doi:10.5598/imafungus.2013.04.02.03

IMA FUNGUS · VOLUME 4 · NO 2: 177–186

ARTICLE

A new species of *Gymnoascus* with verruculose ascospores

Rahul Sharma, and Sanjay Kumar Singh

National Facility for Culture Collection of Fungi, MACS' Agharkar Research Institute, G. G. Agarkar Road, Pune - 411 004, India; corresponding author e-mail: rsharma@aripune.org

Abstract: A new species, *Gymnoascus verrucosus* sp. nov., isolated from soil from Kalyan railway station, Maharashtra, India, is described and illustrated. The distinctive morphological features of this taxon are its verruculose ascospores (ornamentation visible only under SEM) and its deer antler-shaped short peridial appendages. The small peridial appendages originate from open mesh-like gymnothecial ascomata made up of thick-walled, smooth peridial hyphae. The characteristic morphology of the fungus is not formed in culture where it has very restricted growth and forms arthroconidia. Phylogenetic analysis of different rDNA gene sequences (ITS, LSU, and SSU) demonstrates its placement in *Gymnoascaceae* and reveal its phylogenetic relatedness to other species of *Gymnoascus,* especially *G. petalosporus* and *G. boliviensis*. The generic concept of *Gymnoascus* is consequently now broadened to include species with verruculose ascospores. A key to the accepted 19 species is also provided.

Key words:
28S
18S
echinulate ascospores
Gymnoascaceae
ITS
Onygenales
phylogeny

Article info: Submitted: 22 August 2012; Accepted: 15 October 2013; Published: 25 October 2013.

INTRODUCTION

The genus *Gymnoascus* (*Gymnoascaceae, Onygenales*) was established by Baranetzky 1872 with *G. reessii* as the type species. *Gymnoascus* forms yellowish to brownish spherical ascomata having thick-walled, anastomosed, smooth to roughened peridial hyphae, with or without appendages. The ascospores are smooth, irregular, or lumpy. The genus has been the subject of three monographic treatments (Orr *et al*. 1963, Arx 1986, Solé *et al.* 2002) and has now become one of the largest genera of *Onygenales* due to the recent transfer of species from several other genera. Solé *et al*. (2002) merged four genera (viz. *Arachniotus, Gymnascella*, *Gymnoascoideus*, and *Narasimhella*) into *Gymnoascus* based on morphological and molecular data. With this new concept the genus comprised 18 species, distinguished primarily on the basis of ascospore morphology.

These species formed a monophyletic clade in previous ITS sequence-based phylogenetic studies (Solé *et al.* 2002). During a taxonomic study of keratinophilic fungi in India, we isolated an interesting ascomycete (isolate NFCCI 2672) with ascomata similar to those of *Gymnoascus*. In the present study we evaluated its taxonomic placement based on rDNA-based phylogenies and morphology.

MATERIAL AND METHODS

Collection and isolation

A soil sample was collected from Kalyan railway station, Maharashtra, India (19° 14'N, 73° 10'E), and placed in a sterile sealed polythene bag. The soil sample was dry when collected and was stored at room temperature until processed. Hair baiting (Vanbreuseghem 1952) was performed using defatted horse and human hairs as baits; after 1–2 month incubation in the dark at room temperature (28 °C) the ascomata developed in soil. Isolation was by the microdilution drop trail method (Sharma *et al.* 2002) in which a few ascomata were picked up with the help of a fine needle and crushed in sterile distilled water in an Eppendorf tube. Fifty to 100 mL of the ascospore suspension (in small drops) was placed on one side of a PDA plate, which was tilted to spread ascospores with the trail of the drop. To initiate sporulation, the fungus was grown on oatmeal agar (OA), potato carrot agar (PCA), potato dextrose agar (PDA) and Sabouraud dextrose agar (SDA; Crous *et al*. 2009) and incubated at 28 °C.

Microscopy

For microscopic observation and photomicrography, lactophenol mounts were prepared and observed under a Zeiss Axio Imager A.2 light research microscope. Measurements were made with the help of a calibrated scale of the Axiom software at various magnifications. Camera lucida diagrams were prepared using a Nikon E200 microscope fitted with a Nikon-Y-IDT prism. For scanning electron microscopy, ascomata were fixed to stubs with double sided adhesive tape, and sputter coated with platinum. Photomicrographs were taken with JEOL-JSM 6360A scanning electron microscope at various magnifications with 10kV accelerating voltage.

DNA extraction, PCR and sequencing

Genomic DNA was extracted from 2-wk-old fungal colonies grown on PDA by homogenization in a FastPrep 24 tissue

homogenizer (MP Biomedicals, Germany) followed by the salt extraction method of Aljanabi *et al.* (1997). The PCR amplification was performed with universal primer pairs ITS4/ITS5 (ITS regions and 5.8S), 5.8SR/LR7, LROR/LR7, LR7R/LR12 (LSU), NS1/NS4, NS3/NS8 (SSU) (Vilgalys & Hester 1990, White *et al*. 1990, Rehner & Samuels 1994). All PCR reaction mixtures contained: 16 µL PCR grade water (Sigma), 2.5 µL PCR buffer (10X); 1 µL dNTPs (250 mM each); 0.5 µL of each primer (50 pmol μL^{-1}); 1 µL (1 U μl^{-1}) of Taq polymerase (Genei, Bangalore) along with 2.5 µL (10 ng μl^{-1}) of template DNA and was overlaid with one drop of light mineral oil (Genei). PCR was performed in an Eppendorf MasterCycler (Eppendorf, Hamburg). The amplification program included an initial denaturation step at 95 °C for 5 min followed by 30 cycles of denaturation at 95 °C for 1 min, annealing for 1 min at 56 °C and extension for 1 min at 72 °C. A final extension step at 72 °C for 7 min was included at the end of the amplification. This amplification program was used with all primer combinations, except the following ones which used a different annealing temperature: 5.8SR/LR7 (51 °C), NS1/NS4 (54 °C) and NS3/NS8 (65 °C). Sequencing was done with Big Dye Terminator cycle sequencing kit (Applied Biosystems, Foster City, CA) as per the manufacturer's instructions on an ABI 3100 Avant Prism automated DNA sequencer (Applied Biosystems).

