these genera are known and confirmed. This is acceptable and possible in the interim. Monophyletic genera are the goal, but it will be a long time before all fungal genera can be correctly assigned in this way. For a considerable period of time we will need paraphyletic and even polyphyletic genera. These must be recognized as recently emphasized by Gams *et al*. (2012) with whom I fully agree.

As already mentioned, concepts and circumscriptions of genera, including phylogenetic aspects (monophyly, paraphyly, polyphyly) are taxonomic decisions not under the jurisdiction of the *Code*. First priority should be given to the biodiversity at species level. All newly encountered species have to be named so that they are determinable for all users, ranging from ecologists, phytopathologists, physicians engaged in human pathogenic fungi to researchers in fungal genetics and physiology. The correct allocation to an appropriate, whenever possible monophyletic, genus is important, but has only secondary priority.

Facts and problems at the species level and below

Changes in the *Code* become immediately effective when ratified by the final Plenary Sesssion of an International Botanical Congress, unless another date is specified. In the case of dual nomenclature, this ended on 30 July 2012, from which date anamorph-typified and teleomnorph-typified names compete on an equal nomenclatural footing. However, a period of immunity to the end of 2012 was allowed so as not to disrupt works in press which introduced new names for different states of the same species. Thus, as Hawksworth (2011: 158) stressed, “After 1 January 2013, one fungus can only have one name, the system of permitting separate names to be used for anamorphs then ends”. This statement is not wrong, but needs to be clarified as it can cause misunderstandings and confusion since it only refers to new names introduced after 1 January 2013. As already mentioned above, names based on different morphs for the same taxon published before 1 January 2013, are to be considered neither as alternative nor nomenclaturally superfluous names (according to Art. 59 of the Melbourne *Code*). Hence, such names, including those of anamorphs, remain legitimate but compete with teleomorph-typified names.

Another question concerns the conditions applying when one fungus can only have one name in future. The future introduction of alternative names for different morphs is only forbidden if an author definitely states that the morphs concerned belong to one fungus (taxon), independent of the scientific methods that led to this conclusion. If such a statement (or taxonomic treatment) is lacking, possibly due to uncertainty on the part of an author, it will still be possible in future to give two names. Furthermore, other authors could come to a more definite conclusion. They might, for instance, state that the merging of the two morphs in one fungus is incorrect and not justified, e.g. due to different cryptic taxa being involved and confused. Then the statement that only one fungus is present cannot be upheld and the two morphs must be given separate names. This is again solely a taxonomic decision.

Another possible scenario concerns two different morphs independently and validly described by different authors as new species after 1 January 2013. When the two morphs (species) later prove to be conspecific, they have to be merged under application of the priority rule, i.e. the younger name just becomes a heterotypic synonym of the older one but remains legitimate and valid. This is another example where in future, after 1 January 2013, one fungus may have two legitimate and valid names.

Implication of nomenclature and taxonomy

“One fungus = One name” is the premise of the changed Article 59 of the Melbourne *Code*, but the basic question is which criteria should be used to decide whether different morphs actually belong together as one fungus (taxon). It is solely up to the taxonomist to determine these criteria and the methods to answer this question (in this respect previous practices are not different). This question cannot be answered by the *Code*, and it is not the role of the *Code* to define criteria for “one fungus”. These criteria are tightly connected with technical possibilities and methods of taxonomic work that develop continuously and often rapidly. The *Code* simply rules the nomenclature and was not created to interfere in taxonomic questions and decisions. Any method is just a method and not sacrosanct; even molecular results are often debatable and open to interpretation. There are still many unanswered questions, many of which may never be finally answered as they depend on scientific (technical) progress. For instance: is a certain difference in the ITS sequences sufficient? Do we need several markers? If so, which markers and how many at different taxonomic levels? What percentage of genetic similarity of samples (populations) is sufficient to classify them as a single species? Do connections between anamorphs and teleomorphs have to be sufficiently proven by molecular analyses? Etc. Different authors will have different opinions and answers to these questions, and we cannot expect to reach any kind of general agreement on them. Authors will be influenced by differences in circumscriptions of taxa, e.g. whether they are *sensu lato* or *sensu stricto,* the presence of different evaluations of certain characters, the discovery of cryptic species, etc. Taxonomy is always a combination of objective facts and subjective interpretations of results. Hence, even uniform data may result in different taxonomic conclusions by different authors. There are no objective, universal criteria for, and definitions of, taxonomic ranks like order, family or genus, and the most difficult lasting problem concerns the question “what is a species?” Indeed, it is often quoted that a ‘species’ is in the eye of the beholder! There is no general answer, but careful individual taxonomic interpretations are necessary for any particular taxon. Different taxonomic concepts and interpretations are always in competition with each other, and the best solutions prevail, following their eventual adoption by applicants and users of names. We had good and bad taxonomy in previous times and will have it in future, but whether it is good or bad does not depend on the methods applied, and taxonomy

Table 1. Current names in *Erysiphales* proposed for inclusion in a List of accepted names where there is an earlier anamorph-typified name available (placed in **bold** type and listed as a synonym).

Erysiphe arcuata U. Braun, S. Takam. & Heluta**,** *Schlechtendalia* **16**: 99 (2007).
Synonym: **Oidium carpini** Foitzik, *in* Braun, *Powdery Mildews Eur.*: 222 (1995).

Erysiphe azaleae (U. Braun) U. Braun & S. Takam., *Schlechtendalia* **4**: 5 (2000).
Basionym: *Microsphaera azaleae* U. Braun, *Mycotaxon* **14**: 370 (1982).
Synonym: **Oidium ericinum** Erikss., *Meddn Kungl. Landtbr.-Akad. Exper.* **1**: 47 (1885).

Erysiphe buhrii U. Braun, *Česka Mykol.* **32**: 80 (1978).
Synonyms: *Erysiphe pisi* var. *buhrii* (U. Braun) Ialongo, *Mycotaxon* **44**: 255(1992).
Oidium dianthi Jacz., *Karm. Opred. Gribov* **2 (***Muchnisto-rosyanye griby*): 461 (1927).

Erysiphe caricae U. Braun & Bolay, *in* Bolay, *Cryptog. Helv.* **20**: 46 (2005).
Synonyms: *Oidium caricae* F. Noack, *Bol. Inst. Agron. Estado São Paulo* **9**: 81 (1898).
Acrosporum caricae (F. Noack) Subram., *Hyphomycetes*: 835 (1971).
Oidium papayae Marta Sequ., *Garcia de Orta*, *sér. Est. Agron.* **18**: 24 (1992).

Erysiphe catalpae S. Simonyan, *Mikol. Fitopatol.* **18**: 463 (1984).
Synonym: **Oidium bignoniae** Jacz., *Ezhegodnik* **5**: 247 (1909).

Erysiphe celosiae Tanda, *Mycoscience* **41**: 15 (2000).
Synonym: **Oidium amaranthi** R. Mathur *et al.*, *Indian Phytopath.* **24**: 64 (1971).

Erysiphe cruciferarum Opiz ex L. Junell, *Svensk. Bot. Tidskr.* **61**: 217 (1967).
Synonyms: *Erysiphe cruciferarum* Opiz, *Lotos* **5**: 42 (1855), *nom. inval.* (Art. 32).
E. pisi var. *cruciferarum* (Opiz ex L. Junell) Ialongo, *Mycotaxon* **44**: 255 (1992).
Oidium matthiolae Rayss*, Palestine J. Bot.*, *Jerusalem ser.* **1**: 325 (1940) [“1938–1939”].

Erysiphe oehrensii (Havryl.) U. Braun & S. Takam., *Schlechtendalia* **4**: 11 (2000).
Basionym: *Microsphaera oehrensii* Havryl., *Mycotaxon* **49**: 259 (1993).
Synonym: **Oidium robustum** U. Braun & Oehrens, *Mycotaxon* **25**: 268 (1986).

Erysiphe quercicola S. Takam. & U. Braun, *Mycol. Res.* **111**: 819 (2007).
Synonym: ***Oidium anacardii*** Noack, *Bol. Inst. Estado São Paulo* **9**: 77 (1898).

Golovinomyces biocellatus (Ehrenb.) Heluta, *Ukr. bot. Zh.* **45**(5): 62 (1988).
Basionym: *Erysiphe biocellata* Ehrenb., *Nova Acta Phys.-Med. Acad. Caes. Leop.-Carol. Nat. Cur.* **10**: 211 (1821).
Synonyms: *Erysibe biocellata* (Ehrenb.) Link, *Sp. Pl.*, edn 4, **6**(1): 109, 1824) [as ‘*biocellaris*’].
Oidium erysiphoides Fr., *Syst. mycol.* **3**: 432 (1832).

Golovinomyces magnicellulatus (U. Braun) Heluta, *Ukr. bot. Zh.* **45**(5): 63 (1988).
Basionym: *Erysiphe magnicellulata* U. Braun, *Feddes Repert.* **88**: 656 (1978).
Synonyms: *E. cichoracearum* var. *magnicellulata* (U. Braun) U. Braun, *Nova Hedwigia* **34**: 695 (1981).
Oidium drummondii Thüm., *Mycoth. Univ* **12**: no. 1177 (1878).

Golovinomyces sonchicola U. Braun & R.T.A. Cook, *in* Cook & Braun, *Mycol. Res.* **113**: 629 (2009).
Synonym: **Oidium sonchi-arvensis** Sawada, *Bull. Dept. Agric. Gov. Res. Inst. Formosa* **24**: 34 (1927).

Golovinomyces verbasci (Jacz.) Heluta, *Ukr. bot. Zh.* **45**(5): 63 (1988).
Basionym: *Erysiphe cichoracearum* f. *verbasci* Jacz., *Karm. Opred. Gribov* **2 (***Muchnisto-rosyanye griby*): 224 (1927).
Synonyms: *E. verbasci* (Jacz.) S. Blumer, *Beitr. Krypt.-Fl. Schweiz* **7**(1): 284 (1933).
Oidium balsamii Mont., *Ann. Mag. Nat. Hist.*, sér. 2, **13**: 463 (1854).

Leveillula rutae (Jacz.) U. Braun, *in* Braun & Cook, *CBS Biodiversity Series* **11**: 205 (2012).
Basionym: *Leveillula taurica* f. *rutae* Jacz., *Karm. Opred. Gribov* **2 (***Muchnisto-rosyanye griby*): 417 (1927).
Synonyms: *L. rutae* (Jacz.) Durrieu & Rostam, *Cryptog. Mycol.* **5**: 291 (1985) [“1984”]; *comb. inval.* (Art. 33.3).
Oidium haplophylli Magnus, *Verh. zool.-bot. Ges. Wien* **50**: 444 (1900).
Ovulariopsis haplophylli (Magnus) Trav., *Atti Accad. Sci. Veneto-Trentino-Istriana* **6**: 1 (1913).

Table 1. (Continued).

Oidiopsis haplophylli (Magnus) Rulamort, *Bull. Soc. Bot. Centre-Ouest* **17**: 191 (1986).

Phyllactinia ampelopsidis Y.N. Yu & Y.Q. Lai, *Acta Microbiol. Sin*. **19**: 14 (1979).
Synonym: **Ovulariopsis ampelopsidis-heterophyllae** Sawada, *Bull. Dept. Agric. Gov. Res. Inst. Formosa* **61**: 8 (1933).

Phyllactinia chubutiana Havryl. *et al*. *Mycoscience* **47**: 238 (2006).
Synonyms: **Oidium insolitum** U. Braun *et al*., *Sydowia* **53**: 35 (2001).
Ovulariopsis insolita (U. Braun *et al.*) Havryl. *et al.*, *Mycoscience* **47**: 238 (2006).

Phyllactinia gmelinae U. Braun & Bagyan., *Sydowia* **51**: 1 (1999).
Synonyms: *Phyllactinia suffulta* var. *gmelinae* Patil, *Curr. Sci*. **30**: 156 (1961); *nom. inval.* (Art. 36).
P. gmelinae Hosag. *et al.*, *Indian J. Trop. Biol*. **1**: 318 (1993); *nom. inval.* (Art. 37.6).
Ovulariopsis gmelinae-arboreae Hosag. *et al.*, *Indian J. Trop. Biol*. **1**: 316 (1993).

Phyllactinia populi (Jacz.) Y.N. Yu, *in* Yu & Lai, *Acta Microbiol. Sin*. **19**: 18 (1979).
Basionym: *Phyllactinia suffulta* f. *populi* Jacz., *Karm. Opred. Gribov* **2 (***Muchnisto-rosyanye griby*): 439 (1927).
Synonym: ***Ovulariopsis salicis-warburgii*** Sawada, *Bull. Dept. Agric. Gov. Res. Inst. Formosa* **61**: 89 (1933).

based on molecular approaches is not *per se* superior over morphotaxonomy.

