KAVAKA 60(4): 1-12 (2024) DOI: 10.36460/Kavaka/60/4/2024/1-12

Megacapitulaceae, a New Family of *Pleosporales* through Epitypification and Multigene Phylogeny based on Fresh Material from India

Kunhiraman C. Rajeshkumar[1,2*], Sruthi O. Paraparath[1], Harikrishnan K[1], Sinang Hongsanan[3,4], Parayelil A. Ansil[1], Samantha C. Karunarathna[5], Saowaluck Tibpromma[5], Nalin N. Wijayawardene[5,6], Rajnish K. Verma[7] and Rajesh Jeewon[8,9]

[1]*National Fungal Culture Collection of India (NFCCI), Biodiversity and Palaeobiology (Fungi) Gr., Agharkar Research Institute, G.G. Agarkar Road, Pune - 411 004, Maharashtra, India.*
[2]*Faculty of Science, Savitribai Phule Pune University, Ganeshkhind Rd, Ganeshkhind, Pune - 411 007, Maharashtra, India.*
[3]*Shenzhen Key Laboratory of Microbial Genetic Engineering, College of Life Science and Oceanography, Shenzhen University, Shenzhen 518060, People's Republic of China.*
[4]*Guangdong Provincial Key Laboratory for Plant Epigenetics, College of Life Science and Oceanography, Shenzhen University, Shenzhen 518060, People's Republic of China.*
[5]*Center for Yunnan Plateau Biological Resources Protection and Utilization, College of Biology and Food Engineering, Qujing Normal University, Qujing, Yunnan 655011, People's Republic of China.*
[6]*Tropical Microbiology Research Foundation, 96/N/10, Meemanagoda Road, 10230 Pannipitiya, Sri Lanka.*
[7]*Mycology Lab, Department of Plant Pathology, Punjab Agricultural University, Ludhiana - 141 004, Punjab , India.*
[8]*Department of Health Sciences, Faculty of Medicine and Health Sciences, University of Mauritius, Réduit 80837, Mauritius.*
[9]*Department of Zoology, College of Science, King Saud University, Riyadh, Saudi Arabia*.

**Corresponding author Email: rajeshfungi@gmail.com*

(Submitted on September 24, 2024; Accepted on December 9, 2024)

ABSTRACT

This study introduces a new family, *Megacapitulaceae*, to accommodate an enigmatic fungus, *Megacapitula villosa*, hitherto placed under *Pleosporales incertae sedis*. *Megacapitula* is characterised by a large, ellipsoidal, obclavate, or obpyriform, muriform, pigmented, holoblastic conidia with dense hairy apical appendages. This genus has key morphological characteristics that distinctly set it apart from other known genera. The holotype of *Megacapitula* is a desiccated culture, making it incomparable to a freshly collected sporulating specimen and key diagnostic characteristics. Furthermore, the name-bearing type is unavailable for re-examination. Consequently, to comply with the proper nomenclature code and application of this name, a lectotype is assigned using the available illustration in the original protologue. Additionally, we designate a sequenced epitype based on a recent collection from the southern Western Ghats, Kannur (Kerala, India). Phylogenetic analyses of a concatenated LSU-SSU-ITS-*tef1α*-*rpb2* sequence data from the fresh ex-epitype NFCCI 5894 (Epitype AMH 10774) is delineated as a well-supported clade along with the erstwhile *M. villosa* accession, sister to *Phaeoseptaceae*, well within the ordinal classification of *Pleosporales*.

Keywords: *Dothideomycetes*, Kerala, New family, Phylogeny, *Pleosporomycetidae*, Taxonomy.

INTRODUCTION

The genus *Megacapitula* was established by Chen and Tzean (1993) with the type species *M. villosa* based on morphological species concept. The genus was characterised based on asexual morphs reported to date. *Megacapitula* is characterised by mycelium consisting of branched, septate, smooth, roughened, verrucose, hyaline or pigmented hyphae; conidiophores micronematous, semimacronematous, mononematous, simple or branched, pale brown to brown, smooth, roughened or verrucose; conidiogenous cells, integrated, terminal, lateral or rarely intercalary, determinate; conidia holoblastic, solitary, ovoid, obclavate, ellipsoidal or obpyriform, muriform, pigmented with densely packed, branched or unbranched, hair-like appendages at the apex (Chen and Tzean, 1993). This is a monotypic genus with a distinct conidial ontogeny.

