

Auxarthronopsis, a new genus of *Onygenales* isolated from the vicinity of Bandhavgarh National Park, India

Rahul Sharma¹, Yvonne Gräser², and Sanjay K. Singh¹

¹National Facility for Culture Collection of Fungi, MACS' Agharkar Research Institute, G. G. Agarkar Road, Pune - 411 004, India; corresponding author e-mail: singhsksingh@gmail.com

²Institute of Microbiology and Hygiene (Charité), Humboldt University, Dorotheenstr 96, Berlin 10117 Germany

Abstract: An interesting onygenalean ascomycete was isolated from soil collected from a hollow tree near Bandhavgarh National Park situated in central India. The keratinophilic nature associated with a malbranchea-like asexual morph, appendaged mesh-like reticuloperidia, and subglobose to oblate, punctate ascospores, support the inclusion of this isolate in *Onygenaceae*. Further, the pale cream ascospores, punctate ascospores, and swollen septa in the peridial hyphae suggested that this was a new species of *Auxarthron*. However, phylogenetic study of LSU, SSU and ITS sequences, and presence of more than three swollen septa on the peridial appendages, do not support a placement within *Auxarthron*, and the new generic name *Auxarthronopsis* is introduced to accommodate this new fungus. The distinguishing features of this new taxon are the multiple (≥ 10) swollen septa on the appendages attached to its reticulate, loosely mesh-like peridium, the finely and regularly punctate ascospores, and the production of arthroconidial and aleurioconidial asexual forms. Sequence analysis of ITS1-5.8S-ITS2, SSU and LSU regions clearly separate this fungus from monophyletic *Auxarthron* and other taxa bearing some morphological similarity. Phylogenetically, *Auxarthronopsis bandhavgarhensis* gen. sp. nov. is closest to *Amauroascus purpureus*, *A. volatilis-patellis*, *Nannizziopsis albicans*, and *Renispora flavissima*, but differs morphologically.

Key words:

Auxarthron
Knuckle-joints
Molecular phylogeny
Multiseptate appendages
Onygenaceae

Article info: Submitted: 19 February 2012; Accepted: 17 April 2013; Published: 10 June 2013.

INTRODUCTION

Onygenales is an assemblage of fungi which has evolved to utilize keratin that forms part of the integument of birds, reptiles, and mammals. This substrate, like cellulose, is abundant in soil as it is regularly shed by large number of vertebrates either as effete integumental elements or as ingested materials mixed into excreta. Burrows actively inhabited by small mammals are excellent habitats for these fungi since they contain ample substrate, hair – and moisture – at all times of the year, and even in hot dry summers. The unusual form of the ascospores in most onygenalean members, a mesh-like reticuloperidium with various types of appendages, is suggested to be an adaptation to active dispersal via attachment to arthropods, birds, and mammals (Currah 1985). During our studies on keratinophilic fungi from different geographical regions of India, an interesting ascomycete was isolated from a burrow-like habitat, in this case a hollow tree, near Bandhavgarh National Park which has one of the highest densities of wild tigers in the world.

The most recent monographic treatment of *Onygenales* was devoted to evolutionary and molecular phylogenetic studies (Guarro *et al.* 2002). It included four new genera, 14 new species, and six new combinations based on sequence

analysis of one or more rDNA regions (ITS, LSU and SSU). Unlike several other groups of fungi where the rDNA locus fails to resolve the phylogeny of taxa, onygenalean fungal phylogeny was mostly well resolved with rDNA gene comparisons (Gräser *et al.* 1999, Sole *et al.* 2002c, Sugiyama *et al.* 2002). An exception lay in some recently evolved asexual dermatophyte lineages, where little variation is observed even in the ITS region, and where microsatellites or other highly labile loci are necessary for species delimitation by molecular methods.

The order *Onygenales* contains a few genera that have mesh-like peridia, the reticuloperidium (*sensu* Currah 1988), bearing elongate appendages; these include *Auxarthron*, *Pectinotrichum*, and *Uncinocarpus* (Currah 1988). *Castanedomycetes* is another genus of *Onygenales* that forms elongate appendages, but differs in having a membranous peridium similar to that in *Aphanoascus*. The generic name *Auxarthron* was established by Orr *et al.* (1963) for species with “enlargements at the septa in the peridial hyphae; lightly coloured, elongate appendages which are non-septate except for one, two or three basal septa with characteristic swellings at such septa”. The generic name means ‘swollen joints’, and the genus now comprises 13 species (Sole *et al.* 2002a, b, Sigler *et al.* 2002). The monophyly of *Auxarthron*

© 2013 International Mycological Association

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

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

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

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

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

was demonstrated by Sole *et al.* (2002a) based on ITS sequence data. Another morphologically similar genus, *Amauroascus*, has ascomata composed of loosely woven hyphae, and, unlike *Auxarthron*, it forms incompositoperidia¹. That genus was found to be polyphyletic (Sole *et al.* 2002a).

Morphological study of the material from the Bandhavgarh area suggested that it might be accommodated in *Auxarthron*, if the existing circumscription was extended to include species with more than three septate peridial appendages. To determine if such a change was supported by sequence data, we conducted molecular studies using three different regions of rDNA to establish the phylogenetic relationships of our taxon.

MATERIALS AND METHODS

Sample origin and isolation of fungus

A soil sample (S214) was collected from around Bandhavgarh National Park (23.5°N, 80.25°E), Umariya, Madhya Pradesh, India during 2001. Collections were made from the subsurface using sterile spatulas in sterile sealed polythene bags. On return to the laboratory, the samples along with the polythene bags were directly stored in plastic boxes at room temperature (range 10 °C in winter to >40 °C in summer). The present isolate was obtained from the samples in February 2007 by the hair-baiting technique (Vanbreuseghem 1952) and the microdilution drop-trail method (Sharma *et al.* 2002). The growth rate was determined at 28 °C on Sabouraud Dextrose Agar (SDA; peptone 10 g, 40 g dextrose, agar 20 g L⁻¹), dilute Sabouraud Dextrose Agar (dSDA; peptone 1 g, dextrose 4 g, agar 20 g L⁻¹), Oatmeal Agar (OA) (HiMedia, Mumbai), Cornmeal Agar (CMA) (HiMedia, Mumbai), Potato Carrot Agar (PCA) (HiMedia, Mumbai) and Potato Dextrose Agar (PDA; 200 g potato (extract), dextrose 20 g, agar 20 g L⁻¹). The morphological structures were measured in warmed lactophenol mounts.

The specimen is deposited in the Ajrekar Mycological Herbarium (AMH), with pure cultures deposited in the National Fungal Culture Collection of India (NFCCI–WDCM 932), Maharashtra Association for Cultivation of Sciences' Agharkar Research Institute, Pune, India, and at CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands (CBS 134524).

Light and Scanning Electron Microscopy (SEM)

Light microscopy was performed using lactophenol mounts. Photographs were taken either with a Nikon Eclipse E800 research microscope (Nikon, Tokyo) mounted with a Nikon HIII camera or a Zeiss Axio Imager A-2 (Carl Zeiss MicroImaging, Gottingen) research microscope. Stereomicroscopic study of the ascomata was undertaken using a Nikon SMZ1500 microscope with an attached Nikon 8400 camera. For SEM analysis, naturally dried ascomata on baited hair were directly mounted onto stubs and briefly dried under vacuum (15–20

min), then coated with gold-palladium/platinum mixture and visualized under JEOL 610 and JEOL JSM 6360A (JEOL, Tokyo) microscopes at varying magnification and accelerating voltages.