Phylogenetic analysis

DNA sequences obtained from the sequencer were manually edited for inconsistencies using ChromasLite v. 2.01 (http:/www.technelysium.com.au). The sequences of the ITS, LSU and SSU regions of the new isolate were submitted to BLASTn sequence homology searches. On the basis of the results, phylogenetically related species were chosen for the construction of the phylogenetic trees, including 18 *Gymnoascus* spp. and 11 species belonging to the genera *Amauroascus, Arthroderma, Byssoonygena, Ctenomyces, Kraurogymnocarpa, Onygena, Pectinotrichum,* and *Uncinocarpus*. Additionally, related sequences of authentic or ex-type strains of all genera compared were retrieved from GenBank, derived from Solé *et al*. (2002), Sugiyama *et al*. (2002) and Doveri *et al.* (2011). Alignment was performed using CLUSTAL W (http://www.ebi.ac.uk/clustalw/) and tree construction with MEGA v.5 software (Tamura *et al.* 2011). The alignments have been submitted in TreeBASE (http://pirl.org/phylo/treebase/phylows/study/ TB2:S13656). Neighbour-joining (NJ) analysis (Saitou & Nei 1987) was conducted with aligned sequences using Kimura-2 parameter distance model (the Kimura 1980) and complete deletion of gaps option. The robustness of the branches was assessed by bootstrap analysis (Felsenstein 1985) of 1000 replicates. Besides NJ, Maximum Likelihood (ML) and Maximum Parsimony (MP) analyses was also conducted with aligned sequences.

RESULTS

The fungus studied formed minute gymnothecia in soil near the hair-baits. The ascomata superficially resembled those of *Gymnoascus reessii* in having thick-walled, branched, and septate peridial hyphae but without the characteristic boathook-shaped appendages. The asci were globose to subglobose and 8-spored. The spores were smooth under light microscopy. Sequencing of the partial 28S rRNA gene of NFCCI 2672 resulted in a 837 bp long sequence(GenBank–JQ517293) which in BLAST searches showed maximum identity of 93 % with *G. petalosporus* (ATCC 34351; AY176748.1), 90 % with *G. reessii* (CBS 259.61; FJ358284.1, and 89 % with *G. aurantiacus* (ATCC 22394; AY176747.1), *G. littoralis* (CBS 454.73; FJ358272.1) and several other *Onygenaceae* (*Onygenales*) members. For some of the NCBI accessions, current names (Solé *et al.* 2002) are used here instead of those in GenBank which had not been updated. The nrLSU of nine *Gymnoascaceae* and four *Arthrodermataceae* taxa from a previous study (Solé *et al*. 2002) were retrieved from GenBank and aligned with NFCCI 2672. An NJ tree was constructed and yielded distinct clades for members of the three families of *Onygenales* (Fig. 1). The new species, named as *G. verrucosus* below, was placed within the *Gymnoascaceae* clade close to *G. petalosporus*. Sequencing of the partial 18S rRNA gene resulted in a 790 bp long sequence (GenBank–JQ517294) which showed maximum identities of 97 % with *Kraurogymnocarpa trochleospora* (UAMH 10101; AY177295.1), *G. hyalinosporus* (CBS 548.72; AB015775.1), *G. petalosporus* (UAMH 1665; U29392.1), *G. littoralis* (CBS 454.73; FJ358340.1), *G. reessii* (CBS 259.61; FJ358349.1), *G. ruber* (AY177296.1), and 96 % with several other species of *Gymnoascaceae*, *Onygenaceae,* and *Arachnomycetaceae*. The nrSSU of six *Gymnoascaceae*, five *Onygenaceae,* and three *Arthrodermataceae* taxa from previous studies were aligned with NFCCI 2672. The SSU tree resolved the analysed strains into distinct families of *Onygenales* (Fig. 2). The new taxon was placed in the *Gymnoascaceae* clade close to *G. hyalinosporus* and *G. marginosporus*.

Sequencing of the ITS region of NFCCI 2672 resulted in a 555 bp long segment (GenBank–JQ517292). A nucleotide BLAST search with the ITS sequence of NFCCI 2672 showed maximum identity of 86 % with *G. hyalinosporus* (UAMH 7366; AF129853.1; query coverage 95 %) and *G. petalosporus* (NRRL 6001; HM991270.1; query coverage 98 %), 85 % with *G. reessii* (IFM 47419; AB361643.1; query coverage 98 %), and 84 % with several other *Gymnoascus* species. The ITS sequence of *G. verrucosus* differs from its closest neighbour *G. petalosporus* at 98 positions (58 substitutions and 40 indels) out of 548 aligned sites, while it differs from *G. boliviensis* at 101 positions (56 substitutions and 45 indels) out of 555 aligned sites. The next nearest neighbour, *G. hyalinosporus,* differs at 93 positions (62 substitutions and 31 indels) out of 556 aligned sites. The remaining *Gymnoascus* species were even more distant from *G. verrucosus*.

TAXONOMY

Gymnoascus verrucosus Rahul Sharma & S.K. Singh, **sp.nov**.
MycoBank MB800001
(Figs 4–6)