Young conidia initially appear as spherical, subspherical, or ovoid structures, forming primarily

at the terminal ends of conidiophores, or occasionally along intercalary hyphae. During the developmental stage, conidia enlarge, elongate and are of varied shapes including ellipsoidal or muriform. The outer reticulate wall dissolves, splits, and forms a cap. Mature conidia characterized by apical, densely packed, long, hair-like appendages are the key features, that distinguished them from other morphologically similar genera in *Pleosporales* (Chen and Tzean, 1993).

Recently, Boonmee *et al.* (2021) isolated *M. villosa* from submerged decaying wood in a freshwater stream in Thailand. An updated phylogenetic analysis incorporating large subunit (LSU) and internal transcribed spacer (ITS) sequence datasets demonstrated that the newly acquired strain (MFLUCC 16-1231) clustered with other *M. villosa* isolates. Boonmee *et al.* (2021) classified *Megacapitula* under *Pleosporales* genera *incertae sedis* and suggested that this genus may encompass multiple species and could potentially represent a distinct family. Wijayawardene *et al.* (2022) also placed the genus under *Pleosporales* genera *incertae sedis*. Prabhugaonkar and Bhat (2011) first time recorded *M. villosa* (GUFCC 15515) from the decaying fronds of *Caryota urens* (Arecaceae) in India. They isolated the fungus, sequenced DNA, and inferred phylogeny as an undefined lineage in *Pleosporales* (with limited family representatives) based on ITS sequence data. Interestingly, 2 unpublished sequences of *M. villosa* (AL4 and cp053a) are available in GenBank, although they lack associated morphological descriptions and secondary barcode sequences. The present investigation aims to morphologically characterize *Megacapitula villosa* obtained from the southern Western Ghats of India and analyse its DNA sequence based phylogenetic relationships with extant species available using multigene sequence data. We also reevaluate the classification and assign a sequenced epitype for the future application of this name in fungal systematics.

MATERIALS AND METHODS

Sample Collection, Isolation and Morphology

As a part of exploring the lignicolous microfungal diversity of the Western Ghats of India, surveys were carried out to study the diversity of microfungi in decaying litter samples. Samples were collected in December 2023 from decaying leaves of unidentified palm growing in natural forests of the Kottiyoor, Kannur (11.863576°N, 75.907504°E) of Kerala, India. Conidia were isolated directly from the samples and observed using a Nikon binocular stereomicroscope (Model SMZ-1500 with Digi-CAM, Tokyo, Japan). Single conidial cultures were established on 2% malt extract agar plates (MEA; HiMedia, Mumbai, India) following Rajeshkumar *et al.* (2021, 2023). Microscopic observations were made with an Olympus (Model CX-41, Tokyo, Japan) dissecting microscope and Zeiss (AXIO Imager 2, Oberkochen, Germany) compound microscope equipped with Nikon Digital sight DS-Fi1 and AxioCam MRc5 cameras driven by AxioVision Rel 4.8 software (AXIO Imager 2, Oberkochen, Germany). Conidia and conidiophores were mounted in lactic acid cotton blue and measured using an ocular micrometer (confirmed with software available with the Zeiss microscope), with 30 observations per structure. Culture colony characters were recorded after 2 weeks of incubation on two media, including MEA and potato dextrose agar (PDA) (HiMedia, Mumbai, India). Methods for inoculations, incubation conditions and microscopic slide preparations followed Senanayake *et al.* (2020). Colour codes and names used in descriptions refer to Kornerup and Wanscher (1978). The fungal specimen was deposited in the Ajrekar Mycological Herbarium (AMH), and the culture was preserved at the National Fungal Culture Collection of India (NFCCI, WDCM- 932), Agharkar Research Institute, Pune, India. The nomenclatural updates and taxonomic details were submitted to Index Fungorum.