Opinions and proposals to restrict descriptions of new taxa, above all species, in future to those accompanied by data of molecular sequence analyses have been discussed, but they are unrealistic and must be refused. Molecular support of new taxa is advisable, very useful and should be included whenever possible, but its inclusion cannot and should not be mandatory. This would be a kind of unacceptable "molecular censorship" that would inhibit taxonomic work in several parts of the world or would even force certain mycologists to give up taxonomic work. Also, fungi of certain groups cannot be cultivated at all; in other cases it may be very difficult to get cultures or to extract DNA, and further to be confident that the DNA is from the target fungus and not a contaminant. Furthermore, there would be a drastic cut in taxonomic input from amateur mycologists, who study various important fungal groups in, for instance, agaricology, and lichenology. Indeed, we need all available resources for the inventory of worldwide fungal diversity. Demands to insert a particular method like molecular sequence analysis in the *Code* as being essential for valid publication would undoubtedly not gain general acceptance. Such a requirement could only be indirectly applied, outside the *Code*, by particular journals making this a requirement for the acceptance of new species descriptions. However, it is unrealistic to believe that such policies could ever be a way of preventing publication of new taxa not following such a dictat. Editors of other journals will disagree, and publications of new taxa in books would not follow the rule.

Concepts for names in powdery mildews (*Erysiphales*) – an example

A new updated taxonomic monograph of the powdery mildews has recently been published (Braun & Cook 2012). Within this group of obligate plant pathogens, clear connections between anamorph and teleomorph genera (e.g. *Blumeria* with *Oidium s. str.*, *Erysiphe* with *Pseudoidium*, *Golovinomyces* with *Euoidium*) are evident and proven by means of morphology and molecular sequence analyses. All anamorph-typified genera are younger than the corresponding teleomorph-typified genera (except for *Oidium*) and hence will be younger facultative synonyms in future, but nevertheless they will remain legitimate and valid. Anamorph genera play an important role in the taxonomy of powdery mildews and reflect phylogenetic relations within this fungal group. Indeed they provided crucial evidence for the recent re-classification of all the holomorph genera. On the other hand, at species level anamorph species (unlike the anamorph genera) and particularly the conidial stages of powdery mildew species are morphologically often poorly differentiated and of little diagnostic value. Therefore, teleomorphs traditionally prevail in the taxonomy at species level. Hence, in all cases it is proposed to give preference to teleomorph-typified names when they are threatened by anamorph names.

There is only a single generic problem in powdery mildews, viz. the anamorph genus *Oidium* Link 1824, with its type species *Oidium monilioides*, which is the anamorph of *Blumeria graminis*, the type species of the teleomorph genus *Blumeria* Golovin ex Speer 1974. Hence, *Oidium* would be an older name for *Blumeria*, and "*Oidium graminis*" would be the correct name for the powdery mildew of grasses and cereals in future; this is, of course, unacceptable, and *Blumeria* will be proposed as the accepted generic name for this taxon.

Most powdery mildew anamorphs are morphologically poorly differentiated at species level, and it is often difficult to truly distinguish separate species in the absence of the teleomorph. However, their relations to teleomorphic genera are almost always clear. Host switches often occur in glass houses, and also in nature, usually connected with anamorph growth but lacking the teleomorph. Even results of molecular sequence analyses are often not helpful here due to a lack of data from other specimens for comparision or other problems. Hence descriptions of anamorph-typified taxa should be avoided, also in future, but when new descriptions are intended, they should only be based on

striking morphological differences combined, if possible, with molecular data, and the taxa concerned should preferably be assigned to the existing anamorph genera, which can also be used in future as they remain legitimate, valid, and available, as already explained. Descriptions of anamorph-typified new species in *Erysiphe*, *Golovinomyces*, *Neoërysiphe* and other teleomorph-typified genera are in future of course also valid and in accordance with the *Code*, but they should only be proposed in absolutely clear, molecularly proven cases.

A recently found powdery mildew anamorph on *Solanum betaceum* (tamarillo or tree tomato) in India is a striking example. This host is phylogenetically closely related to *S. lycopersicum* (tomato), and the anamorph found on tree tomato is morphologically indistinguishable from *Pseudoidium neolycopersici* (syn. *Oidium neolycopersici*) on tomato (Baiswar *et al*. 2009). Nevertheless, this powdery mildew disease was only recorded as *Oidium* sp. and not as *O. neolycopersici* because reviewers refused the latter denomination without inoculation results and/or molecular analyses. Therefore, cross inoculation tests were later carried out and the tree tomato powdery mildew was subjected to molecular examinations based on amplification of the rDNA ITS region, including the 5.8S rDNA, but, unfortunately, these new results also failed to elucidate its taxonomy. The powdery mildew on *S. betaceum* was unable to infect tomato and several other species of *Solanum*, but the sequence derived from this powdery mildew differed only in one base pair from that of *Pseudoidium neolycopersici*. Is the tree tomato powdery mildew conspecific with the latter species and only a special form? Or is it a separate species, morphologically indistinguishable from *P. neolycopersici*, but biologically distinguished and genetically distinct in one base pair in rDNA ITS sequence data? A final answer cannot yet be given. Incidently, in this case a study of the morphology of this pathogen would now allow it to be referred to the morphospecies *Pseudoidium lycopersici* as listed in the updated monograph (Braun & Cook 2012). As made apparent above, the anamorphic genus *Oidum s. str.* belongs solely to *Blumeria*.

The *Erysiphales* in its current circumscription comprises 873 known species. The number of teleomorph-typified species names threatened by anamorph names is rather limited. Table 1 details the names that come into this category (all of them will be put on a proposed List of accepted names according to the new provisions of the Melbourne *Code* (Art. 14).

ACKNOWLEDGEMENTS

I am much obliged to Roger T.A. Cook, who critically checked the whole text.

REFERENCES

Baiswar P, Braun U, Chandra S, Ngachan SV (2009) First report of an *Oidium* sp. [*neolycopersici*] on *Solanum betaceum* in India. *Australasian Plant Disease Notes* **4**: 32–33.

Baiswar P, Chandra S, Ngachan SV, Braun U, Takamatsu S, Harada M (2012) Molecular Characterization of *Oidium* sp. on *Solanum betaceum* in India. *The Plant Pathology Journal (Korea)*: in press.

Braun U, Cook RTA (2012) *Taxonomic Manual of the Erysiphales (Powdery Mildews)*. [CBS Biodiversity Series no. 11.] Utrecht: CBS-KNAW Fungal Diversity Centre.

Gams W, Humber RA, Jaklitsch W, Kirschner R, Stadler M (2012) Minimizing the chaos following the loss of Article 59: Suggestions for a discussion. *Mycotaxon* **119**: 495–507.

Gams W, Jaklitsch W *et al*. (2011). A critical response to the 'Amsterdam Declaration'. *Mycotaxon* **116**: 501–512.

Hawksworth DL (2011) A new dawn for the naming of fungi: impacts of decisions made in Melbourne in July 1011 on the future publication and regulation of fungal names. *IMA Fungus* **2**: 155–162.

Hawksworth DL, Crous PW, Redhead SA, Reynolds DR, Samson RA, Seifert KA, Taylor JW, Wingfield MJ *et al*. (2011) The Amsterdam Declaration on fungal nomenclature. *IMA Fungus* **2**: 105–112.

Knapp S, McNeill J, Turland NJ (2011) Changes to publication requirements made at the XVIII International Botanical Congress in Melbourne – what does e-publication mean for you? *Mycotaxon* **117**: 509–515.

Norvell LL (2011) Melbourne approves a new Code. *Mycotaxon* **116**: 481–490.

doi:10.5598/imafungus.2012.03.01.09

IMA FUNGUS · VOLUME 3 · NO 1: 87–92

ARTICLE

The importance of fungi and of mycology for a global development of the bioeconomy

Lene Lange*, Lasse Bech, Peter K. Busk, Morten N. Grell, Yuhong Huang, Mette Lange, Tore Linde, Bo Pilgaard, Doris Roth, and Xiaoxue Tong

Institute of Biotechnology and Chemistry, Faculty of Science and Technology, Aalborg University, Denmark; *corresponding author e-mail: lla@adm.aau.dk

Abstract: The vision of the European common research programme for 2014–2020, called *Horizon 2020*, is to create a smarter, more sustainable and more inclusive society. However, this is a global endeavor, which is important for mycologists all over the world because it includes a special role for fungi and fungal products. After ten years of research on industrial scale conversion of biowaste, the conclusion is that the most efficient and gentle way of converting recalcitrant lignocellulosic materials into high value products for industrial purposes, is through the use of fungal enzymes. Moreover, fungi and fungal products are also instrumental in producing fermented foods, to give storage stability and improved health. Climate change will lead to increasingly severe stress on agricultural production and productivity, and here the solution may very well be that fungi will be brought into use as a new generation of agricultural inoculants to provide more robust, more nutrient efficient, and more drought tolerant crop plants. However, much more knowledge is required in order to be able to fully exploit the potentials of fungi, to deliver what is needed and to address the major global challenges through new biological processes, products, and solutions. This knowledge can be obtained by studying the fungal proteome and metabolome; the biology of fungal RNA and epigenetics; protein expression, homologous as well as heterologous; fungal host/substrate relations; physiology, especially of extremophiles; and, not the least, the extent of global fungal biodiversity. We also need much more knowledge and understanding of how fungi degrade biomass in nature.

The projects in our group in Aalborg University are examples of the basic and applied research going on to increase the understanding of the biology of the fungal secretome and to discover new enzymes and new molecular/bioinformatics tools.

However, we need to put Mycology higher up on global agendas, e.g. by positioning Mycology as a candidate for an OECD Excellency Program. This could pave the way for increased funding of international collaboration, increased global visibility, and higher priority among decision makers all over the world.

Key words:
biodiversity
biomass conversion
fungal enzymes
global challenges
new biological solutions
secretomics
teaching
training

Article info: Submitted: 28 May 2012; Accepted: 12 June 2012; Published: 22 June 2012.

INTRODUCTION

Horizon 2020 is a visionary document for the European common research programme 2014–2020 (http://ec.europa.eu/research/horizon2020). The vision is to create a smarter, more sustainable and more inclusive society. However, such endeavor is not only European. It is global. Most importantly, for the members of the International Mycological Association (IMA), it includes a special role for fungi and fungal products. Therefore, it is an agenda of special relevance for mycologists all over the world.

The *Horizon 2020* document emphasizes that the most important goals and objectives for common research programmes are to address the major global challenges. Among the challenges of priority is climate change, the need for increased efficiency in resource utilization, and the urgency of developing renewable substitutes for fossils; and not least to provide for improved human health – combating life- style diseases and ensuring food security for a rapidly growing population. Essential for overcoming much of these challenges is improved use of natural resources; especially biological resources, plant nutrients and water. Regarding the efficient use of bioresources, we can do much better: After harvesting the food and feed, crop residues beyond what is needed to sustain a productive and healthy soil, are left to rot or burned. Further, the potentials of side streams and waste streams from agroindustries often remain unexploited. Also, the organic part of municipality waste is deposited in landfills, burned, or used as combustion feed stock in power plants. However, biomaterials are much too precious for such low value uses. We need more upgraded use of bioresources to both feed the growing population and as a substitute for what we now get from fossil resources.

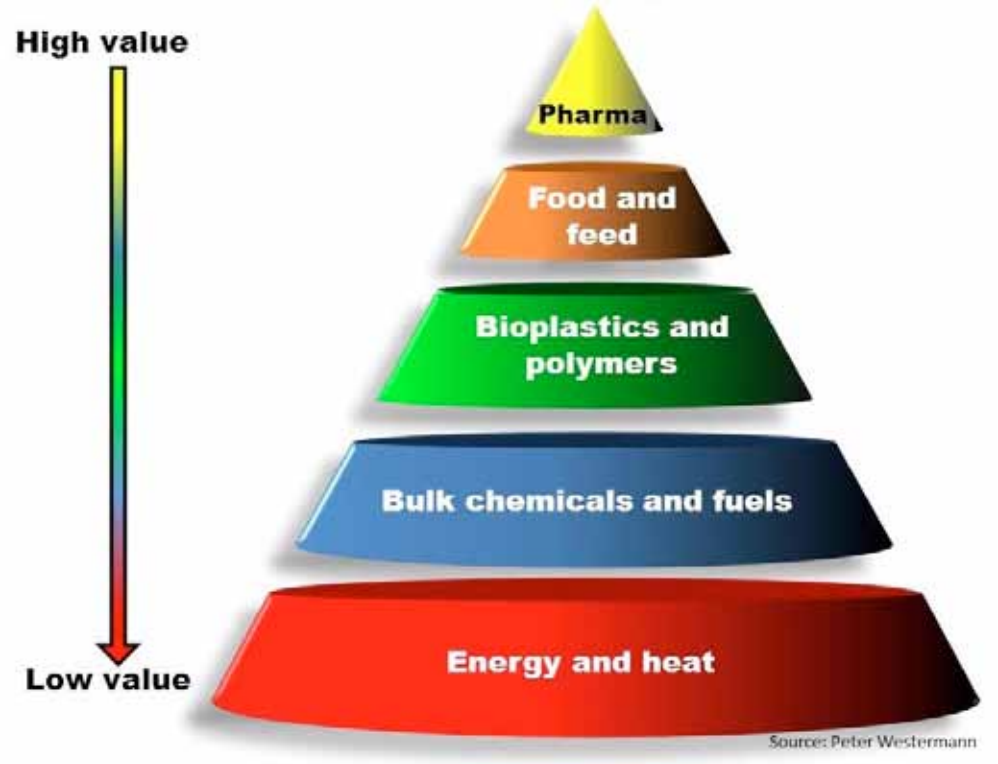


Fig. 1. The Biomass Value Pyramid shows the entire cascade of value adding products which can be produced from agricultural crop residues and other left over bio materials. The lowest value is achieved by burning the biomass and converting it into heat and electricity. Higher value products can be achieved by converting the biomass through treatment with fungal enzymes!