DNA extraction and PCR amplification

DNA was extracted using the CTAB (N-cetyl-N, N, N-trimethylammonium bromide) method (Gräser *et al.* 1999) after the fungus had been grown on Sabouraud glucose agar (Difco Laboratories) for 21 d at 28 °C. The ITS1-5.8S-ITS2, 18S, and 28S rRNA genes were amplified using universal primers. PCR was performed with primer pairs Mass 266/V9D, ITS1/ITS4 (ITS), 5.8SR, LROR, LR7, LR7R, LR12 (LSU), and NS1, NS4, NS3, NS8 (SSU) (Rehner & Samuels 1994, Vilgalys & Hester 1990, White *et al.* 1990). For the ITS region, 50 µL of the PCR reaction mixture contained 28 µL H₂O, 5 µL PCR buffer (10x), 4 µL dNTPs (250 mM each), 1 µL primer (50 pmole µL⁻¹), 0.4 µL Taq polymerase (5 U µL⁻¹) (Applied Biosystems Roche, NJ), and 5 µL genomic DNA (10 ng µL⁻¹). The mixture was overlaid with one drop of light mineral oil (Sigma, Steinheim). PCR was performed in a Perkin Elmer 9600 thermocycler (PerkinElmer, Roche Molecular Systems, Branchburg, NY) with the following reaction conditions: 95 °C for 5 min, (95 °C for 1 min, 55 °C for 1 min, 72 °C for 1 min) ×30; final extension 72 °C for 10 min. For 18S and 28S rRNA, 25 µL of the PCR reaction mixture contained 16 µL H₂O; 2.5 µL PCR buffer (10x); 1 µL dNTPs (250 mM each); 0.5 µL primer (50 pmol µL⁻¹); 1 µL Taq polymerase (1 U µL⁻¹) (Genei, Bangalore); 2.5 µL genomic DNA (10 ng µL⁻¹); and was overlaid with one drop of light mineral oil (Genei, Bangalore). The PCR reaction was performed in an Eppendorf Mastercycler (Eppendorf, Hamburg) with the following conditions: 95 °C for 5 min, (95 °C 1 min, 51 °C or 65 °C for 1 min, 72 °C 1 min) ×30; final extension 72 °C for 10 min. The amplification product was checked on 1.2 % agarose gel stained with ethidium bromide and photographed under UV.

Sequencing and phylogenetic analysis

The resulting amplification products were cleaned with QIAquick PCR purification kit (Qiagen, Hilden) or Axygen PCR Cleanup kit (Axygen Scientific, CA) and sequenced using the same primers (White *et al.* 1990) on an automated sequencer (Beckman-Coulter, Fullerton, California or ABI 3100 Avant, Applied Biosystems, Foster City, California). For phylogenetic analysis, sequences of material used in previous studies and ex-type strains were retrieved in FASTA format from GenBank. Phylogenetic analyses using the Neighbour-Joining (NJ) method (Saitou & Nei 1987) were performed with the MEGA v. 5 computer program (Tamura *et al.* 2011). The phylogenetic tree was constructed using the Kimura two-parameter distance model (Kimura 1980) with the 'pairwise deletion of gaps option'. The robustness of branches was assessed by bootstrap analysis with 1000 replicates. The ITS, LSU, and SSU sequences of our isolate (NFCCI 2185) have been deposited in GenBank (Table 1) and sequence alignments have been submitted to TreeBase (Submission ID 12372).

¹A term used by Currah (1985) for globose ascomata having a loose and incomplete network of hyphae.

Table 1. Fungal species and LSU, SSU and ITS nrDNA GenBank accession numbers used in the study.

| Classification | Taxon | ITS | SSU | LSU | |
|---|-------------------------------|----------------------------------|------------|------------|------------|
| Arthrodermataceae | <i>Arthroderma cajetani</i> | | | AY176736.1 | |
| | <i>A. ciferri</i> | | EF413624.1 | EF413625.1 | |
| | <i>A. curreyi</i> | | AJ315165.1 | | |
| | <i>A. otae</i> | | | AY176735.1 | |
| | <i>Ctenomyces serratus</i> | AJ877222.1 | U29391.1 | AY176733.1 | |
| Gymnoascaceae | <i>Gymnoascus aurantiacus</i> | | AB015772.1 | AY176747.1 | |
| | <i>G. littoralis</i> | | | FJ358272.1 | |
| | <i>G. marginosporus</i> | | AJ315168.1 | | |
| | <i>G. petalosporus</i> | | | AY176748.1 | |
| | <i>G. reesii</i> | | | AY176749.1 | |
| | <i>G. ruber</i> | | AY177296.1 | AY176746.1 | |
| | Onygenaceae | <i>Amauroscopsis reticulatus</i> | AJ271418 | | |
| <i>Amauroascus aureus</i> | | AJ271433 | | AY176705.1 | |
| <i>Am. echinulatus</i> | | AJ271562 | | | |
| <i>Am. mutatus</i> | | AJ271567 | | AB075321.1 | |
| <i>Am. niger</i> | | AJ271563 | | AY176706.1 | |
| <i>Am. oblatum</i> | | AJ271421 | | | |
| <i>Am. purpureus</i> | | AJ271564 | | AY176707.1 | |
| <i>Am. queenslandicus</i> | | AB361646.1 | AJ315175.1 | | |
| <i>Am. volatilis-patellis</i> | | AJ133435 | | AB075324.1 | |
| <i>Aphanoascus fulvescens</i> | | | AJ315172.1 | | |
| <i>A. mephitalis</i> | | | AB015779.1 | AJ176725.1 | |
| <i>Apinisia racovitzae</i> | | AJ271429 | | | |
| <i>Auxarthron alboluteum</i> | | AB361630.1 | AY124494.1 | AB359411.1 | |
| <i>A. californiense</i> | | AF038352.1 | | AY176711.1 | |
| <i>A. chlamydosporum</i> | | AJ271425 | | | |
| <i>A. concentricum</i> | | AJ271428 | | | |
| <i>A. conjugatum</i> | | AJ271573 | | AB075325.1 | |
| <i>A. compactum</i> | | AJ271574 | AB015767.1 | | |
| <i>A. filamentosum</i> | | AY177298.1 | AY124501.1 | AB359417.1 | |
| <i>A. kuehnii</i> | | AB040691 | AB015766.1 | AB040691.1 | |
| <i>A. pseudoauxarthron</i> | | AJ271572 | | | |
| <i>A. pseudoreticulatus</i> | | AJ271420 | | | |
| <i>A. reticulatum</i> | | AJ271568 | | AB359430.1 | |
| <i>A. umbrinum (A. thaxteri)</i> | | AJ271571 | AY124498.1 | | |
| <i>A. zuffianum</i> | | AJ271569 | AY124492.1 | AY176712.1 | |
| <i>Auxarthronopsis bandhavgarhensis</i> | | HQ164436 | JQ048939 | JQ048938 | |
| <i>Byssoonygena ceratinophila</i> | | | AJ315176.1 | | |
| <i>Chlamydosauromyces punctatus</i> | | | AY177297.1 | | |
| <i>Nannizziopsis albicans</i> | | AJ271432 | | | |
| <i>Neogymnomyces demonbreunii</i> | | AJ315842.1 | | AY176716.1 | |
| <i>Onygena corvina</i> | | | AB075364.1 | | |
| <i>O. equina</i> | | | U45442.1 | | |
| <i>Pectinotrichum llanense</i> | | AJ390391.1 | AJ315178.1 | | |
| <i>Renispora flavissima</i> | | AF299348.1 | AB015784.1 | AY176719.1 | |
| <i>Shanorella spirotricha</i> | | AJ271430 | | | |
| <i>Uncinocarpus reesii</i> | | AJ271419 | L27991.1 | AY176724.1 | |
| Trichocomaceae | | <i>Byssochlamys nivea</i> | | GU733368.1 | AY176750.1 |
| | | <i>Eurotium herbariorum</i> | | GU733351.1 | AY176751.1 |
| | | <i>Petromyces alliaceus</i> | | AB002071.1 | AY176752.1 |

Table 2. A comparison of morphological characters of the genus *Auxarthron*, *Auxarthronopsis*, and *Amauroascus*.