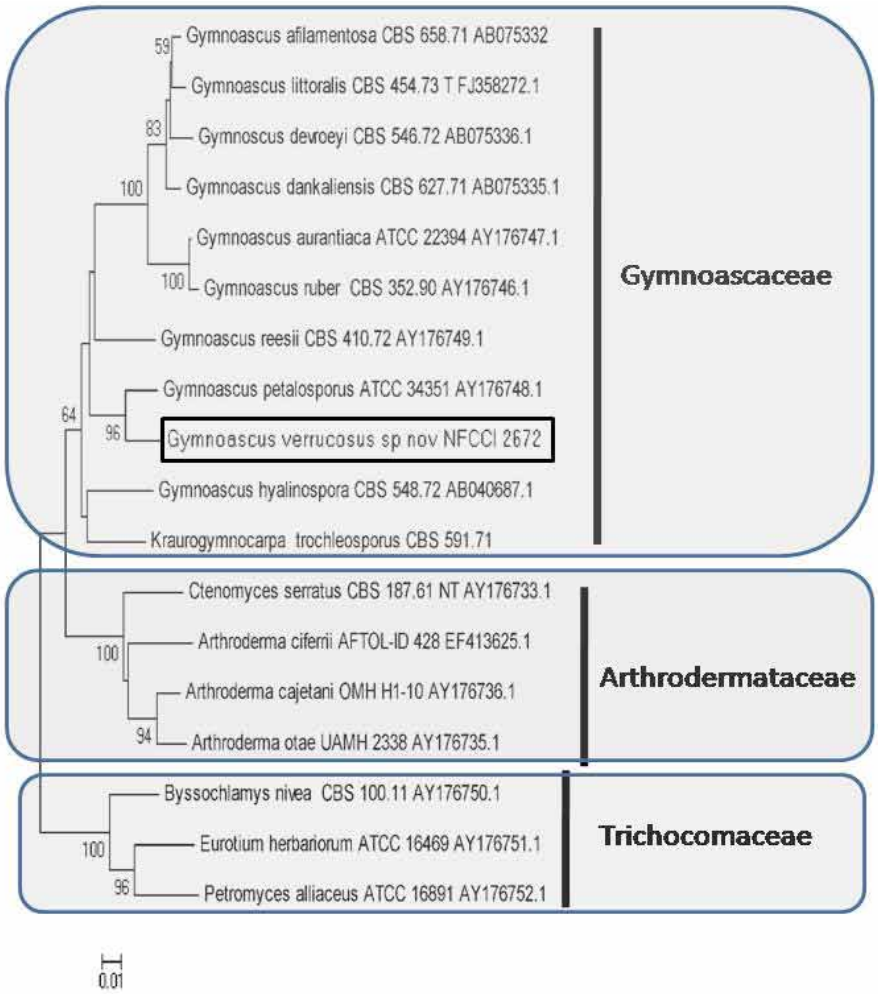

Fig. 1. Neighbour-joining tree of the LSU sequence of *Gymnoascus verrucosus* and 17 other species belonging to the families *Gymnoascaceae, Arthrodermataceae,* and *Trichocomaceae*. The bar indicates distances calculated in MEGA v5.0 and indicates units of base substitution per site. Bootstrap values above 50 % are shown.

Etymology: The species epithet recalls the warted ascospores.

Diagnosis: Deer antler shaped smooth, tapered peridial appendages and warty globose ascospores differentiate the new species from the rest in the genus.

Type: **India:** *Maharashtra*: Kalyan, isol. ex soil sample, 9 Mar. 2009, *Rahul Sharma* (AMH 9454 -- holotype; NFCCI 2672 -- ex-holotype culture).

Description: *Colonies* on PDA extremely slow growing (10 mm diam in 3 wk), initially white but turning yellowish brown, wrinkled, heaped, very hard, reverse brown. *Vegetative hyphae* initially thick-walled, septate, almost every cell giving rise to a new branch; later disintegrating into arthroconidia with rounded ends. When the hyphae transform into arthroconidia, the culture does not remain so rigid or hard and the inocula can be easily lifted with an inoculating needle. *Ascomata* formed only in soil near hair baits, not known in culture, spherical, 50–90 µm diam (including appendages), yellowish to orange-brown, reticulum type open. *Peridial hyphae* deep yellow to brown, smooth, septate, thick-walled, 1.5–2.0 µm wide, originating radially from the centre of the ascoma. *Peridial appendages* deer antler-shaped, smooth, yellow-brown, often bifurcating and tapering towards the apex, 10–15 µm long, 1.0–1.5 µm wide. *Asci* formed at the centre of the ascoma, hyaline to pale yellow, globose to subglobose, 5–6 µm wide, 8-spored. *Ascospores* pale yellowish, globose and 3 µm diam to subglobose and 3.0 × 2.5 µm, walls smooth under LM but minutely tuberculate under SEM.

Asexual morph: Forming thick-walled arthroconidia, smooth, hyaline, irregular in shape and size (1–1.5 × 2–5 µm), globose, subglobose, ellipsoid, cylindrical with rounded ends, constriction at point of septation. Conidia formed by disarticulation of hyphae.

Distribution: Currently known only from the type locality, Kalyan, Maharashtra, India.

DISCUSSION

The yellow to orange-brown gymnothecium (Fig. 4C–D and 5a) is made up of a reticulum of thick-walled hyphae, which shows *Gymnoascus verrucosus* to be a typical member of *Onygenales*