THE POTENTIAL ROLE OF FUNGI

In nature, the breakdown of plant materials is primarily by fungi, by the means of secreted fungal enzymes. Driven by the urge for non-food based bioenergy, industrial scale conversion of biowaste has been researched and developed over the last ten years. After all this research, the conclusion is that the most efficient and gentle way of converting recalcitrant lignocellulosic materials for industrial purposes, is through the use of fungal enzymes (Lange 2010). Through such conversions, the building blocks of the organic materials are kept intact, ready to use in the value cascade (Fig. 1). The enzymatic conversion of biowaste and -sidestreams will provide the basis for an entirely new way for the more efficient use of natural resources, paving the way for a larger bioeconomy sector in a more biobased society:

Plant materials, obtained as crop residues, municipality waste, or from agroindustrial waste streams, will increasingly substitute for fossil carbon from crude oil. Not just substituting fossil energy with bioenergy, but more importantly also substituting the higher value fossil-based materials, such as plastics and chemical building blocks, with biomaterials made from the sugar molecules of the plant cell wall polymers. Thus, the conversion of biowaste is primarily the conversion of plant cell wall materials into higher value products; achieved primarily by a process based on the refined use of fungi and fungal enzymes.

But fungi are providing even more of the solutions for meeting and addressing the various global challenges: Fungi and fungal products are also instrumental in producing fermented foods, to give storage stability and improved health; and it is a fungus (baker's yeast) that is the production organism of choice for producing insulin for the global population of diabetics. Also, cholesterol lowering drugs (the statins), major immunosuppressant drugs (cyclosporins), the cancer drug Taxol, and penicillins are fungal products.

Climate change will lead to increasingly severe stress on agricultural production and productivity, and here the solution may very well be that fungi will be brought in use as a new generation of agricultural inoculants (e.g. mycorrhizas, endophytes, biocontrol agents) to provide more robust, more nutrient efficient, and more drought-tolerant crop plants.

POSITIONING MYCOLOGY IN THE WORLD

Mycologists have over time delivered so much knowledge about fungi (taxonomy, physiology, genetics, host/substrate relations (including plant pathology and studies of biotrophic interactions), molecular biology, metabolites, enzymes and protein expression) that biological products, biological processes, and biological solutions to important problems are already widespread within many industrial sectors. To mention a few: fungal enzymes are instrumental in laundry detergents at lower temperature and in the less polluting production of both paper and textiles, by replacing chemical processing. Thus, our knowledge and insight into fungal growth and fungal products (proteins as well as metabolites) have made biological processes competitive against chemical processes because they have been developed to be both highly efficient and safe. However, much more knowledge is required in order to be able to fully exploit the potentials of fungi, to deliver

what is needed, and to address the major global challenges through new biological processes, products, and solutions.

Additional basic knowledge about fungi is required across an entire spectrum of research fields: The fungal proteome and metabolome; the biology of fungal RNA and epigenetics; protein expression, homologous as well as heterologous; fungal host/substrate relations; physiology, especially of extremophiles; and not least the extent of global fungal biodiversity. Indeed, many of the new applications of fungi and fungal products will be made possible through "unlocking the magic" of fungi we have not yet discovered – let alone described, characterized, or classified.

We also need much more knowledge and understanding of how fungi degrade biomass in nature, and especially on how they interact with each other and with microorganisms, especially bacteria. In order to achieve all this, we need to train next generation of mycologists to be experts in their fields, mastering both the new and the classical methods. Besides researchers, we also need to train the skilled workers in how to handle biological production at the industrial scale. Last but not least, we need skilled and enthusiastic teachers at all levels who can teach about the fascinating world of the fungi, both the friends and the foes, from kindergartens to graduate schools.

THE WAY FORWARD

As a first step forward, we propose a specific global learning loop for knowledge sharing of relevance to speeding up the application of mycology in addressing issues of global concern.

Most importantly, we see that we need to start to change our mindset as mycologists, taking the importance of fungi and fungal products seriously in our personal research agendas. Not with the objective of making all of us to work in applied mycological research in a traditional sense, but recognizing that we also need blue-sky, curiosity-, biodiversity- and exploration- driven research within mycology – perhaps more than ever before, in order to realize the huge potential.

To this end, mycologists in our research group in Aalborg University, Denmark, located on the AAU Copenhagen Campus, now orient our research projects to have a double focus, to: (1) forward the scientific field in which we are working, by increased understanding of the biology of the fungal secretome (regulation, composition and function); and (2) discover new enzymes and new molecular/bioinformatics tools, thereby contributing to the development of new biological products, biological processes, and biological solutions to important problems. Examples of activities with such a double focus, both basic *and* applied are:

The phylogeny of a fungal cellulase

A comparative study of an endoglucanase belonging to protein family GH45, gave surprising results, which lead to a new enzyme discovery approach: A phylogenetic analysis of the GH45 proteins, from all parts of the fungal kingdom, asco-, basidio-, zygo-, and chytridiomycetes (Kauppinen *et al*. 1999), indicated that distantly related fungi, such as the basidiomycete *Fomes fomentarius* and the ascomycete *Xylaria hypoxylon*, had GH45 cellulases in their secretome with an extremely high similarity in the amino acid composition of their active site. Strikingly, both these fungi inhabit and decompose very similar substrates (hard wood). A similar pattern can be seen amongst straw decomposing fungi, for example the basidiomycete *Crinipellis scabella* and the chytrid *Rhizophlyctis rosea*. These two fungi are from two very different parts of the fungal kingdom. Anyway, their GH45 cellulase proteins have an almost identical amino acid composition of their active sites. These observations can tentatively be explained by the following molecular mechanism: Evolution of the fungal GH45 is impacted by gene copying and subsequent gene loss, maintaining the version of the gene which is most suitable for breaking down the cellulose of the substrate of the fungus. This conclusion provided the basis for a new screening approach: select a relevant ecological niche in nature with regard to type of substrate, temperature, and pH; construct a meta-library of the entire microbial (fungal and bacterial) community at such a site; and screen this library for the best enzyme candidates for industrial applications. It also inspired the following hypothesis: evolution of the fungal secretome composition may be interpreted as taking place at the molecular level rather than at the organismal level.

Peptide pattern recognition (PPR)

A new method has been invented for the improved prediction of protein function from protein sequences. It is unique in being non-alignment-based, and permits the comparison of a vast number of sequences with even very low sequence identities. PPR analysis is potent for revealing new protein subfamily groupings, where the subgrouping is correlated with a specific function (Fig. 2). Such new understanding can again be used to understand the biological role of the secreted proteins, interactions between organisms, and interactions between the organism and the substrate. A new subfamily can be described by a list of peptides that is specific to just that subfamily. PPR analysis, moreover, opens the possibility of finding more of a given type of functional proteins belonging to a single subfamily. This can be done by using the conserved peptides for discovering new subfamily members, either by following a bioinformatics approach or by screening biological materials with degenerated primers, constructed based on the list of the identified most conserved peptides (Busk & Lange 2011, 2012).

We analyzed 8138 GH13 proteins represented in the B. Henrissat CAZy database with PPR to generate subfamilies. The subfamily-specific peptide lists were used to predict the function of 541 functionally characterized GH13 proteins. Overall, the function of 85 % of the proteins was correctly predicted (Fig. 2). The figure shows the percentage correct prediction of the enzymatic functions for each of the enzyme classes (new data; P.K. Busk & L. Lange, unpubl.).

Fungal decomposition of specific substrates

Understanding enzymatic degradation of plant cell wall materials is improved by studying in parallel both the plant cell wall composition (by the CoMPP technology, Moller *et al*. 2007) and the fungal secretome enzymes of the fungus responsible for the degradation. The materials under study in a Chinese/Danish research project are duckweed (Cheng & Stomp 2009;

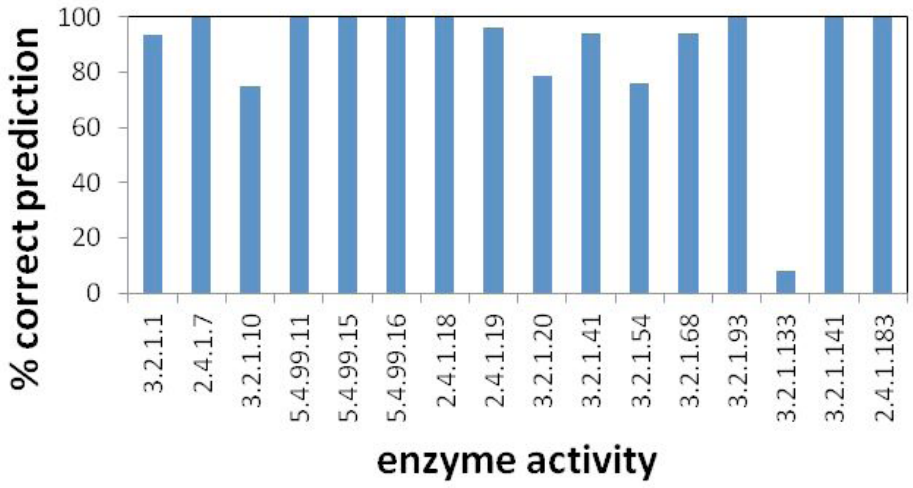


Fig. 2. The Peptide Pattern Recognition, (PPR), generated GH13 protein subfamilies which predicted the enzyme function correctly with 78–100 % accuracy; except for one enzyme class (3.2.1.133) where for so far unknown reasons the PPR subgrouping did not match the function annotations found in the CAZy database.

Fig. 3) and industrial pulp of non-food uses of basic rhizomes such as sweet potato (Zhang *et al*. 2011), cassava, and *Canna edulis*. Using next generation sequencing, the transcriptomes of tropical fungal species, isolated from relevant substrates, are analyzed and novel enzymes are expected to be identified. The secretomes will be further characterized to compare the phylogenetic relationships of the secretome proteins as compared to the phylogenetic relationships of the organisms (L. Bech, Y. Huang, Z. Hai, P.K. Busk, W.G.T. Willats, M.N. Grell, and L. Lange, unpubl.).

Accessory proteins

In 2011 it was discovered that proteins of family GH61 act directly on crystalline cellulose, partially degrading and loosening the structure of the microfibrils, thereby increasing the substrate accessibility for other types of cellulases (Beeson *et al.* 2012, Langston *et al.* 2011, Quinlan *et al*. 2011, Westereng *et al*. 2011). The PPR analysis of all publicly available GH61 sequences resulted in a tentative subgrouping in 16 new subfamilies. We are now studying the possible correlation of such subfamily groupings with the function of the given GH61 proteins, attempting to answer the following biological questions: What is the function and role of the high number of very different GH61 genes, as is so commonly seen among plant cell wall degrading fungi? We wish to increase understanding of the biological role of these non-hydrolytic accessory proteins in nature; and to provide a basis for choosing which GH61 subfamily proteins should be incorporated into new and improved industrial enzyme blends for conversion of lignocellulosic biomasses into free sugars (M. Lange, P.K. Busk, and L. Lange, unpubl.).

Enzymes from thermophilic fungi

Are the enzymes of thermophilic fungi more thermotolerant than those of mesophilic fungi? We are attempting to answer this fundamental physiological question, and at the same time provide a basis for developing a new type of biomass conversion process which can function at high temperatures, in order to improve the efficiency of the added enzymes and to speed up the biomass conversion (Busk & Lange 2011).

Fig. 3. When grown in swine wastewater, some duckweed species such as the *Spirodela polyrhizza* contains up to 40 % protein, which makes it a valuable animal feed source. Picture by courtesy of Armando Asuncion Salmean.