DNA extraction, amplification, and phylogenetic analyses

The colonies were cultured on MEA plates for two weeks, and genomic DNA was extracted using the rapid salt extraction method described by Aljanabi and Martinez (1997). The ITS nrDNA region (internal transcribed spacers 1 and 2 and intervening 5.8S nrRNA gene) was amplified using primer pair ITS5 and ITS4 (White *et al.*, 1990). The 5′ end of the large subunit nrRNA (LSU) gene region was amplified using primer pairs LR0R and LR7 (Vilgalys and Hester, 1990). The 5′ end of the small subunit nrRNA (SSU) gene was amplified using the primers NS1 and NS4 (White *et al.* 1990). Protein coding gene regions *rpb2* (RNA polymerase II second largest subunit) and *tef1α* (translation elongation factor 1- alpha gene) were amplified using the primer pairs fRPB2-5f/fRPB2-7cR (Liu *et al.*, 1999) and 983F/2218R (Rehner and Buckley, 2005) respectively. The amplification was performed

in a 25 µL reaction volume containing 9.5 µL ddH_2O, 12.5 µL 2 × Taq PCR Master Mix with blue dye (Sangon Biotech, Shanghai, China), 1 µL of DNA template and 1 µL of each primer (10 µM). The amplification condition provided by White *et al.* (1990) was followed for ITS and LSU. The amplification condition provided by Liu *et al.* (1999) and Rehner and Buckley (2005) was followed for *tef1α* and *rpb2*. The PCR products were purified with a StrataPrep PCR Purification Kit (Agilent Technologies, TX,97, California, USA) and sequenced using the same primers in the BigDye Terminator v. 3.1 Cycle Sequencing Kit (Applied Biosystems, Massachusetts, USA). Sequencing reactions were run on ABI PRISM® 3100 Genetic Analyzer (Applied Biosystems, Massachusetts, The USA).

The closest matching sequences were identified by searching the NCBI GenBank nucleotide database with MegaBLAST (Morgulis *et al.,* 2008). A sequence dataset was compiled mainly based on sequences published by Hogsanan *et al.* (2020). This dataset was aligned with our newly obtained sequences using MAFFT v. 7.453 (Katoh *et al.,* 2019) by selecting the G-INS-i option. *Asterina phenacis* TH 589, (*Asterinaceae*, *Dothideomycetes*) was chosen as the outgroup for the combined LSU-SSU-ITS-*tef1α*-*rpb2* analyses. Sequence alignments were manually edited where necessary using BioEdit v.7.0.9.0 (Hall, 1999) after which the alignments were concatenated using SequenceMatrix v.1.9 (Vaidya *et al.,* 2011). AliView (Larsson, 2014) was used to transfer the alignment file into PHYLIP and NEXUS format for Maximum Likelihood (ML) and Bayesian analyses, respectively. Phylogenetic analyses of concatenated dataset (LSU-SSU-ITS-*tef1α*-*rpb2*) were performed with the maximum likelihood (ML) method and Bayesian posterior probability (BY-PP) analysis. ML analyses were performed using IQ-TREE v.2.1.3 (Nguyen *et al.,* 2015, Kalyaanamoorthy *et al.,* 2017, Minh *et al.,* 2020) for concatenated dataset (LSU-SSU-ITS-*tef1α*-*rpb2*).

A total of 1000 non-parametric bootstrap replicates were used to calculate node support, while IQ-TREE selected the best-fit model for each data partition via the -m TESTNEW parameter. GAMMA model parameter estimation achieved a precision of 0.1000000000 log-likelihood units. Every 100th tree was saved. The Bayesian posterior probability (PP) analysis was performed using MrBayes v. 3.2.7a (Ronquist *et al.,* 2012), using the parameter settings of two parallel runs of four chains each, run for 100 million generations but with the stop value set at 0.01, the temperature set at 0.2 and the sample frequency every 10th generation. The model of evolution was estimated by using MrModeltest 2.2 (Nylander, 2004). The 50 % majority rule consensus tree was created from the remaining trees after the first 25 % of sampled trees were excluded as burn-in. The resulting trees were illustrated with Figtree v1.3.1 (Rambaut, 2010), and MEGA 7 (Kumar *et al.,* 2016) and the tree layout was created in Microsoft PowerPoint. DNA sequences newly generated in this study were deposited in GenBank. Species were authenticated according to the criteria established by Chethana *et al.* (2021) and Pem *et al.* (2021).