| Character | | <i>Auxarthron</i> | <i>Auxarthronopsis</i> | <i>Amauroascus</i> |
|---------------------|--------|---|--|---|
| Ascomata | Colour | Yellow-brown to brown | White to pale cream | White, yellow or brown |
| | Size | <700 µm | 500–1000 µm | Up to 2.5 mm |
| Ascoma type | | Reticuloperidium | Reticuloperidium | Incompositoperidium |
| Asci | | Subglobose, globose or ovoid, 8-spored, evanescent, | Globose, 8-spored, hyaline evanescent, | Ovoid, 8-spored, up to 14 µm |
| | | 7.2–8 × 10.6 µm | 5 × 5.5 µm | |
| Ascospore | Colour | Hyaline, to yellow or yellow-brown | Hyaline | Hyaline, yellow or reddish brown to dark brown |
| | Shape | spherical to oblate, minutely punctuate to punctate-reticulate | globose to subglobose, finely and regularly punctate, globose in polar view but oblate in equatorial view | spherical with pronounced irregular or regular puncta and ridges |
| | Size | <5 µm | 1.75–2.5 × 2.75–3.2 µm | 3.5–7 µm |
| Peridial hyphae | | Reddish, rusty tan, enlarged at septa, thick cuticularized smooth to minutely to coarsely asperulate or tuberculate | Pale brown, branched and anastomosed form reticulate network, with swollen septa, sparsely asperulate | Usually undifferentiated in culture, occasionally pale brown, smooth & thick walled |
| Peridial appendages | | Branches rare, tips round, sub-acute, larger appendages septate (1, 2 or 3 septa) with simple apices which are bent or slightly coiled or uncinatae | Multiseptate with distinct swollen septa, tapering to acute hyaline apex indistinguishable from vegetative hyphae, upto 400 µm | Appendages lacking |
| Anamorph | | Terminal, intercalary or rarely lateral, pyriform, oblong or cylindrical, aleurioconidia, arthroconidia, keratinolytic | Terminal or intercalary rhexolytically dehiscing arthroconidia and aleurioconidia, slightly keratinolytic | Terminal and/or intercalary rhexolytically dehiscing conidia, keratinolytic |

RESULTS

Hair-baiting of soil sample S214 resulted in isolation of an ascomycete with characteristics found in *Onygenales*. The fungus was isolated six to eight years after the soil was collected, but it was first observed in 2001 when a single ascoma formed in a hair-baiting plate and its unusual form was noted (Sharma 2002). At that time, however, no isolation could be made as the material was destroyed in slide preparation. However, after >6 yr when the soil was re-baited with horse hair, abundant ascomata developed on the hair (Fig. 1a, b). This showed the species' capacity for long-term survival in soil.

Phylogeny

The complete ITS1, ITS2 and 5.8S regions along with partial 18S and 28S rRNA genes, were sequenced for NFCCI 2185 and the sequences are deposited in GenBank with accession nos HQ164436 (ITS), JQ048938 (LSU), JQ048939 (SSU). Sequencing of the partial 28S rDNA gene of NFCCI 2185 resulted in a 1247 bp nucleotide sequence of the 5' end with the following nucleotide composition: 301–A, 293–C, 395–G and 258–T. A nucleotide BLAST search with 689 bp of 5' end of nrLSU showed a maximum homology of 94 % with *Amauroascus purpureus* (IFO 32622, AY176707.1) and *Auxarthron zuffianum* (CBS 219.58, AY176712.1), 93 % with *Neogymnomycetes demonbreunii* (ATCC 18394, AY176716.1), and 92 % with several other onygenalean species. In addition, the nrLSU sequences of onygenaceous (16 taxa), gymnoascaceous (5), and arthrodermataceous (4) from previous studies

were retrieved from GenBank and aligned with NFCCI 2185. Incomplete parts of the sequences from both ends were excluded from the analysis. A neighbour-joining (NJ) tree was constructed using the Kimura–2 parameter nucleotide substitution model. It showed distinct clades for members of the three families of *Onygenales* (Fig. 4). The new isolate was placed within the *Onygenaceae* clade along with *Am. purpureus* and *Am. volatilis-patellis*. Sequencing of the partial 18S rDNA gene of NFCCI 2185 resulted in a 1339 bp nucleotide sequence of the 5' end with the following nucleotide composition: 357–A, 275–C, 360–G, and 347–T. A nucleotide BLAST search with 1339 bp of the 5' end of nrSSU showed a maximum homology of: 98 % with *Chlamydosauromyces punctatus* (UAMH 9990, AY177297.1), *Renispora flavissima* (CBS 708.79, AB015784.1), and *Aphanoascus mephitilis* (CBS 453.75, AB015779.1); and 97 % with several other onygenalean species, including *Auxarthron zuffianum* (UAMH 4098, AY124492.1) and *A. alboluteum* (UAMH 2846, AY124494.1). The nrSSU sequences from previous studies, including 17 onygenaceous, three gymnoascaceous, and three arthrodermataceous, were also aligned with that of NFCCI 2185. Incomplete portions of sequences from both ends were again excluded from the analysis. The neighbour-joining SSU tree resolved the analysed strains into distinct families of *Onygenales* (Fig. 5). The new fungus formed a separate clade that included *Renispora flavissima* and *Chlamydosauromyces punctatus*. Some other related species, such as *Am. purpureus*, *Am. volatilis-patellis*, *Nannizziopsis albicans*, and *Neogymnomycetes demonbreunii* could not be included

in the analysis because SSU sequences were not available in GenBank. The strong LSU and SSU sequence similarity of NFCCI 2185 to other members of *Onygenales* suggests membership in this order.

Sequencing of the ITS region of NFCCI 2185 yielded a 613 bp long nucleotide sequence that included 242 bp of ITS1, 150 bp of 5.8S and 166 bp of ITS2. In contrast to the LSU and SSU, the ITS sequence of the undescribed fungus was distant from other species it was compared with. It showed maximal sequence similarities of only 84 % with two quite distinct species: *Amauroascus purpureus* (IFO 32622, AJ271564) and *Nannizziopsis albicans* (IMI 155645, AJ271432), with 90 % and 88 % query coverage, respectively. The next most closely associated sequences in the BLAST results had similar similarities of 85 %, but show very low query coverage of less than 75 %. These sequences included a mixture of *Eurotiales* and *Onygenales*. The ITS sequence of NFCCI 2185 was also compared with sequences of 29 species from ten genera of related *Onygenaceae* (Table 1) and a phylogenetic tree (NJ) was constructed using Kimura-2 parameter model (Fig. 6).

Phenotypically, the new taxon superficially resembles *Auxarthron* in having peridial hyphae with swollen septa, but it is distinct genetically. This is evident in the LSU, SSU, and ITS trees where *Auxarthron* forms a monophyletic clade (Figs 4–6). A morphological comparison of the newly designated *Auxarthronopsis* with *Auxarthron* and *Amauroascus* is shown in Table 2.

TAXONOMY

Auxarthronopsis Rahul Sharma, Y. Gräser & S.K. Singh, gen. nov.
MycoBank MB563744

Etymology: Named after its phenotypic similarity to the genus *Auxarthron*.

Description: *Ascomata* solitary, globose to subglobose, white to pale cream. *Peridium* made of mesh of interwoven hyphae with swollen septa. *Appendages* straight, tapering at the apex, sometimes branched, with multiple (≥ 10) swollen septa. *Asci* globose to subglobose, hyaline, evanescent, 8-spored. *Ascospores* unicellular, hyaline, oblate, with finely and regularly punctate walls. *Asexual morph* terminal and intercalary hyaline arthroconidia and aleurioconidia.

Type: *Auxarthronopsis bandhavgarhensis* Rahul Sharma et al. (2013).

Auxarthronopsis bandhavgarhensis Rahul Sharma, Y. Gräser & S. K. Singh, sp. nov.
MycoBank MB563745
GenBank HQ164436, JQ048938, JQ048939
(Figs 1–3)

Etymology: *bandhavgarhensis*- referring to the locality where the soil collection was made, Bandhavgarh National Park, India.