Fig. 4. Leaf-cutter ant colony established in the laboratory of JJ Boomsma (University of Copenhagen). The ants have built three fungal gardens under plastic beakers. The beaker has been removed from the garden to the upper right. The gradient of biomass decomposition, from top to bottom, is indicated by the green arrow. The dark material on the surface of the garden is newly incorporated leaf fragments. Non-degraded material is removed by the ants from the bottom of the garden and placed in the refuse dump (upright beaker to the lower right) (photo, Morten N. Grell).

A molecular analysis of biomass conversion in the leaf-cutter ant fungal garden

The fungal garden of leaf-cutter ants constitutes a natural biomass conversion system (Fig. 4). Mediated by fungal secreted enzymes, leaf fragments brought into the nest by the ants are converted to food for the ant larvae as well as serve as substrate for fungal growth. In this study, we investigated which enzymes are produced and their relative expression level along the decomposition gradient of the garden structure (Fig. 4), using the DeepSAGE method. DeepSAGE is a global digital transcript-profiling technology, facilitating measurement of rare transcripts (Nielsen *et al.* 2006). The results of the study have given us interesting new molecular insights into a social insect-fungus symbiosis that relies on conversion of a fresh leaves biomass, recalcitrant to degradation (M.N. Grell, K.L. Nielsen, T. Linde, J.J. Boomsma, and L. Lange, unpubl.). Now the question arises: what can we learn from the type of biomass degradation that the fungus growing leaf cutter ants have developed so successfully?

The subgrouping of esterases and their possible function in biomass conversion in nature

At present we focus on a study of additional and so far almost neglected types of enzymes needed for full biomass conversion, more specifically, on the esterases, especially the ferulic acid esterases (X. Tong, P.K. Busk, M.N. Grell, and L. Lange, unpubl.). A feature of plant cell wall polysaccharides is that they are able to cross-link, and that such cross-links can include phenolic groups represented by ferulic acid (feruloyl). The ferulic acid units can be oxidatively cross-linked by cell wall peroxidases into other polysaccharides, proteins and lignin. This cross-linking increases plant resistance to microbial degradation. The enzymes responsible for cleaving the ester-link between the polysaccharide main chain of xylans and either monomeric or dimeric feruloyl are the ferulic acid esterases (EC 3.1.1.73). The breakage of one or both ester bonds from dehydrodimer cross-links between plant cell wall polymers is essential for optimal action of carbohydrases on substrates such as cellulosic biomass. Subfamily groupings within the field of lipases and esterases are still disputed and unresolved. We attempt to use the PPR method also within these types of enzymes, to provide increased insight in the fungal secretome by achieving function-related subgroupings also of this class of enzymes; and to elucidate further the role also of esterases in biomass conversion.

Studies of secreted enzymes from edible wood-decaying fungi

These studies aim at providing a basis for onsite production of enzyme blends for biomass conversion. Edible basidiomycetes, such as *Pleurotus ostreatus*, are chosen because they do not produce mycotoxins which would prohibit their use as production organisms; and because they have been shown to have the potential to secrete sufficient biomass degrading enzymes, to significantly lower the need for commercial enzyme blends in the production of second generation biofuels. Some even produces secondary metabolites with potential for use in other industries. The combination of these attributes can provide a significant cost reduction of the final products and, most importantly, open for decentralized low-investment use of biorefinery technologies for the production of animal feed, fertilizer, and fuel from crop residues (B. Pilgaard, L. Bech, M. Lange, and L. Lange, unpubl.).

The evolution of obligate insect pathogens, elucidated by studies of their secreted enzymes

In an earlier secretome study of field-collected grain aphids (*Sitobion avenae*) infected with fungi of the order *Entomophthorales* (subphylum Entomophthoromycotina), we identified a number of pathogenesis-related, secreted enzymes (Grell *et al.* 2011). Among these were cuticle

degrading serine proteases and chitinases, involved in fungal penetration of the aphid cuticle, and a number of lipases most likely involved in nutrient acquisition. In a continuation of this study, we are investigating the distribution and variation of selected enzyme-encoding genes within the genera *Entomophthora* and *Pandora*, using fungal genomic DNA originating from field-collected, infected insect host species of dipteran (flies, mosquitoes) or hemipteran (aphid) origin. We anticipate that this study will shed new light on this highly specialized group of enthomophthoralean insect pathogenic fungi and their secreted enzymes (M.N. Grell, A.B. Jensen, J. Eilenberg, and L. Lange, unpubl.).

Evidence for a new biomass conversion role of ectomycorrhizal fungi and their use of a chemical mechanism for biomass conversion

The ectomycorrhizal fungus *Paxillus involutus* converts organic matter in plant litter using a trimmed brown-rot mechanism involving both enzymatic activities and Fenton chemistry (Rineau *et al*. 2012). These results could serve as a model for future industrial biomass conversion, combining chemistry and biology to achieve more efficient biomass conversion.

Studies of the cellulases of the aerobic soil chytrids

Pilgaard *et al.* (2011) have provided insight in the roots and origin of the fungal cellulases by studying the cellulases of aerobic soil inhabiting chytrids; and we are also attempting to further elucidate the aerobic chytrid secretome potentials for industrial exploitation of this unique group of fungi, so far almost totally neglected.

CONCLUSION

In order to achieve the goal of more mycological knowledge brought into use, for a more sustainable world of tomorrow, where the bioeconomy becomes an important pillar for our global society, we need fungi to be recognized with heightened visibility. They need to be higher up on global agendas. One way towards that goal could be to position Mycology as a candidate for an OECD Excellency Program. This could pave the way both for increased (national and international) funding of international collaboration, increased global visibility, and hopefully higher priority among decision makers all over the world. We hope you as mycologists, and the IMA as a global institution, will work together towards realizing this vision.

ACKNOWLEDGEMENTS

Funding for the above mentioned projects have been supplied by: The Danish Council for Strategic Research (BioRef, Bio4Bio; FunSecProt) and Novozymes. We further thank Peter Westermann, Associate Professor at Aalborg University, for letting us use his figure on the biomass cascade, and Armando Asuncion Salmean Ph. D. student at University of Copenhagen, for his picture of duckweed.

REFERENCES

Beeson WT, Phillips CM, Cate JH, Marletta MA (2012) Oxidative cleavage of cellulose by fungal copper-dependent polysaccharide monooxygenases. *Journal of the American Chemical Society* **134**: 890–892.

Busk PK, Lange L (2011) A novel method of providing a library of n-mers or biopolymers. Patent application EP11152232.2

Busk PK, Lange L (2011) Novel glycoside hydrolases from thermophilic fungi. Patent Application EP11152252.0.

Cheng JJ, Stomp A-M (2009) Growing duckweed to recover nutrients from wastewaters and for production of fuel ethanol and animal feed. *CLEAN - Soil, Air, Water* **37**: 17–26.

Grell MN, Jensen AB, Olsen PB, Eilenberg J, Lange L (2011) Secretome of fungus-infected aphids documents high pathogen activity and weak host response. *Fungal Genetics and Biology* **48**: 343–352.

Kauppinen S, Schülein M, Schnorr K, Lassen S.F, Andersen KV, Urs SK, Katila P, Lange L (1999) Comparative analysis of a cellulase gene (Cel45) found in all major fungal groups. *22nd Fungal Genetics Conference at Asilomar*: Abstract.

Lange L (2010) The importance of fungi for a more sustainable future on our planet. *Fungal Biology Reviews* **24**: 90–92

Langston JA, Shaghasi T, Abbate E, Xu F, Vlasenko E, Sweeney MD (2011) Oxidoreductive cellulose depolymerization by the enzymes cellobiose dehydrogenase and glycoside hydrolase 61**.** *Applied and Environmental Microbiology* **77**: 7007–7015.

Moller I, Sørensen I, Bernal AJ, Blaukopf C, Lee K, Øbro J, Pettolino F, Roberts A, Mikkelsen JD, Knox JP, Bacic A, Willats WGT (2007) High-throughput mapping of cell-wall polymers within and between plants using novel microarrays. *The Plant Journal : for cell and molecular biology* **50**: 1118–1128.

Nielsen KL, Hogh AL, Emmersen J (2006) DeepSAGE - digital transcriptomics with high sensitivity, simple experimental protocol and multiplexing of samples. *Nucleic Acids Research* **34**: e133.

Pilgaard B, Gleason F, Lilje O, Lange L (2011) Lignocellulosic enzymes in three species of zoosporic fungi from NSW soils. *2011 Annual Conference of the Ecological Society of Australia*: Abstract.

Quinlan RJ, Sweeney MD, Leggio LL, Otten H, Poulsen J-CN, Johansen KS, Krogh KBRM, Jørgensen CI, Tovborg M, Anthonsen A, Tryfona T, Walter CP, Dupree P, Xu F, Davies GJ, Walton PH (2011) Insights into the oxidative degradation of cellulose by a copper metalloenzyme that exploits biomass components. *Proceedings of the National Academy of Sciences, USA* **108:** 15079–15084.

Westereng B, Ishida T, Vaaje-Kolstad G, Wu M, Eijsink VGH, Igarashi K, Samejima M, Ståhlberg J, Horn SJ, Sandgren M (2011) The putative endoglucanase PcGH61D from *Phanerochaete chrysosporium* is a metal-dependent oxidative enzyme that cleaves cellulose. *PLoS ONE* **6:** e27807.

Zhang L, Zhao H, Gan MZ, Jin YL, Gao XF, Chen Q, Guan JF, Wang ZY (2011) Application of simultaneous saccharification and fermentation (SSF) from viscosity reducing of raw sweet potato for bioethanol production at laboratory, pilot and industrial scales. *Bioresource Technology* **102**: 4573–4579.

doi:10.5598/imafungus.2012.03.01.10

IMA FUNGUS · VOLUME 3 · NO 1: 93–98

ARTICLE

Variation in mitochondrial genome primary sequence among whole-genome-sequenced strains of *Neurospora crassa*

Kevin McCluskey

Fungal Genetics Stock Center, University of Missouri-Kansas City, Kansas City, MO 64110, USA; e-mail: mccluskeyk@umkc.edu

Abstract: Eighteen classical mutant strains of *Neurospora crassa* were subject to whole genome sequence analysis and the mitochondrial genome is analyzed. Overall, the mitochondrial genomes of the classical mutant strains are 99.45 to 99.98 % identical to the reference genome. Two-thirds of the SNPs and three-fourths of indels identified in this analysis are shared among more than one strain. Most of the limited variability in mitochondrial genome sequence is neutral with regard to protein structure. Despite the fact that the mitochondrial genome is present in multiple copies per cell, many of the polymorphisms were homozygous within each strain. Conversely, some polymorphisms, especially those associated with large scale rearrangements are only present in a fraction of the reads covering each region. The impact of this variation is unknown and further studies will be necessary to ascertain if this level of polymorphism is common among fungi and whether it reflects the impact of ageing cultures.

Key words:
SNP
Indel
sequence polymorphism
organelle genome
classical mutant

Article info: Submitted: 26 April 2012; Accepted: 6 June 2012; Published: 21 June 2012.

INTRODUCTION

Widely regarded as being an endosymbiont of ancient protobacterial origin, mitochondria are a defining characteristic of eukaryotic organisms (Gray *et al.* 1999). The availability of *Neurospora* strains carrying mutations in the mitochondrial genome enabled the first studies of maternal inheritance in *Neurospora* (Mitchell & Mitchell 1952), and mitochondrial inheritance has been shown to be primarily maternal for *Neurospora* (Mannella *et al.* 1979) as well as for other filamentous fungi (Griffiths 1996). In rare cases mitochondrial genome markers are transmitted by the fertilizing cytoplasm or in unstable heterokaryons (Collins and Saville 1990). Mitochondrial genome analysis has been used both to understand fundamental aspects of evolution (Gray *et al.* 1999) and as a source of markers for population and species delimitation (Moore 1995). Some recent analysis of a rapidly expanding pool of information has led to re-evaluation of some of the assumptions of early studies of mitochondrial genetics (Galtier *et al.* 2009). Moreover, mitochondrial biology has seen a resurgence of interest as degraded mitochondria were reported in brain tissue from Alzheimer's (Sultana & Butterfield 2009), and Huntington's (Damiano *et al.* 2010) patients.