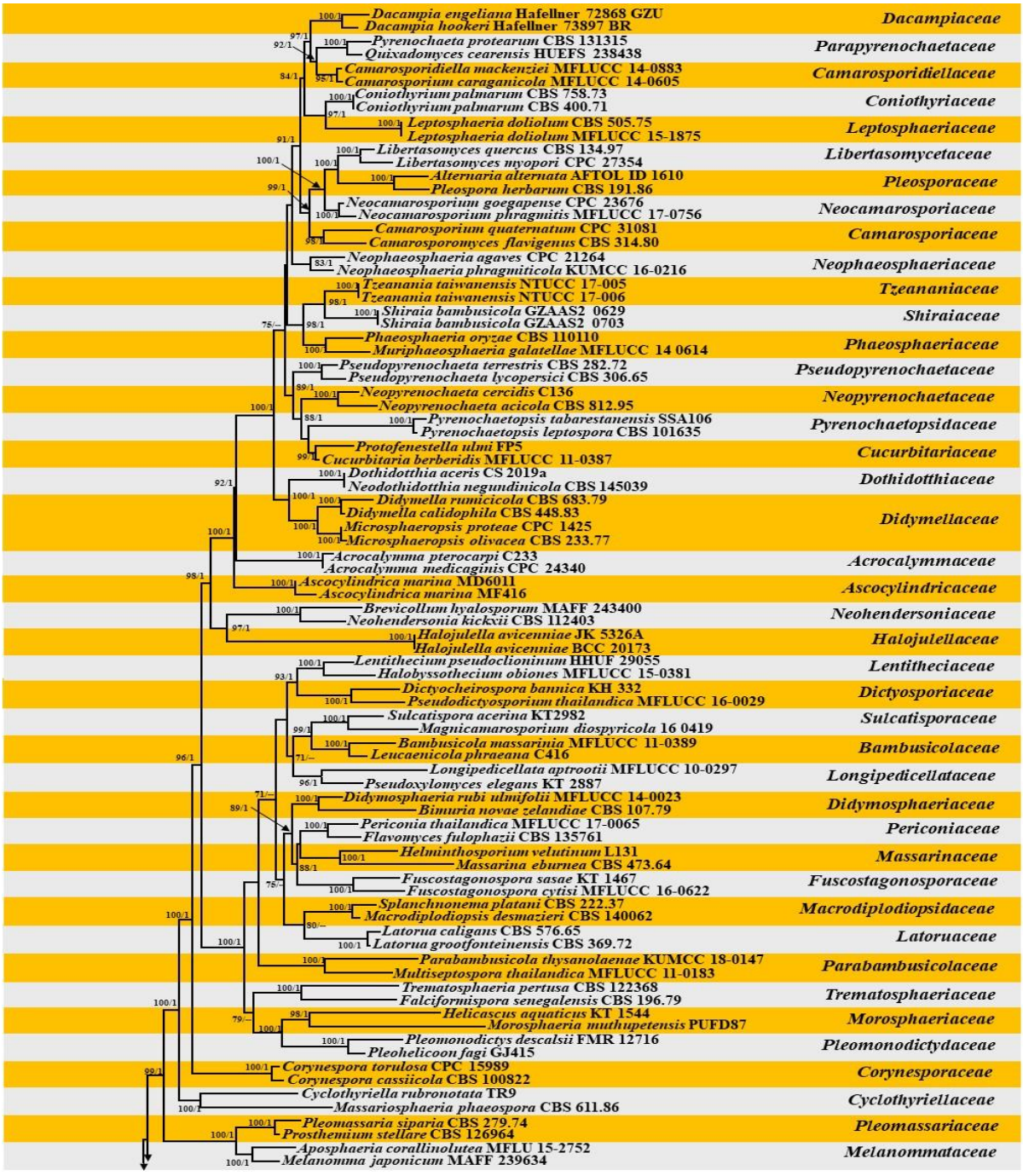
RESULTS

Phylogeny

Based on a MegaBLAST search of our *Megacapitula villosa* sequence in NCBI's GenBank nucleotide database, the closest matches using ITS were; *M. villosa* GUFCC 15515 [GenBank JN128868; Identities = 505/505(100%), Gaps = 0/505(0%)], *M. villosa* GZAAS 24-0040 [GenBank PP902467; Identities = 504/504(100%), Gaps =0/504(0%)] and *M. villosa* LS03 [GenBank MZ538513; Identities =499/499(100%), Gaps =0/499(0%)]. The closest hits using LSU were *M. villosa* SNC122M [GenBank PP621038; Identities = 870/871(99%), Gaps = 0/871(0%)], *M. villosa* SNC122 [GenBank PP621037; Identities = 872/874(99%), Gaps = 1/874(0%)], *M. villosa* GZAAS 24-0040 [GenBank PP657317; Identities = 835/837(99%), Gaps = 0/837(0%)]. The closest hits using SSU were *M. villosa* SNC122M [GenBank PP627301; Identities = 973/974(99%), Gaps = 1/974(0%)], *M. villosa* SNC122 [GenBank PP627300; Identities = 947/948(99%), Gaps = 1/948(0%)], *Preussia* sp. CCF3831 [GenBank FJ430777; Identities = 938/975(96%), Gaps = 4/975(0%)]. The closest hits using *rpb2* were *M. villosa* SNC122 [GenBank PP780225; Identities = 923/932(99%), Gaps = 3/932(0%)], *Lophiostoma compressum* KT 534 [GenBank JN993492; Identities = 763/931(82%), Gaps = 9/931(0%)], *L. caespitosum* OF:256902 [GenBank MW752383; Identities = 759/930(82%), Gaps = 11/930(1%)]. The closest hits using *tef1α* were *M. villosa* SNC122 [GenBank PP740447; Identities = 943/947(99%), Gaps = 0/947(0%)], *M. villosa* LS03 [GenBank MZ567115; Identities = 932/934(99%), Gaps = 0/934(0%)], *Pleopunctum ellipsoideum* MFLU 19-