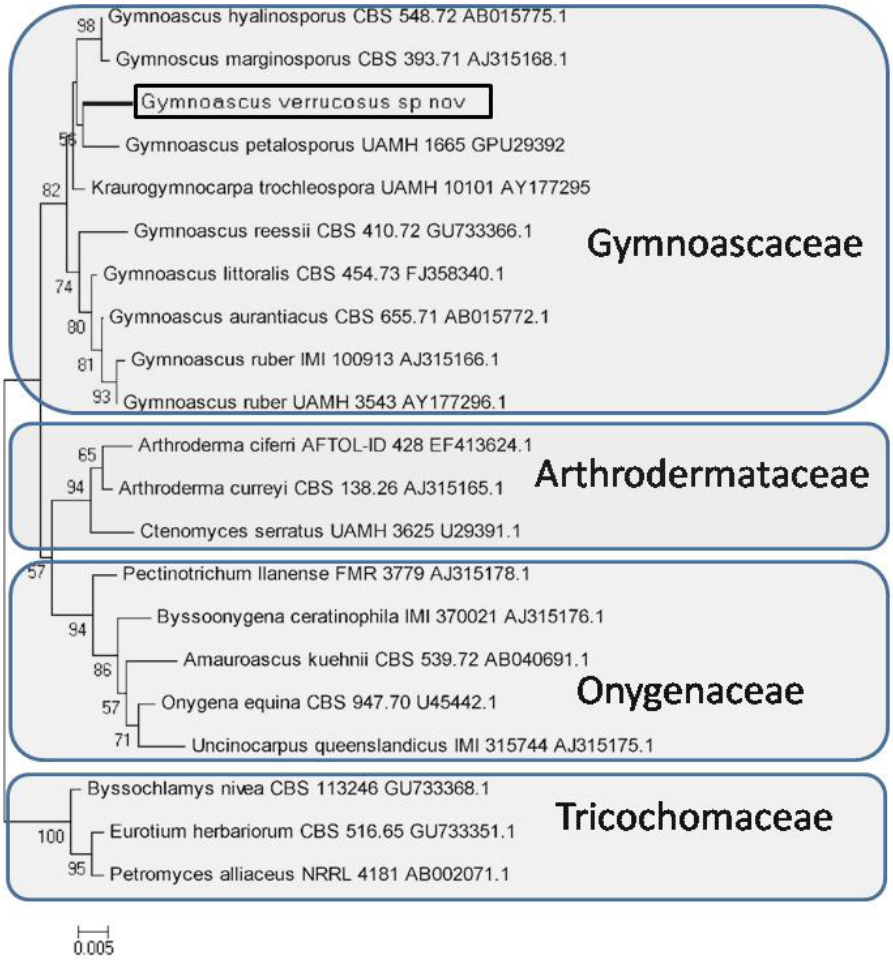

Fig. 2. Neighbour-joining tree of the SSU sequence of *Gymnoascus verrucosus* and 17 other species belonging to families *Gymnoascaceae*, *Arthrodermataceae,* and *Onygenaceae*. The bar indicates distance calculated in MEGA v. 5.0 and indicates units of base substitution per site. Bootstrap values above 50 % are shown.

(Currah 1985) with affinity to *Gymnoascaceae*. However, due to the minute echinulations or verruculose structures on the ascospore walls it was difficult to place it within *Gymnoascus,* whose species have smooth, irregular, or lumpy ascospores. The ascospores of *G. verrucosus* superficially resemble those of *Mallochia* whose species possess globose, echinulate ascospores. *Mallochia echinulata* forms ascospores with blunt spines apart from coarse echinulations. The other species of the genus, *M. endodonta,* possess two-walled ascospores unlike *G. verrucosus*. Also, *Mallochia* forms a telaperidium (ascomata made of an envelope of thin-walled hyphae not differentiated from vegetative hyphae) while *G. verrucosus* forms a reticuloperidium (ascomata mesh-like made of richly branched and more or less anastomosed, thick walled hyphae, mostly with thick-walled appendages). However, molecular studies using various rDNA regions suggest that our fungus belongs in the monophyletic genus *Gymnoascus.* The inclusion of this species in the genus now means that the generic circumscription needs to be amended to include species with verruculose ascospores. Phylogenetic analysis of the ITS region of the rDNA places this new species in subclade Ia of *Gymnoascus,* comprising *G. petalosporus, G. boliviensis, G. reessii* and *G. uncinatus* (Fig. 3). Morphologically, the gymnothecium of *G. verrucosus* superficially resembles that of *G. reessii* in having a thick-walled reticulum, albeit an open one. The thick-walled, septate, peridial hyphae seen in *G. verrucosus* are also found in *G. petalosporus,* but the latter species has a loose mesh of such hyphae (not a true reticuloperidium), which are branched at right angles, while those of the former do not. Also, *G. verrucosus* possesses small, pointed appendages along with a completely different type of ascospores, unlike *G. petalosporus*. Two other genera of *Onygenales*, *Auxarthon* and *Acitheca* (*Onygenaceae*) possess pointed appendages, but unlike those of *G. verrucosus,* they are elongate with or without septation. Also, *Auxarthron* forms an inverted Y-shaped arch at the base of the elongate appendage(s), unlike *G. verrucosus* whose appendages are short and branched like deer antlers. Both the genera *Acitheca* and *Auxarthron,* however, have a different type of ascospore morphology and never have the verruculose type of sculpturing seen in *G. verrucosus*. The other species of the clade, *G. boliviensis* (Guarro *et al.* 1992), differs from *G. verrucosus* in having smooth, discoid ascospores with an equatorial rim, which are arranged within the ascus in a petaloid manner. A depression is seen on one side of some of the ascospores of *G. verrucosus* under SEM (Fig. 5C); this may be an artefact due to collapse of the ascospore wall under high vacuum during SEM observation; typical globose