Filamentous fungi have been described as providing a good model for the study of mitochondrial inheritance and biology (Griffiths 1996). In one instance a fungal mitochondrial genome project emphasized high level comparisons and used one representative of each major phylogenetic lineage (Paquin *et al.* 1997). More recent pan-fungal phylogenetic analysis, however, did not include mitochondrial markers (James *et al.* 2006) and recent fungal genome analysis does not emphasize mitochondrial biology (Martin *et al.* 2011), although some authors have described mitochondrial genomes as part of their whole genome sequence projects (Torriani *et al.* 2008). The *N. crassa* mitochondrial genome is 64,800 bases and it encodes twenty-eight protein coding genes, as well as two rRNAs and twenty-eight tRNA genes (Borkovich *et al.* 2004). Among these are genes for the electron transport chain, subunits of the mitochondrial ATPase, protein synthesis, and genes of unknown function. Compared to other mitochondrial genomes, the *N. crassa* mitochondrial genome is larger than many, but still near the middle of the 19 to 109 Kb range for fungi as well as for the overall range of 16 to 366 Kb from human to *Arabidopsis* (Bullerwell & Lang 2005). The *Neurospora* mitochondrial genome is a circular molecule and it varies somewhat in size depending on the presence or absence of optional intron sequences (Griffiths 1996; Collins & Lambowitz 1983). Additionally, an aberrant version of the NADH dehydrogenase was characterized in the *Neurospora* mitochondrial genome (de Vries *et al.* 1986) and this was ultimately associated with a duplication that includes two tRNA genes as well as the mutant version of the NADH dehydrogenase subunit 2 (Agsteribbe *et al.* 1989). Mitochondrial genome rearrangements were associated with the intermittent cessation of growth phenotype known as 'stopper' and these rearrangements involved the NADH dehydrogenase gene fragment (de Vries *et al.* 1986). Additionally, while the *Neurospora* mitochondria has been known to harbor various plasmids, the Varkud satellite plasmids were recently shown to be phenotypically neutral (Keeping & Collins 2011). Other mitochondrial plasmids

Table 1. Strains employed in the current analysis.

FGSC #	Gene*	Mutagen	Genetic background‡	Reference
106	*com*	UV	SL3	(Perkins & Ishitani 1959)
305	*amyc*	?	SL3	(Atwood & Mukai 1954)
309	*ti*	X-rays	SL3	(Perkins 1959)
322	*ty-1*	spontaneous	M	(Horowitz *et al*. 1961)
821	*ts*	spontaneous	M	(Nakamura & Egashira 1961)
1211	*dot*	spontaneous	SL3	(Perkins 1962b)
1303	*fi*	spontaneous	M	(Perkins 1962b)
1363	*smco-1*	Mustard	L	(Garnjobst & Tatum 1967)
2261	*do*	UV	SL2	(Perkins 1962b)
3114	*Sk-2*	Introgression	SL	(Turner & Perkins 1979)
3246	*fs-n*	spontaneous	M	(Mylyk & Threlkeld 1974)
3562	*mb-1*	UV	M	(Weijer & Vigfusson 1972)
3564	*mb-2*	UV	M	(Weijer & Vigfusson 1972)
3566	*mb-3*	UV	M	(Weijer & Vigfusson 1972)
3831	*ff-1*	spontaneous	M	(Tan & Ho 1970)
3921	*tng*	spontaneous	SL2	(Springer & Yanofsky 1989)
7022	*fld*	spontaneous	M	(Perkins 1962b)
7035	*per-1*	UV	SL3	(Howe & Benson 1974)

*Gene refers to the genetically characterized locus that was putatively identified by whole genome analysis in McCluskey *et al*. (2011)

‡In the genetic background field, SL is used to indicate the reference genome background (St Lawrence) and the following number indicates how many generations of backcrosses to a reference strain were carried out. L indicates the Lindegren background while M is used when the background is mixed or not documented.

are known to induce senescence, presumably through recombination with the mitochondrial genome (Court *et al.* 1991). Self-splicing introns of the 25S rRNA gene were identified in *Neurospora* mitochondria (Garriga & Lambowitz 1983) and led to the characterization of the mechanism of self splicing of the group I introns in *Neurospora* (Garriga *et al.* 1986). Whole genome resequencing has been used to analyze the nuclear genome of numerous *N. crassa* classical mutant strains (McCluskey *et al.* 2011) and that dataset provides unprecedented insight into *Neurospora* mitochondrial genetics and biology. Because most whole genome data includes mitochondrial sequence it is likely that analysis of mitochondrial genomes will be available for many fungal taxa and this suggests a renaissance of interest in mitochondrial genetics in fungi.

MATERIALS AND METHODS

Total DNA from *Neurospora* strains (Table 1) was prepared as described (McCluskey *et al.* 2011). Most strains were preserved on anhydrous silica gel (Perkins 1962a) since their original deposit into the FGSC collection without multiple passages. For example, strain FGSC 1303 was preserved in 1966 and strain FGSC 1363 was preserved in 1967. Some of these strains have morphological abnormalities and for these strains, the cultures were macerated with sterile glass tissue grinder and resuspended in fresh culture medium to allow production of enough tissue for DNA extraction. Genome sequencing was carried out at the US DOE JGI using the Illumina platform as described (McCluskey *et al.* 2011).

SNP and indel analysis was carried out using the MAQ software platform, version 0.7.1 (Li *et al.* 2008) . Larger indels and rearrangements were assessed using Breakdancer (Chen *et al.* 2009b). Comparative analysis of polymorphisms was carried out as previously described (McCluskey *et al.* 2011).

RESULTS

Among all the resequenced strains 129 single nucleotide variants (SNV) occurring at 67 different positions in the mitochondrial genome were detected. Of these, 48 were found in only one strain each while nineteen were found in two or more strains (Table 2). Two variants were present in fourteen and seventeen strains respectively. The SNV found in fourteen strains is a C to G at position 2,246 in non-coding sequence. The SNV found in seventeen strains occurs at position 17,478 just downstream from the mitochondrial ribosomal protein S5 (S3). All of the SNVs are non-coding except one that encodes a synonymous substitution in NCU16015 in strain FGSC 821 (Table 3). One strain, FGSC 3566, had the most SNVs in its mitochondrial genome, with 36 SNVs, of which 23 are unique to this strain. With the exception of the C to G at position 17,478 all of the SNVs in this strain are ambiguous with alternate bases making up 2 to 49 % of reads. In every case, among these variants in strain 3566 the primary call at each variant site was identical with the reference genome. At

Table 2. Number of mitochondrial polymorphisms in each of 18 strains of *Neurospora crassa*.

Strain	SNP	Indel	CDS indel
106	2	14	0
305	4	65	5
309	3	23	0
322	7	83	5
821	6	133	8
1211	4	69	4
1303	4	53	4
1363	6	11	0
2261	2	10	0
3114	4	51	2
3246	3	28	1
3562	6	27	1
3564	3	70	4
3566	36	322	21
3831	16	153	10
3921	3	18	2
7022	11	92	3
7035	9	29	1

CDS indels occur within the coding sequence of an ORF and are also included among the total count of indels.

the other extreme, several strains had fewer than three or four SNVs and all of these strains included the C to G mutation at position 17,478. Strains FGSC 106 and FGSC 2261 each had only two SNVs and these were both shared and had no significant alternate base calls.

A total of 1,250 insertions and deletions were identified among the strains. These occur as 553 different unique changes relative to the reference genome. These occur at 475 positions and of all of the independent iterations of all indels, 1,080 were annotated as being homo-allelic while 170 were identified as multi allelic (that is, different reads were recovered for the same location in one strain). In total, 662 deletions and 588 insertions were characterized. Three hundred and twenty-five indels occur only once in the dataset while 228 indels occur among two to eighteen strains. Sixty-six sites have two different variants (insertions or deletions of a different base, or of a different number of bases) and 4 sites have 3 variants.

One position with an indel in all eighteen strains occurs at position 12,228. This position, falling in intergenic space between the full-length NADH Dehydrogenase (NCU16004) and the mitochondrial ribosomal protein S5 (NCU16005), has sixteen deletions of one T and two insertions of one T and these all occur adjacent to a stretch of nine Ts. Most of the indels that are found in multiple strains occur among stretches of five or more repeats of the same base as the specific indel.

Among indels occurring in gene coding sequence the indel at 1,532 (NCU16002), is seen in fourteen strains. The deletion of one A from this position is homoallelic and strongly supported in thirteen strains, while the addition of one A is less well supported in strain FGSC 3246. Four strains are identical with the reference genome at this position. The deletion at 1,532 causes numerous stop codons in the NCU16002 ORF, beginning with a TAG at amino acid residue 203, which removes 121 residues from the full length conserved hypothetical protein encoded at NCU16002. In strains FGSC 3566 and 3831 this ORF has additional indels including the insertion of GG at position 1356. Position 1481 has an insertion of one G in strain 3566 and one C in strain 3831.

NCU16001 encodes a truncated version of NADH dehydrogenase subunit 2, with the full-length version encoded by NCU16006. The truncated NCU16001 ORF is 705 nucleotides in length and has multiple indels in five strains and all of these indels induce frameshift errors. The deletion of the C at position 616 is found in strains FGSC 3114 and FGSC 3566 and is homoallelic in both strains. This deletion causes a frameshift and introduces multiple stop codons, the first being a TAG codon at triplet 120 of the 235 amino acid protein. Similarly, the deletion of one G at position 630 in strains 322 and 3921 causes a frameshift that introduces a stop codon at position 121, as well as multiple stops after that position. There are no indels in the full-length version of NADH Dehydrogenase subunit 2 (NCU16006) in any of the strains sequenced in this program.

In all, twenty mitochondrial ORFs have indels (Table 3) and of these, nine ORFs have indels within the protein coding region of the gene. Eleven ORFs have insertions or deletions in an intron and four have indels directly adjacent (3' or 5') to the ORF. Five ORFs have no insertions or deletions and these include the full-length version of the NADH dehydrogenase subunit 2 (NCU16006), two hypothetical proteins (NCU16011, NCU16023), and two endonucleases (NCU16014 and NCU16021).

Seventy-two larger rearrangements with both endpoints within the mitochondrial genome were detected among

Table 3. Mitochondrial open reading frames (ORF) with polymorphisms relative to the reference genome.

ORF	Name	Type
SNPs		
NCU16007	NADH dehydrogenase subunit 3	Intron SNPs
NCU16008	NADH dehydrogenase subunit 4L	Intron SNPs
NCU16009	hypothetical protein	Intron SNPs
NCU16012	NADH dehydrogenase subunit 5	Intron SNPs
NCU16015	laglidadg endonuclease	5' and CDS SNPs
INDELS		
NCU16001	NADH dehydrogenase subunit 2	FS
NCU16002	conserved hypothetical protein	FS
NCU16003	cytochrome c oxidase subunit 3	FS
NCU16004	NADH dehydrogenase subunit 6	FS
NCU16005	mitochondrial ribosomal protein S5 (S3)	FS
NCU16007	NADH dehydrogenase subunit 3	int, 5', 3'
NCU16008	NADH dehydrogenase subunit 4L	int, 5'
NCU16009	hypothetical protein	int
NCU16010	laglidadg endonuclease	int
NCU16012	NADH dehydrogenase subunit 5	int
NCU16013	cytochrome b	int
NCU16015	laglidadg endonuclease	FS
NCU16016	cytochrome c oxidase subunit 1	5', 3'
NCU16017	hypothetical protein	FS
NCU16018	NADH dehydrogenase subunit 1	int
NCU16019	group I intron endonuclease	FS
NCU16020	NADH dehydrogenase subunit 4	int
NCU16022	hypothetical protein	FS
NCU16024	ATPase subunit 8	int
NCU16025	ATPase subunit 6	int

*FS = frameshift inducing indel, int = intron indel

these 18 strains using Breakdancer (Chen *et al.* 2009a). An additional 37 rearrangements have one endpoint on a chromosome in the nuclear genome. All of the polymorphisms detected with Breakdancer are unique although nineteen have shared endpoints with another variant. Of these, all consisted of different variants within one strain with one shared endpoint. Twenty-five of the polymorphisms detected with Breakdancer were deletions while twelve were insertions. The average deletion was 2,925 bases, although three putative deletions of over 20 Kb were identified in different stains. The average insertion was 123 bases with a range of 96 to 159 bases.

DISCUSSION

Overall there is a very low level of SNVs in the mitochondrial genomes of the eighteen strains characterized by whole genome sequence analysis. Even the strain with the most SNVs, FGSC 3566, had only 36 SNVs and most of these were at positions where both the reference genome base and an alternate base were detected. Interestingly, several of the strains characterized in the present study are related to those used in the pioneering work clearly showing uniparental inheritance of *Neurospora* mitochondria (Mannella *et al.* 1979). Strain FGSC 821 was deposited as a spontaneous mutant arising in strain 4A, which is the designation used for the Abbott strain in Mannella *et al.* (1979). In this earlier work, Abbott strains were described as mitochondrial genome type I. Similarly, strains in the Lindegren and St Lawrence backgrounds are described as having type II mitochondria. On the deposit form submitted with the strain, FGSC 1363 was explicitly listed as being in a Lindegren background. Other strains in the current analysis were backcrossed into the St Lawrence background (for example, FGSC 7035). The possibility that the strains in the current study contain the same mitochondrial genome as those described in Mannella et al (1979) is supported by the presence of the G for C SNP at position 2,246 in both St Lawrence type genome (FGSC 7035) and the Lindegren derived strain (FGSC 1363) as well as thirteen additional strains, but not in strain FGSC 821 (the Abbott strain).