0685 [GenBank MK828510; Identities = 886/947(94%), Gaps = 0/947(0%)].

The final combined LSU, SSU, ITS, *tef1α*, and *rpb2* dataset comprised a total of 8347 characters, including all alignment gaps for 166 sequences of strains belonging to 83 families of *Pleosporales*, including the outgroup. The maximum likelihood (ML) tree obtained from the concatenated alignment (LSU-SSU-ITS-*tef1α*-*rpb2*) with IQ-TREE was constructed with a log-likelihood of -105666.293. The matrix had 3514 distinct alignment patterns, with 2123 parsimony-informative, 823 singleton sites, and 5401 constant sites. Parameters for the SYM+I+G4 model of the combined LSU-SSU-ITS-*tef1α*-*rpb2* dataset were as follows: estimated base frequencies A = 0.250, C = 0.250, G = 0.250, T = 0.250; substitution rates AC = 1.40835, AG = 3.92948, AT = 1.66837, CG = 1.24270, CT = 7.61140, GT = 1.00000; gamma distribution shape parameter α = 0.584. The final ML concatenated tree with superimposed PP values is shown in **Figure 1.** Phylogenetic analyses of the LSU-SSU-ITS-*tef1α*-*rpb2* concatenated dataset and individual datasets supported the placement of *Megacapitula villosa*, in the proposed new family *Megacapitulaceae* with statistical support (BS = 100% / PP = 1.00) as a sister lineage to *Phaeoseptaceae*.