Description: *Ascomata* discrete, globose, white to pale cream, reticuloperidium 500–1000 μm diam, with appendages. *Peridial hyphae* branched and anastomosed to form reticulate network with knuckle-joints 2.0–2.5 μm wide. *Peridial appendages* pale, numerous, elongated, straight or bifurcated, multiseptate (≥ 10) with distinct swollen septa 2–2.5 μm wide, tapering to acute hyaline apex indistinguishable from vegetative hyphae, up to 400 μm long. *Asci* globose, eight-spored, hyaline, evanescent 5 \times 5.5 μm . *Ascospores* unicellular, hyaline, globose to subglobose, smooth under light microscopy, finely and regularly punctate under SEM, globose in polar view but oblate in equatorial view, 1.5–2.5 \times 2.5–3.0 μm .

Asexual morph: Conidia malbranchea-like, arthric, intercalary or terminal on short branches, abundant, hyaline, solitary, aseptate, 2.0–4.5 \times 4.5–11.5 μm , separated by autolytic connective cells.

Cultures: Colonies after 3 wk at 28 °C (Figs 2a, b) on SDA, white, slow growing (3 cm), cottony, slightly raised at the centre; reverse uncoloured to pale brown; on CMA, OA, and dilute SDA, slow growing (3 cm diam), mostly submerged with sparse mycelium, pale brown, reverse uncoloured to pale brown; on PCA and PDA slow growing (2 cm diam), pale coloured at the centre and white in the peripheral region, slightly cottony, and the reverse pale brown. *Ascomata* produced sparsely on PCA and abundantly on PDA (Figs 2c, d), but without elongate appendages.

Type: **India**: *Madhya Pradesh*: Umariya, buffer zone of Bandhavgarh National Park, ascomata growing on horse hair (keratin bait) in soil S214 collected from inside a big hollow tree, 16 June 2001, R. Sharma (AMH 9405 – **holotype**; NFCCI 2185 = CBS 134524 – cultures ex-type).

Substratum: Isolated on horse hair from soil.

Distribution: Known only from the type locality, Bandhavgarh, India.

DISCUSSION

The typical mesh-like peridium of *Auxarthronopsis bandhavgarhensis* and other features, including the ability to grow on horse hair (keratinophilic nature), make this fungus a relatively typical member of *Onygenales* (Currah 1985). However, it could not be placed in the morphologically similar genus *Auxarthron*, which never forms multi-septate appendages. The new genus *Auxarthronopsis* shows a resemblance to *Auxarthron* in its mesh-like reticulo-peridium and in its possession of knuckle joints; however, the ascomata of *Auxarthron* are mostly yellow to brown at maturity, while the ascomata of the new taxon are white to pale cream. The peridial hyphae of *Auxarthron* species are dark coloured, rigid, and relatively broad, while those of the new taxon are pale, narrow, and flexible. Also, the ascospores of the new species are finely and regularly punctate (with circular punctae), a morphology that differs from the minutely

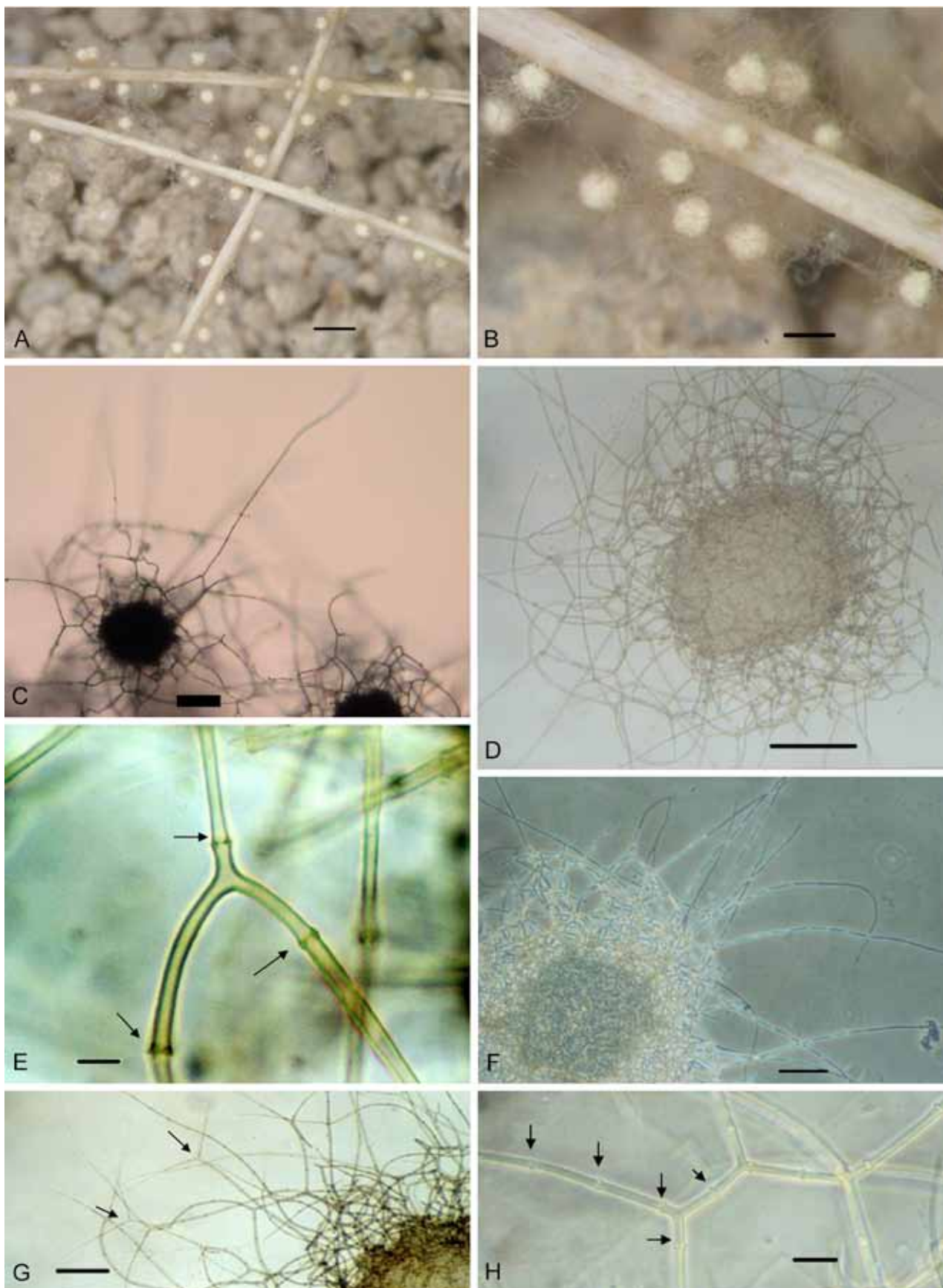


Fig. 1. *Auxarthronopsis bandhavgarhensis* (AMH 9405). **A–B.** Stereomicroscopic-view of mature ascoma growing on horse hair. **C.** Light microscopic view of unmounted ascomata with elongate appendages picked up from hair bait. **D.** Mesh-like reticuloperidium with central ascospore mass. **E.** Base of elongate appendage showing inverted Y-shaped arch with swollen septa (arrows). **F.** Phase contrast image of ascoma showing multiseptate peridial appendages. **G.** Bifurcate branching of peridial appendages (arrows). **H.** Dichotomously branched peridial hyphae showing knuckle joints (arrows). Bars: A = 600 μm ; B = 200 μm ; C = 100 μm ; D = 80 μm ; E = 6 μm ; F = 80 μm ; G = 80 μm ; H = 10 μm .



Fig. 2. *Auxarthronopsis bandhavgarhensis* (NFCCI 2185^T). Colonies at 28 °C after 3 wk of incubation. **A.** Colony front on different media. **B.** Reverse of colony on different media. **C.** Enlarged view of the colony on PDA with abundant ascomata near peripheral region. **D.** Developing ascomata on the periphery of colony on PDA. Bars: C = 1 cm; D = 300 μ m.