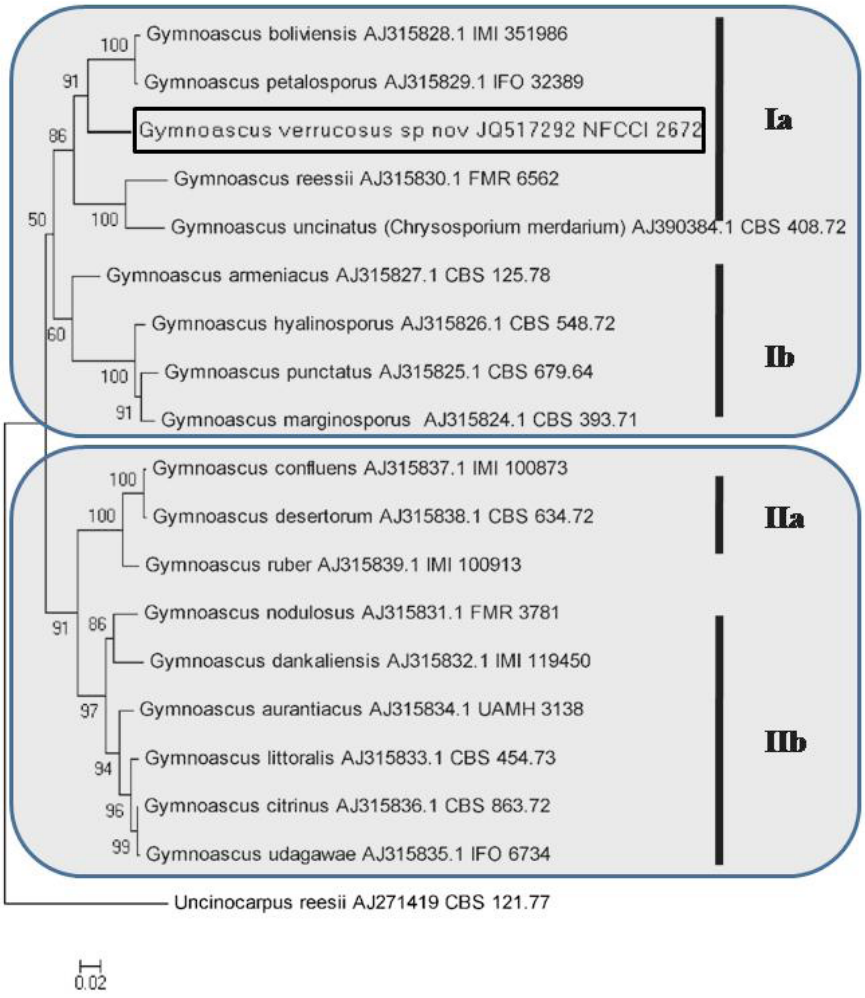

Fig. 3. Neighbour-joining tree of ITS sequences showing the phylogenetic position of *Gymnoascus verrucosus* within the genus *Gymnoascus*.

ascospores are seen in Fig. 5D (without a depression).

The generic name *Kraurogymnocarpa* was introduced by Udagawa & Uchiyama (1999) with the new species *K. lenticulospora* as type. The new genus was distinguished from *Gymnoascus* on the basis of distinct bivalvate ascospores having warts on the convex surface and the lack of enlargements or swellings at the septa on peridial appendages. A second species, *K. trochleospora* (syn. *Pseudoarachniotus trochleosporus*) was added to the genus based on the similar, but not identical, ascospores. Only one species of *Gymnoascus* forms bivalvate ascospores, *G. ruber,* whose ascospores are smooth, unlike *Kraurogymnocarpa*. The original authors did not perform a molecular analysis, but stated in their paper that such studies may supply more evidence on their relationships (Udagawa & Uchiyama 2001). Sigler *et al.* (2002) sequenced the SSU region of an ex-type culture of *Kraurogymnocarpa trochleospora* and showed that it was distinct from five species of five different genera of *Gymnoascaceae*, all of which are now placed in *Gymnoascus* (Sole *et al.* 2002). When we included the sequence of *K. trochleospora* in our SSU and LSU analysis, it was also placed within the monophyletic *Gymnoascus* clade (Figs 1–2). The ascospores of *K. trochleospora* have a combination of characters found in two different species of *Gymnoascus, viz*. *G. verrucosus* which has verruculose ascospore surfaces, and *G. ruber* with bivalvate ascospores. The SSU and LSU results, therefore, do not support the recognition of *K. trochleospora* as distinct from *Gymnoascus*.

Species delimitation in the broad generic concept of *Gymnoascus* (*sensu* Solé *et al*. 2002) is primarily based on shape, size, and sculpturing of ascospores. The verruculose ascospores (with small tubercules over the entire surface) of *G. verrucosus* make it distinct from all currently known species of the genus. Only *Gymnoascus udagawae* forms ascospores that seem to have warts, which are sporadic but do not resemble the prominent verruculose pattern of *G. verrucosus.*

The treatment of the new isolate as a new species of *Gymnoascus* is supported by the sequence analysis of the ITS, LSU and SSU regions of rDNA, which places it near *G. petalosporus, G. boliviensis, G. reessii,* and *G. uncinatus,* all of which form thick-walled differentiated peridial hyphae suggesting they evolved from an ancestor having a thick-walled reticuloperidium. The genus now comprises 19 species, which form discoid (with or without equatorial rim), bivalvate, globose, subglobose, irregular or lumpy ascospores with smooth, irregular or verruculose walls. A key to the known species is given below, adapted from Solé *et al.* (2002) to accommodate the new species.