Two of the indels in NCU16001 (the truncated NADH dehydrogenase subunit 2) occur in multiple strains and are well supported although both of these indels occur in short strings of the same base. NCU16002 encodes a conserved hypothetical protein and has multiple unique and shared indels

including the second most common indel in the mitochondrial genome among these strains. The deletion of one A from position 1,532 in this ORF removes 121 amino acids from the final putative protein product. This ORF, also known as uflLM (D'Souza *et al.* 2005), has little orthology to other proteins in the PUBMED NR protein database, and has no conserved protein domains. The finding of these frameshift inducing indels suggests that these two genes are both pseudogenes resulting from an ancestral partial duplication within the mitochondrial genome (Agsteribbe *et al.* 1989). While many mitochondrial ORFs have indels, these do not follow the same pattern of bias towards indels that do not disrupt the reading frame as was seen for indels in nuclear genes (McCluskey *et al.* 2011). Although the observation of the same indel in multiple strains lends credence to the fact that they are an accurate representation of the underlying sequence, indels are commonly seen occurring in runs of the same base and it cannot be determined from these data whether these are changes in the mitochondrial genome or systematic errors in the sequencing process. Although intrachromosomal rearrangements have been previously implicated as being responsible for the start-stop growth phenotype of so called stopper mutants, the rearrangements found in the present study do not correspond to those described for the stopper E35 mutant (de Vries *et al.* 1986). Indeed, the rearrangements typically only comprise a fraction of the reads for a given region. The anomalous characterization of interchromosomal recombination between nuclear and mitochondrial genomes by the Breakdancer program suggests either artifacts from library construction or *in silica* in the subsequent analysis. The possibility that mitochondrial sequences are found in the nuclear genome or that nuclear sequence is present in the mitochondrial genome is impossible to assess without additional investigation.

While a traditional view of the mitochondria is that of individual cell-like organelles (Luck 1963), recent study suggests more of a filamentous or syncytial structure (Bowman *et al.* 2009) with the mitochondrial DNA organized into nucleoids (Gilkerson *et al.* 2008, Basse 2010). Moreover, recent analysis of the mitochondrial proteome is adding to the understanding of the role of nuclear and mitochondrial encoded genes (Keeping *et al.* 2011). While it may be attractive to suggest that the deleterious mutations detected in a fraction of the reads in the whole genome sequencing of *Neurospora* strains represent defective mitochondrial genomes present in an otherwise healthy background, the present level of analysis does not allow this conclusion. The fact that most of the indels were homoallelic contrasts markedly with the observation that most of the SNVs were multiallelic. By way of contrast, larger scale rearrangements detected by the Breakdancer algorithm were mostly multiallelic. Whether these observations provide insight into fundamental aspects of mitochondrial genome maintenance cannot be determined with the present dataset. Additional experiments, for example comparing sequence from freshly germinated conidia to that generated from stationary-phase cultures, may allow insight into the nature of these polymorphisms. Future studies may take advantage of the information presented here to, for example, amplify unique DNA fragments only generated by deletions or large-scale rearrangements. Recent advances in whole genome sequencing may enable experimental analysis of mutation and rearrangements of mitochondrial genome in *Neurospora*, other fungi, and indeed all organisms.

ACKNOWLEDGEMENTS

The FGSC is supported by grant 0742713 from the US National Science Foundation.

REFERENCES

Agsteribbe E, Hartog M, de Vries H (1989) Duplication of the tRNA(MMet) and tRNA(Cys) genes and of fragments of a gene encoding a subunit of the NADH dehydrogenase complex in *Neurospora crassa* mitochondrial DNA. *Current Genetics* **15**: 57–62.

Atwood KC, Mukai F (1954) Survival and mutation in *Neurospora* exposed at nuclear detonations. *American Naturalist* **88**: 295–314

Basse CW (2010) Mitochondrial inheritance in fungi. *Current Opinion in Microbiology* **13**: 712–719.

Borkovich KA, Alex LA, Yarden O, Freitag M, Turner GE, Read ND, Seiler S, Bell-Pedersen D, Paietta J, Plesofsky N, Plamann M, Goodrich-Tanrikulu M, Schulte U, Mannhaupt G, Nargang FE, Radford A, Selitrennikoff C, Galagan JE, Dunlap JC, Loros JJ, Catcheside D, Inoue H, Aramayo R, Polymenis M, Selker EU, Sachs MS, Marzluf GA, Paulsen I, Davis R, Ebbole DJ, Zelter A, Kalkman ER, O'Rourke R, Bowring F, Yeadon J, Ishii C, Suzuki K, Sakai W, Pratt R (2004) Lessons from the genome sequence of *Neurospora crassa*: tracing the path from genomic blueprint to multicellular organism. *Microbiology, Molecular Biology Reviews* **68**: 1–108.

Bowman BJ, Draskovic M, Freitag M, Bowman EJ (2009) Structure and distribution of organelles and cellular location of calcium transporters in *Neurospora crassa*. *Eukaryotic Cell* **8**: 1845–1855.

Bullerwell CE, Lang BF (2005) Fungal evolution: the case of the vanishing mitochondrion. *Current Opinion in Microbiology* **8**: 362–369.

Chen CH, Ringelberg CS, Gross RH, Dunlap JC, Loros JJ (2009a) Genome-wide analysis of light-inducible responses reveals hierarchical light signalling in *Neurospora*. *EMBO Journal* **28**: 1029–1042.

Chen K, Wallis JW, McLellan MD, Larson DE, Kalicki JM, Pohl CS, McGrath SD, Wendl MC, Zhang Q, Locke DP, Shi X, Fulton RS, Ley TJ, Wilson RK, Ding L, Mardis ER (2009b) BreakDancer: an algorithm for high-resolution mapping of genomic structural variation. *Nature Methods* **6**: 677–681.

Collins RA, Lambowitz AM (1983) Structural variations and optional introns in the mitochondrial DNAs of *Neurospora* strains isolated from nature. *Plasmid* **9**: 53–70.

Collins RA, Saville BJ (1990) Independent transfer of mitochondrial chromosomes and plasmids during unstable vegetative fusion in *Neurospora*. *Nature* **345**: 177–179.

Court DA, Griffiths AJ, Kraus SR, Russell PJ, Bertrand H (1991) A new senescence-inducing mitochondrial linear plasmid in field-isolated *Neurospora crassa* strains from India. *Current Genetics* **19**: 129–137.

D'Souza AD, Sultana S, Maheshwari R (2005) Characterization and prevalence of a circular mitochondrial plasmid in senescence-prone isolates of *Neurospora intermedia*. *Current Genetics* **47**: 182–193.

Damiano M, Galvan L, Deglon N, Brouillet E (2010) Mitochondria in Huntington's disease. *Biochimica et Biophysica Acta* **1802**: 52–61.

de Vries H, Alzner-DeWeerd B, Breitenberger CA, Chang DD, de Jonge JC, RajBhandary UL (1986) The E35 stopper mutant of *Neurospora crassa:* precise localization of deletion endpoints in mitochondrial DNA and evidence that the deleted DNA codes for a subunit of NADH dehydrogenase. *EMBO Journal* **5**: 779–785.

Galtier N, Nabholz B, Glemin S, Hurst GD (2009) Mitochondrial DNA as a marker of molecular diversity: a reappraisal. *Molecular Ecology* **18**: 4541–4550.

Garnjobst L, Tatum EL (1967) A survey of new morphological mutants in *Neurospora crassa*. *Genetics* **57**: 579–604.

Garriga G, Lambowitz AM (1983) RNA splicing in *Neurospora* mitochondria. The large rRNA intron contains a noncoded, 5'-terminal guanosine residue. *Journal of Biological Chemistry* **258**: 14745–14748.

Garriga G, Lambowitz AM, Inoue T, Cech TR (1986) Mechanism of recognition of the 5' splice site in self-splicing group I introns. *Nature* **322**: 86–89.

Gilkerson RW, Schon EA, Hernandez E, Davidson MM (2008) Mitochondrial nucleoids maintain genetic autonomy but allow for functional complementation. *Journal of Cell Biology* **181**: 1117–1128.

Gray MW, Burger G, Lang BF (1999) Mitochondrial evolution. *Science* **283**: 1476–1481.

Griffiths AJF (1996) Mitochondrial Inheritance in filamentous fungi. *Journal of Genetics* **75**: 403–414.

Horowitz NH, Fling M, Macleod H, Sueoka N (1961) A genetic study of two new structural forms of tyrosinase in *Neurospora*. *Genetics* **46**: 1015–1024.

Howe HB jr, Benson EW (1974) A perithecial color mutant of *Neurospora crassa*. *Molecular and General Genetics* **131**: 79–83.

James TY, Kauff F, Schoch CL, Matheny PB, Hofstetter V, Cox CJ, Celio G, Gueidan C, Fraker E, Miadlikowska J, Lumbsch HT, Rauhut A, Reeb V, Arnold AE, Amtoft A, Stajich JE, Hosaka K, Sung GH, Johnson D, O'Rourke B, Crockett M, Binder M, Curtis JM, Slot JC, Wang Z, Wilson AW, Schussler A, Longcore JE, O'Donnell K, Mozley-Standridge S, Porter D, Letcher PM, Powell MJ, Taylor JW, White MM, Griffith GW, Davies DR, Humber RA, Morton JB, Sugiyama J, Rossman AY, Rogers JD, Pfister DH, Hewitt D, Hansen K, Hambleton S, Shoemaker RA, Kohlmeyer J, Volkmann-Kohlmeyer B, Spotts RA, Serdani M, Crous PW, Hughes KW, Matsuura K, Langer E, Langer G, Untereiner WA, Lucking R, Budel B, Geiser DM, Aptroot A, Diederich P, Schmitt I, Schultz M, Yahr R, Hibbett DS, Lutzoni F, McLaughlin DJ, Spatafora JW, Vilgalys R (2006) Reconstructing the early evolution of *Fungi* using a six-gene phylogeny. *Nature* **443**: 818–822.

Keeping A, Deabreu D, Dibernardo M, Collins RA (2011) Gel-based mass spectrometric and computational approaches to the mitochondrial proteome of *Neurospora*. *Fungal Genetics and Biology* **48**: 526–536.

Keeping AJ, Collins RA (2011) Evidence for the phenotypic neutrality of the *Neurospora* Varkud (V) and Varkud satellite (VS) plasmids. *Fungal Genetics and Biology* **48**: 741–746.

Li H, Ruan J, Durbin R (2008) Mapping short DNA sequencing reads and calling variants using mapping quality scores. *Genome Research* **18**: 1851–1858.

Luck DJ (1963) Genesis of mitochondria in *Neurospora crassa*. *Proceedings of the National Academy of Sciences, USA* **49**: 233–240.

Mannella CA, Pittenger TH, Lambowitz AM (1979) Transmission of mitochondrial deoxyribonucleic acid in *Neurospora crassa* sexual crosses. *Journal of Bacteriology* **137**: 1449–1451.

Martin F, Cullen D, Hibbett D, Pisabarro A, Spatafora JW, Baker SE, Grigoriev IV (2011) Sequencing the fungal tree of life. *New Phytologist* **190**: 818–821.

McCluskey K, Wiest A, Grigoriev IV, Lipzen A, Martin J, Schackwitz W, Baker SE (2011) Rediscovery by whole genome sequencing: classical mutations and genome polymorphisms in *Neurospora crassa*. *G3, Genes, Genomes, Genetics* **1**: 303–316.

Mitchell MB, Mitchell HK (1952) A Case of "Maternal" Inheritance in *Neurospora crassa*. *Proceedings of the National Academy of Sciences, USA* **38**: 442–449.

Moore WS (1995) Inferring phylogenies from mtDNA Variation: mitochondrial-gene trees *versus* nuclear-gene trees. *Evolution* **49**: 718–726.

Mylyk OM, Threlkeld SF (1974) A genetic study of female sterility in *Neurospora crassa*. *Genetics Research* **24**: 91–102.

Nakamura K, Egashira T (1961) Genetically mixed perithecia in *Neurospora*. *Nature* **190**: 1129–1130.

Paquin B, Laforest MJ, Forget L, Roewer I, Wang Z, Longcore J, Lang BF (1997) The fungal mitochondrial genome project: evolution of fungal mitochondrial genomes and their gene expression. *Current Genetics* **31**:380-395.

Perkins DD (1959) New markers and multiple point linkage data in *Neurospora*. *Genetics* **44**: 1185–1208.

Perkins DD (1962a) Preservation of *Neurospora* stock cultures with anhydrous silica gel. *Canadian Journal of Microbiology* **8**: 591–594.

Perkins DD, Ishitani C (1959) Linkage data for Group III markers in *Neurospora*. *Genetics* **44**: 1209–1213.

Perkins DD, Glassey M, Bloom BA (1962) New data on markers and rearrangements in *Neurospora*. *Canadian Journal of Genetics and Cytology* **4**: 187–205.