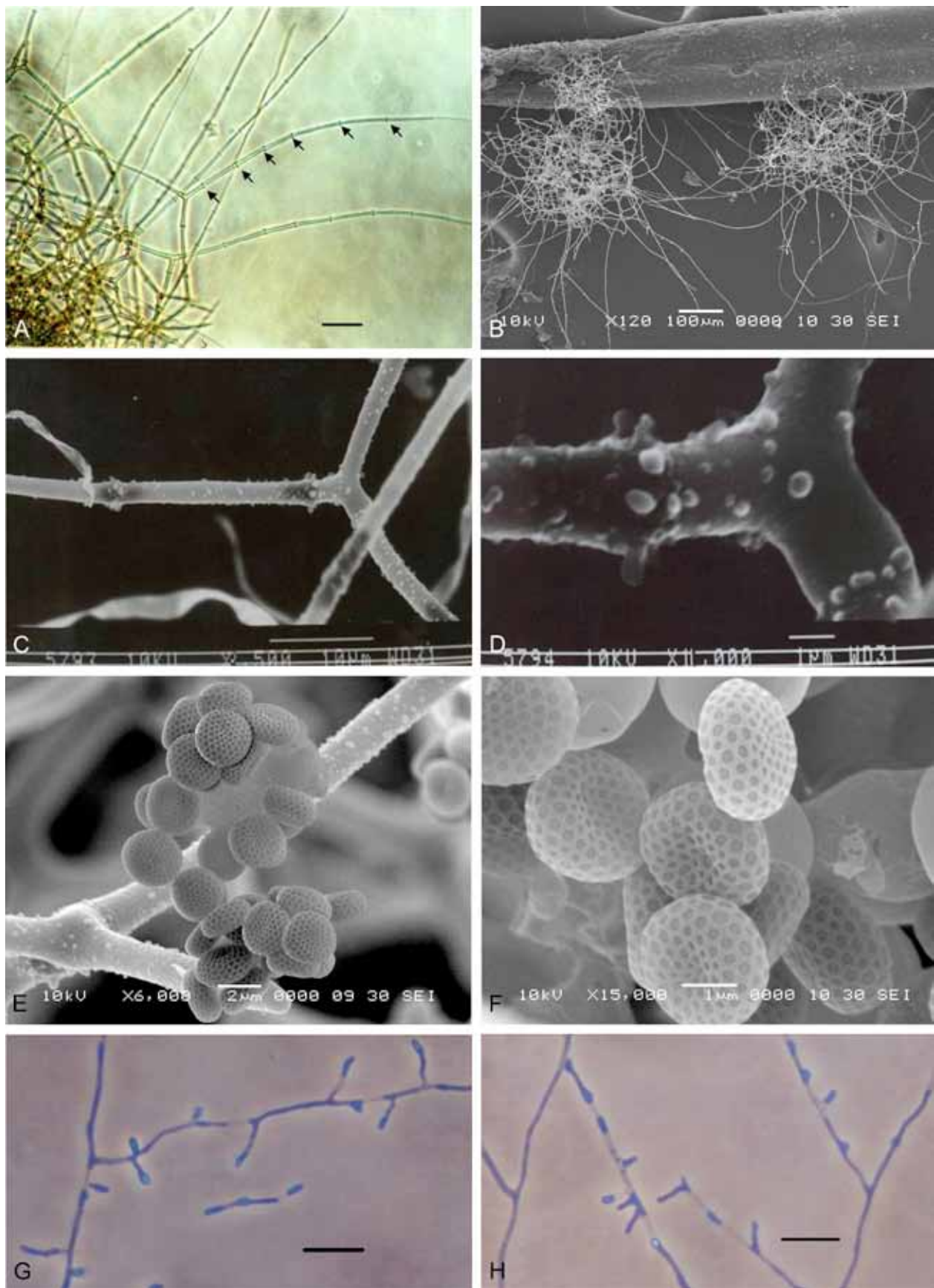


Fig. 3. *Auxarthronopsis bandhavgarhensis* (AMH 9405). Peridial appendages, ascospores and asexual morph. **A.** Multiple septa on peridial appendages (arrows). **B.** Elongate appendages radiating from reticuloperidia on horse hair. **C.** Sparsely asperulate basal portion of peridial appendage with two swollen septa. **D.** Enlarged portion of septa with ring of tubercles. **E.** Asci and ascospores. **F.** Finely and regularly punctate ascospores showing circular punctae. **G, H.** Slide culture preparation showing sessile and stalked aleurioconidia and arthroconidia (NFCCI 2185). Bars: A = 40 μm ; B = 100 μm ; C = 10 μm ; D = 1 μm ; E = 2 μm ; F = 1 μm ; G–H = 20 μm .