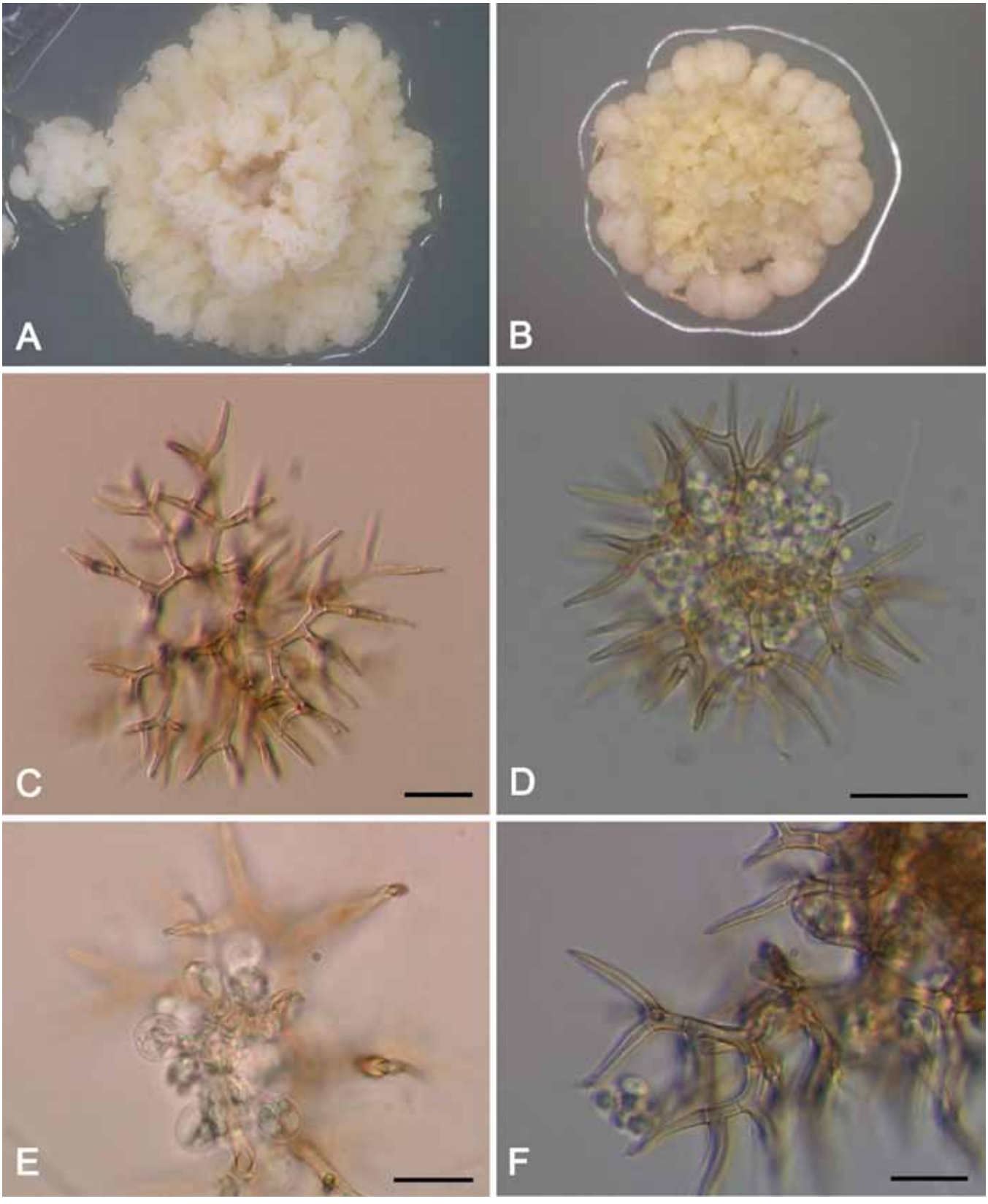

Fig. 4. *Gymnoascus verrucosus* (ex-type NFCCI 2672, holotype AMH 9454) **A**, **B.** Colonies on PDA after 4 wk at 28 °C. **C.** Peridium bearing thick-walled dichotomously branched peridial hyphae with short bifurcating spine like appendages. **D.** Globose open reticulum-type gymnothecium with ascospore mass in the centre. **E.** Developing asci and ascospores. **F.** Typical spine-like radially projecting small peridial appendages resembling deer antlers. Bars: C = 20 µm; D = 30 µm; E, F = 10 µm.

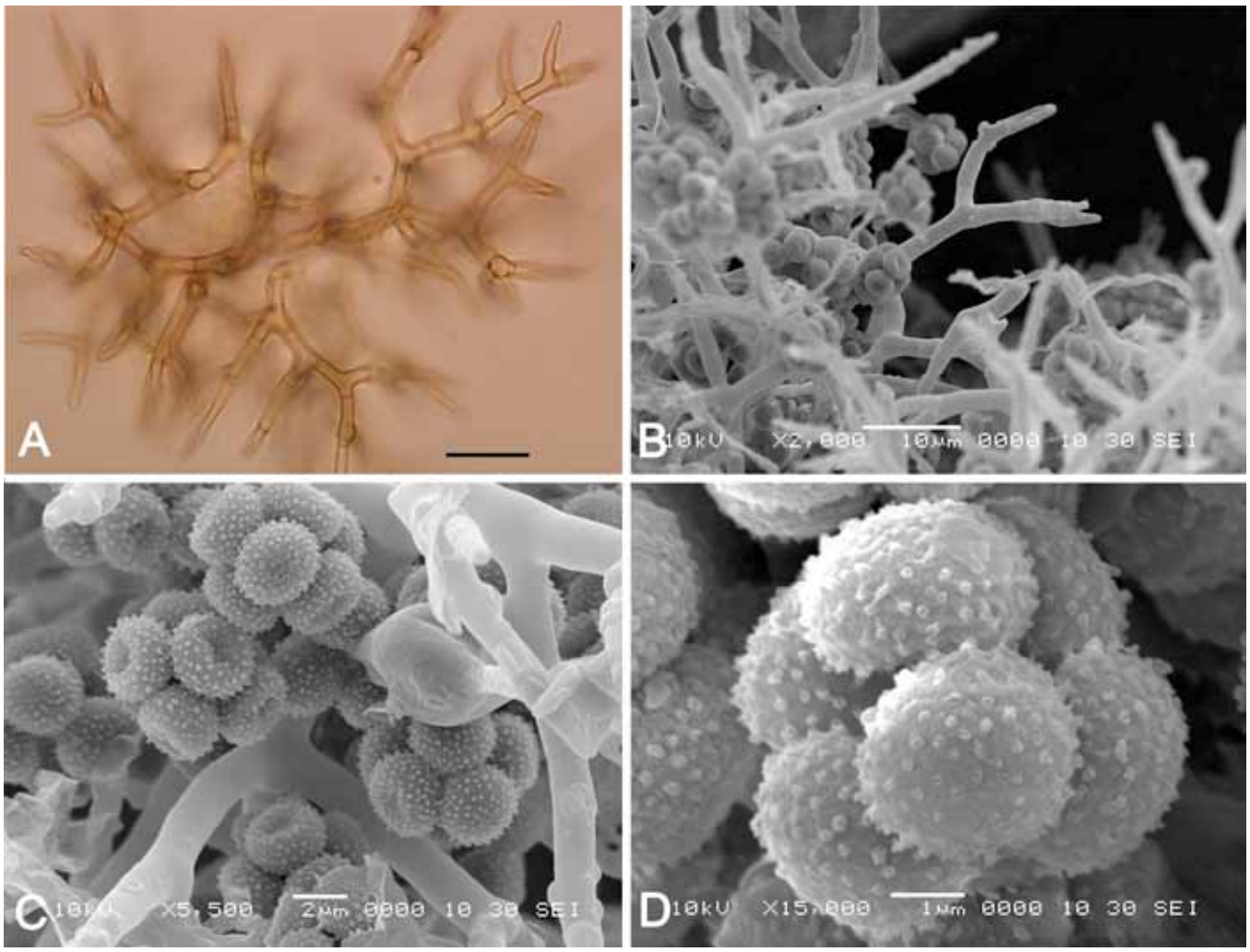

Fig. 5. *Gymnoascus verrucosus*, (AMH 9454) **A–D.** Peridial hyphae, asci and ascospores. **A.** Thick-walled, septate peridial hyphae. **B.** Portion of peripheral region of ascoma showing bi- or trifurcating peridial appendages. **C.** Mature asci, note the smooth peridial hyphae. **D.** Tuberculate ascospores compactly arranged. Bar: A = 10 µm.