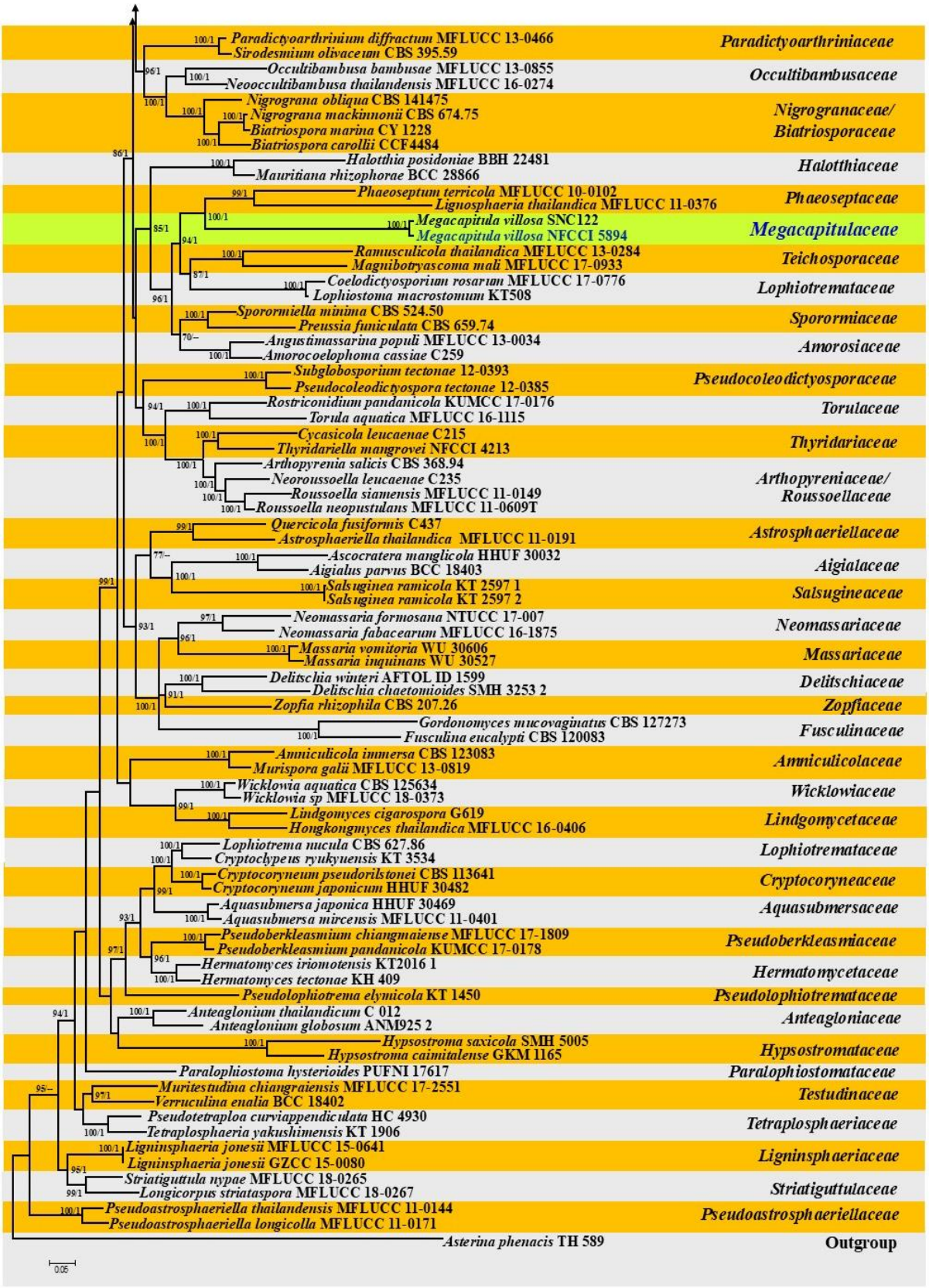


Figure 1: Phylogram generated from maximum likelihood analysis (IQ-TREE) of *Pleosporales* (including 83 families) based on combined LSU, SSU, ITS, *tef1α*, and *rpb2* sequence data. Maximum likelihood bootstrap values (≥70%) and Bayesian posterior probabilities (≥0.90) are provided at the nodes, with the species name followed by the corresponding original isolate number. *Asterina phenacis* (TH 589) is selected as the outgroup. Hyphen (-) represents support values below 70 % MLBS and 0.90 BYPP.

Taxonomy

Megacapitulaceae Rajeshk., Sruthi OP, Hongsanan, Jeewon, Karuna, Harik. **fam. nov.**

Index Fungorum identifier: IF 902119

Etymology: referring to the name of the type genus name, *Megacapitula*.