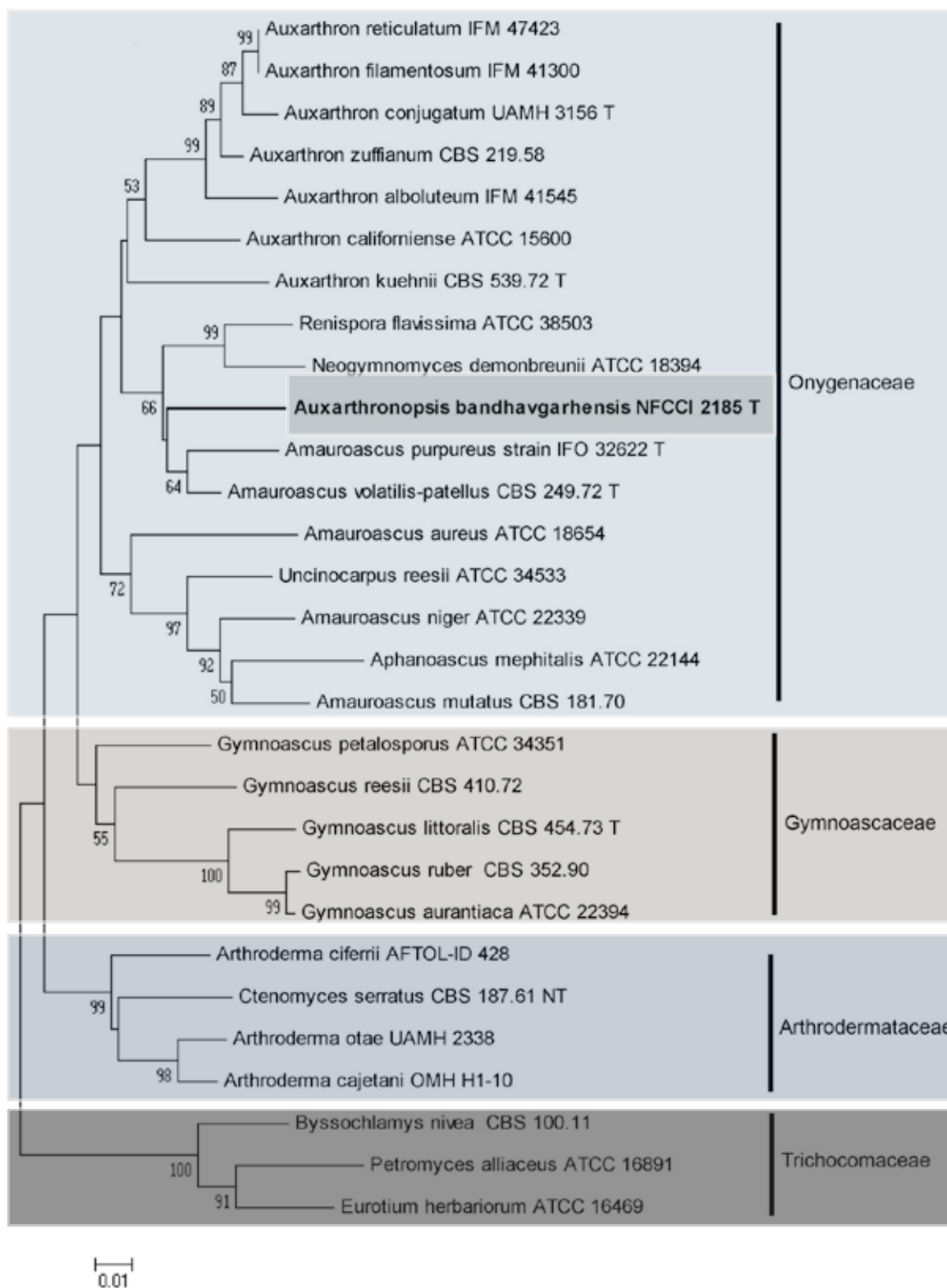


Fig. 4. Neighbour-joining tree based on nucleotide sequences of 28S rDNA gene of the 25 stains of *Arthrodermataceae*, *Gymnoascaceae* and *Onygenaceae* listed in Table 1 along with *Auxarthronopsis bandhavgarhensis* (NFCCI 2185^T). Three strains belonging to *Trichocomaceae* *Byssoschlamys nivea* (AY176750.1), *Eurotium herbariorum* (AY176751.1), and *Petromyces alliaceus* (AY176752.1) served as outgroup. The branch lengths are proportional to distance values calculated in MEGA 5 and values at nodes represents bootstrap percentage of 1000 replicates. Bootstrap values above 50 % are shown.

asperulate to punctate-reticulate ascospores of *Auxarthron*. Ascospores of *A. pseudoauxarthron* possess similar circular pits on the ascospore walls, but in that species the spores are

spherical and the ascomatal appendages lack knuckle joints. In general, species of *Auxarthron* do not possess more than three septa in the peridial appendages, whereas those of

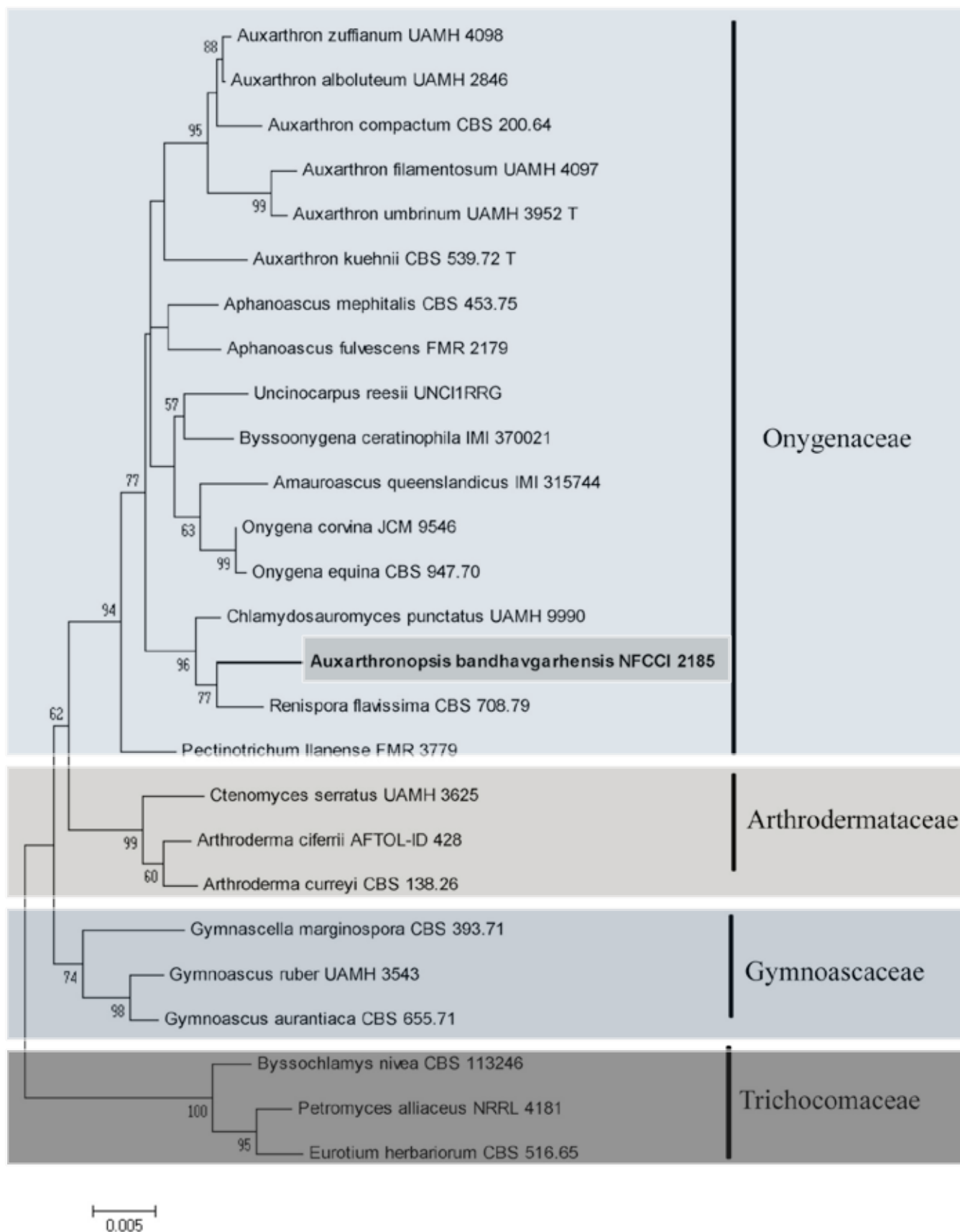


Fig. 5. Neighbour-joining tree based on nucleotide sequences of 18S rDNA gene of the 22 stains of *Arthrodermataceae*, *Gymnoascaceae* and *Onygenaceae* listed in Table 1 along with *Auxarthronopsis bandhavgarhensis* NFCCI 2185^T. Three strains belonging to *Trichocomaceae* *Byssochlamys nivea* (GU733368.1), *Eurotium herbariorum* (GU733351.1), and *Petromyces alliaceus* (AB002071.1) served as outgroup. The branch lengths are proportional to distance values calculated in MEGA 5 and values at nodes represents bootstrap percentage of 1000 replicates. Bootstrap values above 50 % are shown.