Springer ML, Yanofsky C (1989) A morphological and genetic analysis of conidiophore development in *Neurospora crassa*. *Genes and Development* **3**: 559–571.

Sultana R, Butterfield DA (2009) Oxidatively modified, mitochondria-relevant brain proteins in subjects with Alzheimer disease and mild cognitive impairment. *Journal of Bioenergetics and Biomembranes* **41**: 441–446.

Tan ST, Ho CC (1970) A gene controlling the early development of protoperithecium in *Neurospora crassa*. *Molecular and General Genetics* **107**: 158–161.

Torriani SF, Goodwin SB, Kema GH, Pangilinan JL, McDonald BA (2008) Intraspecific comparison and annotation of two complete mitochondrial genome sequences from the plant pathogenic fungus *Mycosphaerella graminicola*. *Fungal Genetics and Biology* **45**: 628–637.

Turner BC, Perkins DD (1979) Spore killer, a chromosomal factor in *Neurospora* that kills meiotic products not containing it. *Genetics* **93**: 587–606.

Weijer J, Vigfusson NV (1972) Sexuality in *Neurospora crassa*. I. Mutations to male sterility. *Genetics Research* **19**: 191–204.

doi:10.5598/imafungus.2012.03.01.11

IMA FUNGUS · VOLUME 3 · NO 1: 99–102

ARTICLE

The status of mycology in Africa: A document to promote awareness

Marieka Gryzenhout[1*], Joyce M. Jefwa[2], and Nourou S. Yorou[3]

[1]Department of Plant Sciences, University of the Free State, Bloemfontein, South Africa, 9301; Corresponding author email: Gryzenhoutm@ufs.ac.za

[2]International Centre for Tropical Agriculture (CIAT), P.O. Box 823-00621, Nairobi, Kenya

[3]Faculty of Agronomy, University of Parakou, BP 123, Parakou, Benin

Abstract: The African Mycological Association (AMA) promotes mycology amongst members in Africa and globally. The AMA has about 200 members, mostly from African states but also with strong representation from Europe and USA, amongst others. Recent efforts by members of the AMA focused on reviving and developing mycological research and networking in Africa. A great deal must, however, still be done to promote the AMA under African mycologists, and those elsewhere with interests in Africa. African mycologists also experience challenges typical of the developing world and a great deal of fungi still needs to be discovered. This can also be seen as representing great opportunities for research and collaboration. Several issues pertinent to mycology in Africa were discussed during Special Interest Group sessions of the 9th International Mycological Congress in 2010, and through several opinion pieces contributed by AMA members in the AMA newsletter, *MycoAfrica*. This contribution serves as a document to summarise these in a form that can be presented to fellow mycologists, biologists and other scientists, relevant government departments, funding bodies and Non-Governmental Organizations and that pins down the importance of mycology, the status thereof in Africa and the need to promote it more.

Key words:
Africa
challenges
mycology
opportunities
threats
uses

Article info: Submitted: 27 May 2012; Accepted: 12 June 2012; Published: 27 June 2012.

THE IMPORTANCE OF FUNGI CONTRASTED TO THE LIMITED CAPACITY TO STUDY THEM

In the wide and diverse field of biology, it is necessary to study fungi. Fungi are an incredibly speciose, and biologically and morphologically diverse group, with estimates indicating at least a predicted 1.5 million species on Earth. Some of these fungi are visible to humans as larger fungi, such as mushrooms, but others are microbial with incredibly high numbers of species and individuals present in any substrate on earth, for instance soil, water, air, or in dead or living plants. They exist as saprotrophs that break down organic material, parasites causing diseases, and various types of symbionts of all types of larger organisms. It is evident that fungi play an irreplaceable role in the ecology and micro-ecology of any ecosystem, and contribute to the health of living organisms in either a positive or negative way. More directly in the lives of humans, fungi play an incredibly important role as sources of food or in processing food, novel sources of industrially important enzymes and compounds, human, animal and plant pathogens, spoiling or contaminating food with mycotoxins, agents for biological control, and ecological indicators.

In Africa that is endowed with high biodiversity and unique but vulnerable ecosystems, mycology is an endangered discipline. Fungal components of any ecosystem are seldom characterised and almost never included in biodiversity data. Proper fungal inventories and databases are largely non-existent, while those that exist contain only scanty and basic information. Due to the lack of human capacities, national monographs of biodiversity in many African countries rarely encompass fungi. This not only leads to an unfortunate bias in the complete assessment of biodiversity, but also pertains to the unawareness of public and decision makers of fungi as important organisms. Needless to say fungal biodiversity does not feature in biological checklists and red data listings of countries.

The problem is worsened due to large numbers of new taxa still awaiting description and numerous areas and niches that are unexplored. In South Africa alone, over 171 000 fungal species are estimated to exist based only on the modest assumption that an average of seven new fungal species are associated with each of the plant species known (Crous *et al.* 2006). Of these, only 780 represented new species (Crous *et al.* 2006). In Egypt, only 4.6% of the recorded fungi were newly described (Abdel-Azeem 2011). This dilemma is largely compounded by an enormous lack of human capacity and resources.

Africa is third-world with numerous typical problems such as poverty and overpopulation. These have put excessive pressures on the environment and also on already limited food sources, compounded by threats of plant pathogens and pests. Livestock and humans are equally threatened by fungal diseases or fungal toxins. Even for quarantine purposes against plant pathogens, comprehensive lists of pathogens do not exist for most African countries. Despite these threats, the study of pathogenic fungi is also generally

neglected despite their importance, and again limited and pressured human and resource capacity exist to address this.

Mycology presents vast opportunities to address the various challenges in Africa. Sustainable ethnomycology provides valuable food and medicines and conserves such knowledge (e.g. Lowore & Boa 2001, Bloesch & Mbago 2008). The cultivation of mushrooms on waste products such as straw is practised in numerous rural communities and provides valuable sources of protein and income. Africa's fungal biodiversity represents potential opportunities to develop valuable products or by-products to address third world environmental and food security related problems, or to discover biological control methods to combat diseases.

CAPACITY - WHAT ARE THE NEEDS AND RESOURCES FOR MYCOLOGY

Mycology is a diverse biological field. It can be broadly divided into medical mycology, food mycology, industrial mycology, aspects of plant pathology, symbioses, ecology, biodiversity, and systematics, with numerous overlaps between fields and expertise. Specific needs and processes for each of these fields may differ. However, the following broad activities underlie each.

Specific fungi need to be collected and isolated using the specialised techniques known for the different groups of fungi and for different fields of mycology, unless these are obligate parasites that cannot be cultured. Collecting trips largely involve the same procedures as those for other organisms, but for many groups of fungi, a great deal of additional effort is necessary to first isolate and purify these fungi in culture before identification can be attempted. Whereas the larger fungi, such as mushrooms, are more tangible and countable, it is especially these cultured fungi that are more numerous but difficult to comprehend by non-mycologists. Furthermore, even with the larger fungi, the absence of fruiting bodies does not necessarily imply the absence of the fungus, but are merely tied to the absence of special environmental conditions conducive to the production of fruiting bodies.

Ideally, living isolates and biologically inactive herbarium specimens of fungi should be preserved in culture collections and herbaria, respectively, where these will continue to be available for study by other mycologists. The needs to do this are quite different from those of preserving various types of animals and plants, and in general are more costly, labour and time intensive, and specialised. Sadly, very few such official collections exist, while large numbers of these fungi are kept in private research collections under immense financial pressure and largely linked to the presence of the particular researcher or the particular research project.

Various steps of identification and special expertise are necessary to either identify known fungi, for instance in the case of pathogens where the correct identity is vital, or to be able to obtain a sensible designation for unknown fungi. Such identifications are often very difficult due to the large degree of variation in the fungal kingdom, the specialization and techniques required to identify different groups, and limited human expertise. Even internationally, fewer mycologists and funding are available to actually identify and describe new fungi.

Several specific needs and resources have been identified for mycology in Africa, although these may by no means be unique to this continent. Physical needs such as funding and infrastructure are quite delimiting, and both are usually necessary for sustainable research. Even with increasing interest from global funding organizations in Africa, mycologists usually have to compete with other biologists working with more understandable and visible organisms. Resources such as checklists are lacking. Whereas physical needs can be met, human needs such as support by experts and mentors to provide guidance and training, and available students interested and funded to do projects, are more difficult to meet. Experts willing to assist in identifications and coaching are difficult to find, usually overloaded themselves, and are often not on the African continent.

THREATS TO FUNGI IN AFRICA

The same problems existing in Africa affecting diversity and numbers of animals and plants, apply to fungi. These problems include slash and burn, overgrazing, alien plant invasions, reforestations with non-native trees, encroachment, fragmentation, poor land management, degradation, and transformation (Gryzenhout *et al.* 2010, Ngala & Gryzenhout 2010). However, the difference is that fungi are not at all included in any such assessments, nor are the effects and impact on these fungi known. Their diversity and functionality are understudied, and hence the impact of anthropogenic activities is unknown and the need for conservation overlooked. The invasiveness of non-native fungi is seldom studied, except for plant pathogens proven to be introduced, and the displacements of native fungi are thus unknown.

Numbers of microfungi and larger, visible fungi are disappearing without being noticed. Due to pressures to produce food, indiscriminate spraying of especially non-selective fungicides by farmers is detrimental to fungi occurring on non-agricultural crops. Africa prizes a number of local edible mushrooms, such as species of the truffle genus *Terfezia* and chanterelles (*Cantharellus*), but these are occasionally overharvested or traded illegally. The loss of habitat due to deforestation for settlement and cultivation in Africa is alarming and associated with the loss of local fungi. Furthermore, trees are destroyed for firewood, the making of charcoal, timber, and tourist ornaments, and thus the fungi occurring with them as natural pathogens or endophytes inside plant tissues, also disappear. Numerous fungal taxa are thus undergoing threats of extinction, along with their symbiotically associated plant species and generally in small sized hotspots. A recent Red List of threatened larger fungi of Benin totaling 30 species within two hotspots (Yorou & De Kesel 2011), gives evidence of the impact of human activities on fungal biodiversity and the need to elaborate ecosystems-based conservation strategies.

Africa has a rich tradition of ethnomycology that has not yet been documented in most parts. The traditional knowledge and practices are, however, declining from one generation to another, with older generations often still remaining the sole custodians. Simultaneously, a number

of negative or ignorant perceptions also exist, often among the public, other biologists or in government circles. These factors are compounded by poverty, land allocation, and land use practices, which usually take preference above the need to also study fungi.

The lack of capacity in mycology is due to several challenges. While funding is already difficult to obtain, funding for basic mycology is even scarcer, and can usually only be obtained for applied projects in fields where fungi play an important role, such as plant pathology, food microbiology, bioactive compounds, and/or applications in forest regeneration. Herbaria and living culture collections are battling to maintain high standards or even to survive, due to lack of interest for investment and scarcity of funds. The large numbers of cultures also housed in private culture collections and that are carried by research funds and the dedication of the particular researchers, are also under threat. These collections are in danger of being lost when these funds become unavailable or the researchers discontinue their work. Such private collections are not sponsored by government or other funding bodies.

Whereas institutes and research programmes with state-of-the-art infrastructure, capacity and excellence do exist in some African countries, these are usually absent. Training of new or practising mycologists is thus difficult. All of these issues are usually the reasons for the “brain drain” of talented mycologists to other continents, from which some never return.

There is a general consensus that there should be more scientific input by mycologists in political issues. This is difficult due to a lack of interest and ignorance in government, biodiversity, conservation, and public circles. Legislation and permits are often very difficult for fungi due to ignorance of the special needs of mycologists and the lack of general checklists, and these processes are also often coupled with corruption by officials. Political upheaval is a reality in many African countries, making the practice of any science difficult. Moreover, inner politics within scientific communities are also debilitating for significant progress and large scale projects. Often there is also a lack of collaboration and communication with other mycologists.

MANY OPPORTUNITIES IN AFRICA

The rich and unique biodiversity of Africa and indigenous knowledge systems present numerous opportunities for fungal bio-exploration, characterization of species, bio-prospecting, and potential downstream applications paired with capacity building. For fungi, this biodiversity is virtually untapped. Unlike the more developed, Northern Hemisphere countries with their extensive histories of mycology, Africa in a sense can start with a clean slate with not too many problems, such as taxonomical ambiguities, to first resolve. The majority of biodiversity data can also immediately be fed to the current global biodiversity initiatives and need not be harvested first from previous and extensive lists. Metagenetic approaches such as direct environmental sequencing or the use of other biochemical or molecular typing techniques of microbial communities, also present novel techniques to investigate these communities, albeit these are usually costly.