Key to accepted *Gymnoascus* species

Note: The substrate and distributional data included here are based on the information provided in Currah (1985) and Guarro *et al.* (2012).

1 Colonies restricted; ascomata yellow, ascospores irregular in shape; isolated from pasture soil in Japan ..... **japonicus**
Above characters not combined 2

2 (1) Ascospores with an equatorial depression or furrow 3
Ascospores without such features 4

3 (2) Equatorial depression of the ascospores distinctly deep; isolated from soil, plant litter, and dung, apparently worldwide ..... **ruber**
Equatorial depression of the ascospores shallow; isolated from soil, rat or carnivore dung, Canada, Kuwait, Pakistan, and USA ..... **confluens**

4 (2) Ascospores 3–4 µm long 5
Ascospores 4–8 µm long 12

5 (4) Ascospores hyaline to pale yellow 6
Ascospores yellow, yellow-orange or reddish brown 7

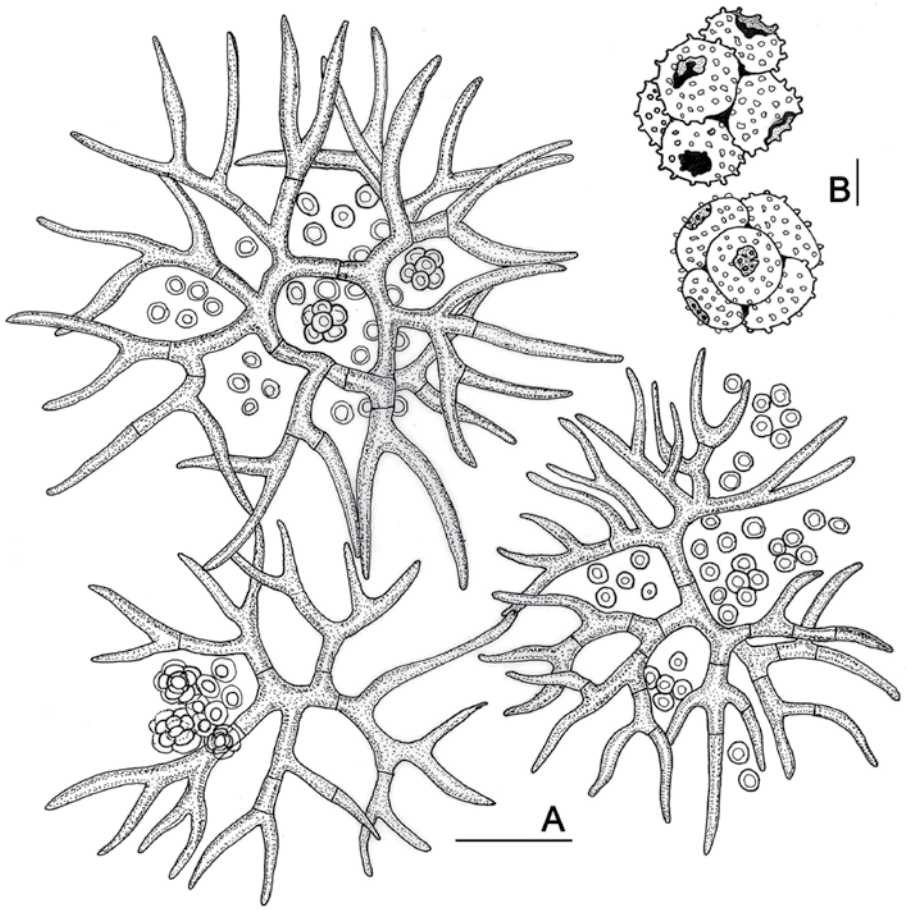

Fig. 6. *Gymnoascus verrucosus.* (AMH 9454) **A.** Ascomata with loose central mass of asci and ascospores and thick-walled, septate peridial hyphae radiating towards periphery. Note typical bi- or trifurcating peridial appendages resembling deer antlers. **B.** Two asci traced from SEM photomicrographs showing minute tuberculations on the entire surface of the ascospores. Bars: A = 20 µm; B = 2 µm.

6 (5) Ascospores appearing smooth, with scattered warts under oil; isolated from soil and also dung of various aninals, including fox, guinea pig, lizard, goat, and rat, apparently worldwide ..... **hyalinosporus**
Ascospores appearing smooth, with small tubercles under SEM; isolated from soil, India ..... **verrucosus**

7 (5) Ascospores arranged in a petaloid pattern in the ascus; arthroconidia absent 8
Ascospores not arranges as above; arthroconidia scarce or lacking 9

8 (7) Ascomata forming dense, orange-white aggregations; ascospores with a distinct equatorial thickening; isolate from chicken-yard soil, Bolivia ..... **boliviensis**
Ascomata discrete to confluent, brown to greenish brown; ascospores with no equatorial thickening; isolated form soil, rat dung, horn, horse ringworm, a human lesion, pigeon or duck feathers, apparently worldwide ..... **petalosporus**

9 (7) Ascomata formed by thick-walled, well differentiated peridial hyphae with appendages 10
Ascomata formed by peridial hyphae poorly differentiated from vegetative hyphae and without appendages 11

10 (9) Ascomata with uncinate appendages; asexual state- terminal aleurioconidia and intercalary arthroconidia (chrysosporium-like); isolated from dung of various animals, including chicken, dog, deer, rat, racoon, and Hyrax, and also owl pellets, Canada, Uganda, USA ..... **uncinatus**
Ascomata with curved, hooked and branched appendages; asexual state unknown; isolated from dung of various animals, including coyote, deer, elephant, fowl, fox, hare, lizard, porcupine, and sheep, apparently worldwide ..... **reessii**

11 (9) Ascospores yellow-orange, smooth or slightly rough, with an equatorial thickened band; isolated from soil, marine sludge, bat, rabbit, and tiger dung, and also linen and buried cables, apparently worldwide ..... **marginosporus**
Ascospores hyaline, smooth, without equatorial thickening; isolated from kangaroo dung, India ..... **armeniacus**