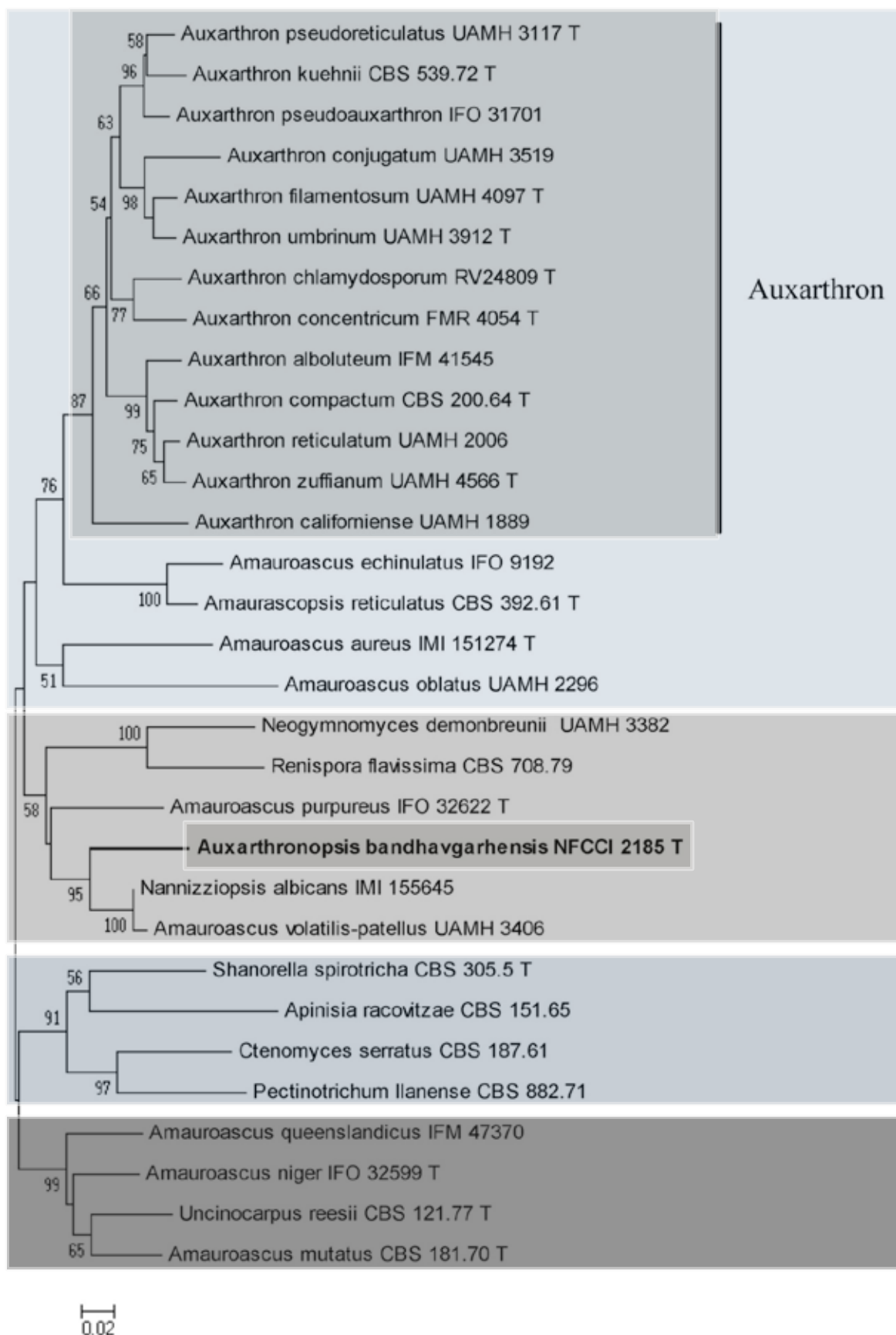


Fig. 6. Neighbour-joining bootstrap consensus tree based on nucleotide sequences of internal transcribed spacer (ITS) region and 5.8S rDNA gene of the 30 strains listed in Table 1 which includes reference strains of *Auxarthron* species and other onygenalean genera along with *Auxarthronopsis bandhavgarhensis* (NFCCI 2185T). The unrooted phylogenetic tree was drawn using 611 nucleotide of the ITS1, 2 and 5.8S rRNA gene using MEGA 5 software. The branch lengths are proportional to distance values calculated in MEGA 5 and values at nodes represents bootstrap percentage of 1000 replicates. Bootstrap values above 50 % are shown.

Auxarthronopsis have more than 10 septa. The phylogenetic interrelationships of *Auxarthron* and *Amauroascus* have been studied by Sole *et al.* (2002a) using ITS sequences, who found the former genus to be monophyletic and the latter polyphyletic. Our nrSSU and nrLSU trees also showed *Auxarthron* as monophyletic. The new taxon was excluded from the *Auxarthron* clade (Figs 4–5), and the ITS sequence comparisons also showed *Auxarthronopsis* as distinct from *Auxarthron*, but closer to *Amauroascus purpureus*, *Nannizziopsis albicans*, and *Amauroascus volatilis-patellis*. The ITS similarity of *Auxarthronopsis bandhavgarhensis* to the nearest neighbour, *Amauroascus purpureus*, was less than 85 % (119 bp out of 620 bp; 92 substitutions and 27 indels). Morphologically, *A. purpureus* is also distinct in possessing purple ascospores that lack knuckle joints and peridial appendages, and forming globose, frilled ascospores (Ito & Nakagiri 1995). The other phylogenetically closest neighbour, *Nannizziopsis albicans* (Fig. 6), differs from the new taxon in having a peridium of undifferentiated peridial hyphae without appendages, and in lacking a well-developed reticuloperidium (Guarro *et al.* 1991). Phylogenetically these two species differ at 128 of 627 nucleotide positions, including 74 substitutions and 54 indels. Another phylogenetically related species, *Amauroascus volatilis-patellis*, differs from *Auxarthronopsis bandhavgarhensis* in forming a peridium of undifferentiated hyphae lacking appendages, and in producing ascospores which are punctate-reticulate. It also differs at 166 of 623 nucleotide positions, 102 of which are substitutions and 64 of which are indels.

In the nrLSU and nrSSU trees (Figs 5–6), *Auxarthronopsis* was placed close to *Amauroascus*, *Renispora*, and *Neogymnomyces*. However, the new taxon is morphologically inconsistent with these genera in the distinctness of the ascospores, with a mesh-like reticuloperidium with swollen septa. *Renispora flavissima* has ascospores of poorly differentiated hyphae, which lack appendages, and forms finely pitted reniform or bacilliform ascospores. The type species of *Neogymnomyces*, *N. demondreunii*, forms discrete, spherical golden-yellow ascospores with hyaline, thick-walled, smooth and septate peridial hyphae, along with long, hyaline, peridial appendages. Recently, a new species of *Neogymnomyces*, *N. virgineus*, was described by Doveri *et al.* (2011) based on morphological and molecular data; however, it could not be included in our analysis as its ITS and LSU sequences (JN038187, JN038186) were not available to us. *Neogymnomyces virgineus* forms irregularly globose to pulvinate ascospores with pale yellow, fairly thick walled, verruculose septate hyphae along with two kinds of

peridial appendages: one long, tapering and smooth, and the other comparatively short, often branched, verruculose, and rounded at the tips. *Neogymnomyces* also differs in lacking swollen septa in the peridial hyphae and the appendages, and in possessing an arthro- and aleurioconidial asexual morph characterised by frequent swollen cells.

Another genus that possesses knuckle joints is the monotypic *Pectinotrichum* (Varsavsky & Orr 1971). *Pectinotrichum llanense* forms a reticuloperidium with long appendages and knuckle joints, but differs from *Auxarthronopsis* in having pectinate hyphae, large tubercles on the appendages and peridial hyphae, a *Chrysosporium* asexual morph, and almost smooth ascospores. Currah (1994) transferred *Pectinotrichum llanense* to *Auxarthron*, but its distance from *Auxarthron* was substantiated by the molecular studies of Sugiyama *et al.* (1999). Phylogenetically that species is distant from *Auxarthronopsis* as it separates out from the main *Onygenaceae* clade in SSU analysis (Fig. 5) and clusters with *Ctenomyces serratus*. Varsavsky & Orr (1971), who originally noted that the pectinate hyphae of *P. llanense* resemble the ctenoid appendages of *Ctenomyces serratus*, suggested that genetic studies of these genera would provide more evidence of a close relationship between them (Varsavsky & Orr 1971).

Knuckle-joints are a common feature in species with well developed reticuloperidia, *viz.* *Gymnoascus reesii*, *Pectinotrichum llanense*, and almost all *Auxarthron* species. Their purpose may be to provide strength to the mesh-like spherical peridium which protects the central ascospore mass from mite grazing in nature (Summerbell 2000). The swollen septa in *Auxarthronopsis* are slightly different in that they have a ring of tubercles visible under SEM (Fig. 3c, d) making knuckle joints relatively prominent in LM even though they are found on thin, smooth or sparsely asperulate, peridial hyphae.

Currah (1985) suggested that the morphological discontinuity seen in several genera of *Onygenales* might be bridged by as yet unrecorded forms. Perhaps *Auxarthronopsis* represents one such intermediate genus.

The order *Onygenales* now comprises 18 genera that have peridial appendages. These are usually borne on either a membranous cleistoperidium or a completely hyphal, mesh-like reticuloperidium. They may also be formed on an incomplete hyphal mesh-like incompositoperidium. A key to all appendaged genera of the order is provided, including *Ctenomyces*, the peridium of which includes membranous as well as hyphal elements.