Although numerous challenges exist, incredibly talented, passionate and diverse mycologists are practising their science with few resources, and often at an international level. A number of international centres of expertise already exist within some African countries. Much of this is achieved through a system of African and global connections and collaborations. Such expertise is actually playing a pioneering role to perpetuate mycological research and teaching locally. One most important but discrete opportunity is that numerous local students can be motivated and engaged to make a career in mycology, as fungal science is perceived to be a new and promising biological field for them. Numerous students also do their pre- and postgraduate studies at African centres or abroad, and often at acclaimed research groups where they actually act to promote mycology (brain gain) and, if they return, the expertise they gained is brought back (brain circulation). In some African countries, emerging good will towards biological sciences also may include more opportunities for mycology.

Several initiatives already exist in Africa to promote awareness of fungi. The use of ethnomycology is promoted under communities in attempts not to lose those skills and knowledge, and these are useful initiatives to show the value of mycology for government officials and funders. A number of groups for communities and amateur mycologists are driven by individuals or groups of mycologists, who are usually already pressured. These are essential to nurture interest in this poorly represented field or to promote awareness of the importance of including fungi in biodiversity and conservation initiatives. These groups often include biologists from other disciplines, non-governmental organizations (NGO’s), nature conservationists, and from government, and thus play the important role of promoting mycology in the broader scientific community and disseminating interesting, useful or essential data.

CONCLUSION

When building a discipline on a continental scale, several things are essential. These include leading research, to promote the particular field of science across general scientific fields, to attract students with pertinent and stimulating teaching and research projects, and to build capacity through post-graduate training. Due to relatively limited capacity in Africa, strong ties must be sought and fostered with international collaborators, institutions and societies.

A choice must be made if mycology in Africa is to grow stronger and become more prominent, or if it will merely continue to exist. Strong and continued action with clear goals will be necessary to build mycology in Africa, and these must not be from a few individuals, but from numerous people and teams. Common goals for groups of mycologists from several countries may be a way to stimulate this.

In the open world we live in today, numerous ways of communicating, networking and collaborating is possible. Novel, different ways of doing things must be sought if current systems are not working. Ways must be found to reward passion and energy. This is especially true to retain young

talent and to enable it to become able mycologists. Ways must be sought to solve the challenges. These are difficult, but if even one of these challenges can be addressed and a solution established, it will make it possible to solve the next challenge and do more in future. For these, continuous advice is needed from fellow mycological societies, experienced mycologists, and those outside the field of mycology.

ACKNOWLEDGMENTS

We thank the various contributors to the "Opinion" features of the official newsletter of the AMA, *MycoAfrica* [vol 2(4), vol. 3 (1, 2, 4), vol. 4 (1, 2); www.africanmycology.org], for their views and stimulating further discussions. These include Levi Yafetto, Rosemary Tonjock (University of Buea, Cameroon), George Ngala (Bamenda University of Science and Technology, Cameroon), Jo Taylor (National Botanical Garden of Edinburgh) and Jonathan Jansen (Rector, University of the Free State, South Africa). Features published in the various issues of *MycoAfrica* also contributed to the content of this commentary and include various useful references. Referencing for this commentary was thus deliberately not comprehensive. We are also thankful to the Organising Committee of the 9th International Mycological Congress for presenting the AMA with an opportunity to have a stimulating session on mycology in Africa.

REFERENCES

Abdel-Azeem AM (2011) The history, fungal biodiversity, conservation, and future perspectives for mycology in Egypt. *IMA Fungus* **1**: 123–142.

Bloesch U, Mbago F (2008) *The potential of wild edible mushrooms in the miombo woodlands of the Selous-Niassa Wildlife Corridor for the livelihood improvement of the local population.* Commissioned by the Deutsche Gesellschaft fur Technische Zusammenarbeit and the Association for the Development of Protected Areas.

Crous PW, Rong IH, Wood A, Lee S, Glen HF, Botha W, Slippers B, De Beer ZW, Wingfield MJ, Hawksworth DL (2006) How many species of fungi are there at the tip of Africa? *Studies in Mycology* **55**: 13–33.

Gryzenhout M, Roets F, de Villiers R (2010) Fungal conservation in Africa. *Mycologica Balcanica* **7**: 53–58.

Lowore J, Boa E (2001) *Bowa markets in Malawi: Local practices and indigenous knowledge of wild edible fungi.* Egham, UK: CAB International.

Ngala GN, Gryzenhout M (2010) Biodiversity and conservation in Cameroon. *Mycologica Balcanica* **7**: 65–72.

Yorou NS, De Kesel (2011) Larger fungi. In: *Nature Conservation in West Africa: Red List for Benin* (P Neuenschwander, B Sinsin & G Goergen, eds): 47–60. Ibadan: International Institute of Tropical Agriculture.

All submitted materials must be digitized and submitted electronically to d.hawksworth@nhm.ac.uk and p.crous@cbs.knaw.nl, with the manuscript ideally in Microsoft Word. Illustrations (Line drawings (600 dpi or higher) and half tone pictures (300 dpi or higher) should be submitted separately, never embedded in Word files. Phylogenetic trees will only be accepted when submitted as Powerpoint files, or in Adobe (never as pictures).

The corresponding author should confirm that: (a) all named authors have agreed to publication of the work; and (b) the manuscript does not infringe any personal or other copyright or property rights.

Papers cited as "in press" should be provided for the benefit of the referees.

The content should be structured as follows: ABSTRACT, INTRODUCTION, MATERIALS AND METHODS, RESULTS, TAXONOMY, DISCUSSION, ACKNOWLEDGEMENTS and REFERENCES. Key words and a Running Head should also be provided.

English-English is preferred, and used for all non-article material. Authors of articles can use American-English, provided that it is consistent within their contributions. Words of non-English origin, like *bona fide*, *prima facie*, *in vitro*, *in situ*, should be placed in *italic*, together with scientific names of any rank (e.g. *Ascomycota*, *Dothideales*, *Mycosphaerellaceae*, *Mycosphaerella nubilosa*).

Common abbreviations are as follows: h, min, s, mL, µL, mg/L, °C, Fig., d, wk, but also ITS, RPD, RFLP, rDNA, 18S etc.

Authorities of fungal taxa should be omitted from the general text, unless novelties and synonymies are listed, or nomenclatural issues discussed. In these cases, authorities for taxa should follow the list of authors' names, see http://www.speciesfungorum.org/AuthorsOfFungalNames.htm, followed by the year of publication of the name. Journal abbreviations in the text (species synonymies, descriptions, etc.) should follow the International Plant Name Index (see http://www.ipni.org/index.html).

Experimental procedures must be reproducible and must follow *Good Cultural Practice* (Mycological Research 106: 1378–1379), with sequences lodged at *GenBank*, alignments in *TreeBASE*, voucher specimens in a public reference collection recognized in Index, or another recognized in *Index Herbariorum* (online) or the *World Directory of Collections and Cultures of Microorganisms* (online), with acronym and accession numbers (where allocated), and ex-type and other representative cultures in long-term genetic resource depositaries or collections. For new scientific names a *MycoBank* number is required (see www.MycoBank.org), and should be obtained on acceptance for publication.

Collections must be cited as:

Specimens examined. **Country**: *State/Province/County*: location, substratum, date (e.g. 10 Dec. 1993), *collector* (e.g. *S. Uper & F. Ungus*) (COLLECTION ACRONYM and ACCESSION NUMBER -- holotypus; cultures ex-holotype COLLECTION ACRONYM(S) and ACCESSION NUMBER(S)).

Reference citation in text: References in the text should be chronological, and given in the following form: "Smith & Jones (1965) have shown ...", or, "some authors (Zabetta 1928, Taylor & Palmer 1970, Zabetta 1970) consider that ...". The names of collaborating authors are joined by an ampersand (&). Where there are three or more authors, names should be cited by the first name only, adding "*et al.*", e.g. "Bowie, Black & White (1964)" are given as "Bowie *et al.* (1964)" or "(Bowie et al. 1964)". Where authors have published more than one work in a year, to which reference is made, they should be distinguished by placing a, b, etc. immediately after the date, e.g. "Dylan (1965a, b)". Reference citations in text should be in ascending order of year first, followed by authors' names. Each reference should include the full title of the paper and journal, volume number, and the final as well as the first page number. In the case of chapters in books, the names of editors, first and last page numbers of the articles, publisher and place of publication are needed.

Examples:

Black JA, Taylor JE (1999a) Article title. *Studies in Mycology* **13**: 1–10.

Black JA, Taylor JE (In press) Article title. *Fungal Biology*.

Black JA, Taylor JE, White DA (1981) Article title. In: *Book Title* (KA Seifert, W Jones & P. Jones, eds): 11–30. Town (and country if not well-known): Press.

Simpson H, Seifert KA (2000) *Book Title*. 2nd edn. Town (and country if not well-known): Press.

White DA (2001) *Dissertation Title*. PhD thesis, Department, University, Country.

Editorial

Holistic mycology – back to biology! (1)

"Stop press -- Registries of names and the new Code" by Lorelei Norvell (2)

News

IMA Fungus full content available in PubMed (2010 onwards) – Fear of Fungi – White-nose fungus kills around six million bats – The Top 10 fungal pathogens in molecular plant pathology – Fungus makes the Top 10 species 2012 – 2013 CBS Spring Symposium – One Fungus : Which Gene(s) (1F = ?G) – Special issues of journals – Mycophily, mycophilogy, and insect conservation – Global Mycology Initiatives – A network of European scientists investigating endophytic microorganisms: a new COST programme – New funding for Australian medical mycology – China establishes State Key Laboratory of Mycology – FungalDC: a database on fungal diversity in genetic resource collections (3)

Reports

ONE FUNGUS = WHICH NAME ? – IMA Executive Committee Meeting – International Commission on the Taxonomy of Fungi (ICTF): 2012 General Meeting – CBS Course Medical Mycology: Chinese edition – Hidden Danger, Bright Promise: 4th Meeting of the ISHAM Working Group on Black Yeasts – International cooperation in zygomycete research (10)

Awards and Personalia

CBS-KNAW Fungal Biodiversity Centre Awards (Johanna Westerdijk Award; Josef Adolf von Arx Award) (24)

IMA Young Mycologist Awards 2011 (Elias Magnus Fries Medal; Carlos Luis Spegazzini Medal) (25)

Anton de Bary Medaille: Walter Gams (26)

Queen's Award for Forestry: Jolanda Roux meets the Queen of England (26)

In Memoriam: Vernon Ahmadjian (1930–2012); Ovidiu Constantinescu (1933–2012); Walter Friederich Otto Marasas (1941–2012); Erast Parmasto (1928–2012) (27)

Research News

Archaeorhizomycetes: a new class for a major clade of soil fungi – Inter-specific sex in grass smuts – Prions and phenotypic inheritance in wild yeasts – Different fungal and algal genotypes demonstrated within one lichen specimen – Fungi that can transform lead – Nutritional value of fungi in animal diets – Archaeolichenology: a novel use of lichens (29)

Book News

(34)

Forthcoming Meetings

(40)

Articles

"*Pilidiella tibouchinae* sp. nov. associated with foliage blight of *Tibouchina granulosa* (quaresmeira) in Brazil" by Bruno E.C. Miranda, Robert W. Barreto, Pedro W. Crous, and Johannes Z. Groenewald 1

"Reappraisal and neotypification of *Phyllachora feijoae*" by Lilian C. Costa, Davi M. Macedo, and Robert W. Barreto 9

"Managing and coping with names of pleomorphic fungi in a period of transition" by David L. Hawksworth 15

"*Afrocantharellus* gen. stat. nov. is part of a rich diversity of African *Cantharellaceae*" by Donatha D. Tibuhwa, Sanja Savić, Leif Tibell, and Amelia K. Kivaisi 25

"The identity of *Cintractia disciformis*: reclassification and synonymy of a southern Asian smut parasitic on *Carex* sect. *Aulocystis*" by Marcin Piątek 39

"*Ceratocystis eucalypticola* sp. nov. from *Eucalyptus* in South Africa and comparison to global isolates from this tree" by Marelize van Wyk, Jolanda Roux, Gilbert Kamgan Nkuekam, Brenda D. Wingfield, and Michael J. Wingfield 45

"*Aspergillus* section *Versicolores*: nine new species and multilocus DNA sequence based phylogeny" by Zeljko Jurjevic, Stephen W. Peterson, and Bruce W. Horn 59

"The impacts of the discontinuation of dual nomenclature of pleomorphic fungi: the trivial facts, problems, and strategies" by Uwe Braun 81

"The importance of fungi and of mycology for a global development of the bioeconomy" by Lene Lange, Lasse Bech, Peter K. Busk, Morten N. Grell, Yuhong Huang, Mette Lange, Tore Linde, Bo Pilgaard, Doris Roth, and Xiaoxue Tong 87

"Variation in mitochondrial genome primary sequence among whole-genome-sequenced strains of *Neurospora crassa*" by Kevin McCluskey 93

"The status of mycology in Africa: A document to promote awareness" by Marieka Gryzenhout, Joyce M. Jefwa, and Nourou S. Yorou 99