12 (4) Ascospores with equatorial thickenings 13
Ascospores without distinct equatorial thickenings 15

13 (12) Ascospores 6–8 × 4.5–5.5 µm, with a distinct narrow equatorial thickening and polar thickenings; isolated from a human ear, foot, skin and toenail, dog hair or tick, and bat, bird, fox, and lizard dung, apparently worldwide .... **dankaliensis**
Ascospores smaller, without polar thickenings 14

14 (13) Colonies restricted; ascomata yellow to orange-brown; ascospores with narrow equatorial thickening; isolated from conch, crab or snail shell, red alga, sea anemone, and soil, Canada (BC), Italy, Mexico, and the USA ..... **littoralis**
Colonies expanding; ascomata pale to bright pink becoming rusty orange; ascospores with a broad equatorial band; isolated from paddy soil, India ..... **punctatus**

15 (12) Ascospores lemon-yellow, with polar thickenings; isolated from dog dung and soil, India and the USA ..... **citrinus**
Ascospores a different colour, without polar thickenings 16

16 (15) Ascospores oblate, yellow-orange to orange or reddish brown; arthroconidia present; isolated from soil, bat and rabbit dung, India and Somalia ..... **devroeyi**
Ascospores discoid, yellow to golden-yellow; aleurioconidia present or not 17

17 (16) Colonies on PDA orange to ochraceous; ascospores with irregular polar surfaces; isolated from plant debris, bat, dog and sheep dung, owl pellets, and soil, apparently worldwide ..... **aurantiacus**
Colonies on PDA lemon-yellow or pale; ascospores smooth 18

18 (17) Colonies restricted; peridial hyphae with irregularly thick-walled broad swellings and constrictions at the septa; ascospores 4–5 × 2–2.5 µm, arranged in a petaloid pattern in the ascus; isolated from fox, guinea pig, and mouse dung, India and the USA ..... **nodulosus**
Colonies expanding; peridial hyphae undifferentiated from vegetative hyphae; ascospores 4– 5 × 2.5–3.5 µm, not arranged in a petaloid pattern in the ascus; isolated from soil, Japan ..... **udagawae**

ACKNOWLEDGEMENTS

We thank Sugat. V. Shende (Pune University) for assistance with the scanning electron microscopy, the Department of Science and Technology (DST, Government of India, New Delhi) for financial support to set up a National Facility for Culture Collection of Fungi (No. SP/SO/PS-55/2005) at MACS'Agharkar Research Institute, Pune, India, and its Director, Maharashtra Association for Cultivation of Sciences' Agharkar Research Institute for providing facilities. We declare that we have no conflicts of interest.

REFERENCES

Aljanabi SM, Martinez I (1997) Universal and rapid salt extraction of high quality genomic DNA for PCR based techniques. *Nucleic Acids Research* **25**: 4692–4693.

Arx JA Von (1986) The ascomycete genus *Gymnoascus*. *Persoonia* **13**: 173–183.

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

Currah RS (1985) Taxonomy of the *Onygenales*: *Arthrodermataceae, Gymnoascaceae, Myxotrichaceae* and *Onygenaceae*. *Mycotaxon* **24**: 1–216.

Doveri F, Pecchia S, Vergara M, Sarrocco S, Vannacci G (2011) A comparative study of *Neogymnomyces virgineus*, a new keratinolytic species from dung, and its relationships with the *Onygenales*. *Fungal Diversity*, **52**: 13–34.

Felsenstein J (1985) Confidence limits in phylogenies: an approach using the bootstrap. *Evolution* **39**: 783–791.

Guarro J, Ulfig K, Vroey CD (1992) A new keratinophilic *Gymnoascus* from Bolivian soil. *Mycotaxon* **45**: 317–322.

Guarro J, Gené J, Stchigel AM, Figueras MJ (2012) *Atlas of Soil Ascomycetes*. [CBS Biodiversity Series 10.] Utrecht: Centraalbureau voor Schimmelcultures.

Kimura M (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* **16**: 111–120.

Orr GF, Kuehn HH, Plunkett OA (1963) The genus *Gymnoascus* Baranetzky. *Mycopathologia et Mycologia Applcata* **21**: 1–18.

Rehner SA, Samuels GS (1994) Taxonomy and phylogeny of *Gliocladium* analysed for nuclear large subunit ribosomal DNA sequences. *Mycological Research* **98**: 625–634.

Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* **4**: 406–425.

Sharma R, Rajak RC, Pandey AK (2002) Teaching techniques for mycology: 19. A micro-dilution drop-trail method for isolating onygenalean ascomycetes from hair baits. *Mycologist* **16**: 153–157.

Sigler L, Hambleton S, Pare JA (2002) *Chlamydosauromyces punctatus* gen. & sp. nov. (*Onygenaceae*) from the skin of a lizard. *Studies in Mycology* **47**: 123–129.

Solé M, Cano J, Pitarch LB, Stchigel AM, Guarro J (2002) Molecular phylogeny of *Gymnoascus* and related genera. *Studies in Mycology* **47**: 141–152.

Sugiyama M, Summerbell RC, Mikawa T (2002) Molecular phylogeny of onygenalean fungi based on small subunit (SSU) and large subunit (LSU) ribosomal DNA sequences. *Studies in Mycology* **47**: 5–23.

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, and Kumar S (2011) MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution* **28**: 2731–2739.

Udagawa S, Uchiyama S (1999) Taxonomic studies on new or critical fungi of non-pathogenic *Onygenales* 1. *Mycoscience* **40**: 277–290.

Udagawa S, Uchiyama S (2001) Taxonomic studies on new or critical fungi of non-pathogenic *Onygenales* 4. *Mycoscience* **42**: 281–287.

Vanbreuseghem R (1952) Techniques biologique pour l'isolement des dermatophytes du sol. *Annales de la Société Belge de Médecine Tropicale* **32**: 173–178.

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 S, 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 method and applications*: 315–322. San Diego: Academic Press