Key to the genera of *Onygenales* with peridial appendages

- | | | |
|------|---|-----------------------|
| 1 | Ascospores cleistoperidium type | 2 |
| | Ascospores reticuloperidium or incompositoperidium type | 3 |
| 2(1) | Ascospores made of only cleistoperidium | Castanedomyces |
| | Ascospores made of inner layer cleisto-type, outer layer reticulo-type | Ctenomyces |
| 3(1) | Peridial appendages straight with simple or curved apices, branched or unbranched, uncinata, never coiled | 4 |
| | Peridial appendages coiled | 11 |

| | | |
|---------|--|---|
| 4(3) | Peridial hyphae or appendages with knuckle joints | 5 |
| | Peridial hyphae or appendages without knuckle joints | 7 |
| 5(4) | Peridial appendages without or up to 3 septa | Auxarthron |
| | Peridial appendages multi-septate | 6 |
| 6(5) | Peridium with short pectinate appendages | Pectinotrichum |
| | Peridium lacking pectinate appendages, swollen septa on appendages | Auxarthronopsis |
| 7(4) | Peridial appendages straight, rarely branched | 8 |
| | Peridial appendages, if present, branched or uncinata | 9 |
| 8(7) | Peridial appendages with acute apices, ascospores smooth | Acitheca |
| | Peridial appendages with blunt apices, ascospores ornamented | Nannizziopsis |
| 9(7) | Ascospores smooth | 10 |
| | Ascospores pitted, pitted-reticulate | Neogymnomyces |
| 10(9) | Peridial appendages if present, smooth, roughened (uncinate or boat-hook shaped apices) | Gymnoascus |
| | Peridial appendages ornamented with numerous short fine hairs, dichotomously branched, with blunt apices | Bifidocarpus |
| 11(3) | Peridial appendages coiled | 12 |
| | Peridial appendages uncinata | Uncinocarpus |
| 12(11) | Ascospores hyaline | 13 |
| | Ascospores yellow to orange | 14 |
| 13 (12) | Ascospores smooth | Histoplasma (syn. Ajellomyces) |
| | Ascospores irregularly ridged | Kuehniella |
| 14(12) | Ascospores ellipsoidal or oblate | 15 |
| | Ascospores globose, finely echinulate | Apinisia |
| 15(14) | Peridial hyphae made up of ossiform cells | Arthroderma |
| | Peridial hyphae not made up of ossiform cells | 16 |
| 16(15) | Peridial appendages thin walled, ascospores minutely roughened, pitted | Shanorella |
| | Peridial appendages thick walled | 17 |
| 17(16) | Ascospores smooth | Spiromastix |
| | Ascospores regularly punctate-muricate | Polytolypa |

ACKNOWLEDGEMENTS

R.S. is indebted to Tiger Trust, India, for organising a two-day Workshop on Tiger Conservation at Tiger Lodge, near Bhandhavgarh National Park, that made the collection of soil samples possible, to Madan L. Sharma (Regional Sophisticated Instrumentation Centre, Panjab University, Chandigarh) and Sugat V. Shende (SEM Facility, Department of Physics, University of Pune, Pune) for assistance with the scanning electron microscopy. Ram C. Rajak and Akhilesh K. Pandey are acknowledged for support during the initial stages of this study. We thank the Department of Science and Technology (DST), Government of India, New Delhi for financial support (No. SR/FT/L-36/2005 and No. SP/SO/PS-55/2005), and the Director, MACS'Agharkar Research Institute, Pune, for extending facilities.

REFERENCES

- Currah RS (1985) Taxonomy of the *Onygenales*: *Arthrodermataceae*, *Gymnoasaceae*, *Myxotricaceae*, and *Onygenaceae*. *Mycotaxon* **24**: 1–216.
- Currah RS (1988) An annotated key to the genera of the *Onygenales*. *Systema Ascomycetum* **7**: 339–350.
- Currah RS (1994) Peridial morphology and evaluation in the prototunicate ascomycetes. In: *Ascomycete Systematics: problems & perspectives in the nineties* (Hawksworth DL, ed.): 281–293. [NATO ASI Series no. 269.] New York: Plenum Press.
- Doveri F, Pecchia S, Vergara M, Sarrocco S, Vannacci G (2011) A comparative study of *Neogymnomyces virgineus*, a new keratinophilic species from dung, and its relationships with the *Onygenales*. *Fungal Diversity* **52**: 13–34.

- Gräser Y, El Fari M, Vilgalys R, Kuijpers AFA, de Hoog GS, Presber W, Tietz HJ (1999) Phylogeny and taxonomy of family *Arthrodermataceae* (dermatophytes) using sequence analysis of the ribosomal ITS region. *Medical Mycology* **37**: 105–114.
- Guarro J, Cano J, de Vroey Ch (1991) *Nanniziopsis* (*Ascomycotina*) and related genera. *Mycotaxon* **42**: 193–200.
- Guarro J, Summerbell RC, Samson RA (eds) (2002) *Onygenales: the dermatophytes, dimorphics and keratin degraders in their evolutionary context*. *Studies in Mycology* **47**: 1–220.
- Ito T, Nakagiri A (1995) *Amauroascus purpureus*, a new species of the *Amauroascaceae* (*Ascomycotina*). *Mycotaxon* **55**: 347–352
- 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) A new genus of the *Gymnoascaceae* with swollen peridial septa. *Canadian Journal of Botany* **41**: 1439–1456.
- Rehner SA, Samuels GS (1994) Taxonomy and phylogeny of *Gliocladium* analyzed for nuclear large subunit ribosomal DNA sequences. *Mycological Research* **98**: 625–634.
- Sharma R, Rajak RC, Pandey AK (2002) Teaching technique for mycology 19: A micro-dilution drop-trail method for isolating onygenalean ascomycetes from hair baits. *Mycologist* **16**: 153–158.
- Sharma R (2002) *Taxonomic studies on keratinolytic fungal diversity of Central India*. PhD thesis, Rani Durgavati University, Jabalpur.
- Sautou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* **4**: 406–425.
- Sigler L, Hambleton S, Flis AL, Pare JA (2002) *Auxarthron* teleomorphs for *Malbranchea filamentosa* and *Malbranchea albolutea* and relationships within *Auxarthron*. *Studies in Mycology* **47**: 111–112.
- Sole M, Guarro J, Cano J (2002a) Molecular phylogeny of *Amauroascus*, *Auxarthron* and morphologically similar onygenalean fungi. *Mycological Research* **106**: 388–396.
- Sole M, Cano J, Stchigel AM, Guarro J (2002b) Two new species of *Auxarthron* morphologically and genetically close to *A. kuehnii*. *Studies in Mycology* **47**: 103–110.
- Sole M, Cano J, Pitarch LB, Stchigel AM, Guarro J (2002c) Molecular phylogeny of *Gymnoascus* and related genera. *Studies in Mycology* **47**: 141–152.
- Sugiyama M, Ohara A, Mikawa T (1999) Molecular phylogeny of onygenalean fungi based on small subunit ribosomal DNA (SSU) sequences. *Mycoscience* **40**: 251–258.
- Sugiyama M, Summerbell RC and 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.
- Summerbell RC (2000) Form and function in the evolution of dermatophytes. In: *Biology of Dermatophytes and other Keratinophilic Fungi* Kushwaha RKS, Guarro J, eds): 30–43. Bilbao: Revista Iberoamericana de Micología.
- Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution* **28**: 2731–2739.
- Vanbreuseghem R (1952) Technique biologique pour l'isolement des dermatophytes du sol. *Annales de la Societe Belge de Medicine Tropicale* **32**: 173–178.
- Varsavsky E, Orr GF (1971) A new genus of the *Gymnoascaceae*. *Mycopathologia et Mycologia Applicata* **43**: 229–234.
- 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: *PCR Protocols: a guide to methods and applications* (Innis MA, Gelfand DH, Sninsky JJ, White TJ, eds): 315–322. San Diego: Academic Press.