Classification: *Pleosporales*, *Dothideomycetes*, *Ascomycota*

Mycelium composed of branched, septate, smooth, roughened, verrucose, hyaline or pigmented hyphae. *Conidiophores* micronematous, semi-macronematous, mononematous, simple or branched, pale brown to brown, smooth, roughened or verrucose. *Conidiogenous cells* integrated, terminal, lateral or rarely intercalary, determinate. *Conidia* holoblastic, solitary, ovoid, obclavate, ellipsoidal or obpyriform, muriform, pigmented with densely packed, branched or unbranched, hairlike appendages at the apex.

Type genus: *Megacapitula* Chen & Tzean

Notes: The genus *Megacapitula* is unique due to its exceptional morphological characteristics. Even though the taxon was collected from several habitats in Asia, such as Taiwan, China, Thailand and India, the fresh collection and epitypification was missing. That is the reason for the placement of this genus in *incertae sedis* under *Pleosporales*. In this study, we designated a lectotype based on the illustrations (which is, therefore, part of the original material and available for lectotypification) that is *Code* compliant and further assigned a sequenced epitype AMH 10774. This study based on concatenated alignment (LSU-SSU-ITS-*tef1α*-*rpb2*) positioned *Megacapitulaceae* clade, closely related to *Phaeoseptaceae*, in a robustly supported clade (BS = 100 / PP = 1.00), adjacent to *Teichosporaceae* and *Lophiotremataceae* (BS 94 / PP 1.00). *Sporomiaceae* and *Amorosiaceae* constituted an additional basal clade with strong support (BS = 96 /PP = 1.00) well within *Pleosporales*.

Megacapitula Chen & Tzean *Mycological Research* **97(3)**:347 (1993)

Index Fungorum identifier: IF **25546**

Mycelium composed of branched, septate, smooth, roughened, verrucose, hyaline or pigmented hyphae. *Conidiophores* micronematous, semi-macronematous, mononematous, simple or branched, pale brown to brown, smooth, roughened or verrucose. *Conidiogenous cells* integrated, terminal, lateral or rarely intercalary, determinate. *Conidia* holoblastic, solitary, ovoid, obclavate, ellipsoidal or obpyriform, muriform, pigmented with densely packed, branched or unbranched, hair-like appendages at the apex.

Type species: *Megacapitula villosa* Chen & Tzean

Megacapitula villosa Chen & Tzean *Mycological Research* **97(3)**:347 (1993)

Index Fungorum identifier: IF **359484**

Typus: TAIWAN, Taipei, on fallen, decayed petiole of broadleaved trees, 31 March 1991, J. L. Chen & S. S. Tzean (Holotype PPH17 (dried culture) &: ex type PPH17E (living culture) were deposited in Department of Plant Pathology and Entomology, National Taiwan University, Taipei, Taiwan, R.O.C. PPH17E was also deposited in Culture Collection and Research Center (CCRC 32736), Hsinchu, Taiwan, R.O.C. isotypes in NY and as IMI 353413).

Mycelium partly superficial, partly immersed, composed of branched, septate, smooth, roughened, or verrucose, hyaline, pale brown, to dark brown or olive brown, 1.3–4.2 µm wide hyphae, *Conidiophores* micronematous, semi-macronematous, mononematous, simple or branched, straight or flexuous, pale brown to brown, smooth, roughened or verrucose, 5–6.3 × 3–6.7 µm, *Conidiogenous cells* integrated, terminal, lateral or occasionally intercalary, determinate. *Conidia* holoblastic, solitary, ovoid, obclavate, ellipsoidal or obpyriform, muriform, 79–230 × 47–120 µm, brown to dark brown or black, smooth, often outer wall reticulate in young stage, capped when mature, with apical densely packed hairy appendages which are branched or unbranched, septate, smooth, up to 555 µm long, base brown or dark brown, thick-walled, 3–6 µm wide, tapering and paler toward the apex which is hyaline, 1–1.7 µm wide.

Note: The holotype is a desiccated culture, and the name-bearing type is incomparable with fresh sporulating species from nature. To comply with the code, a lectotype is designated using the illustration available in the original protologue and a sequenced epitype is designated using the fresh collection. Phylogenetic analyses using concatenated LSU-SSU-ITS-*tef1α*-*rpb2* from our recent collection from Kannur delineate a well-supported clade sister to *Phaeoseptaceae* under *Pleosporales*, named as a new family, *Megacapitulaceae*.

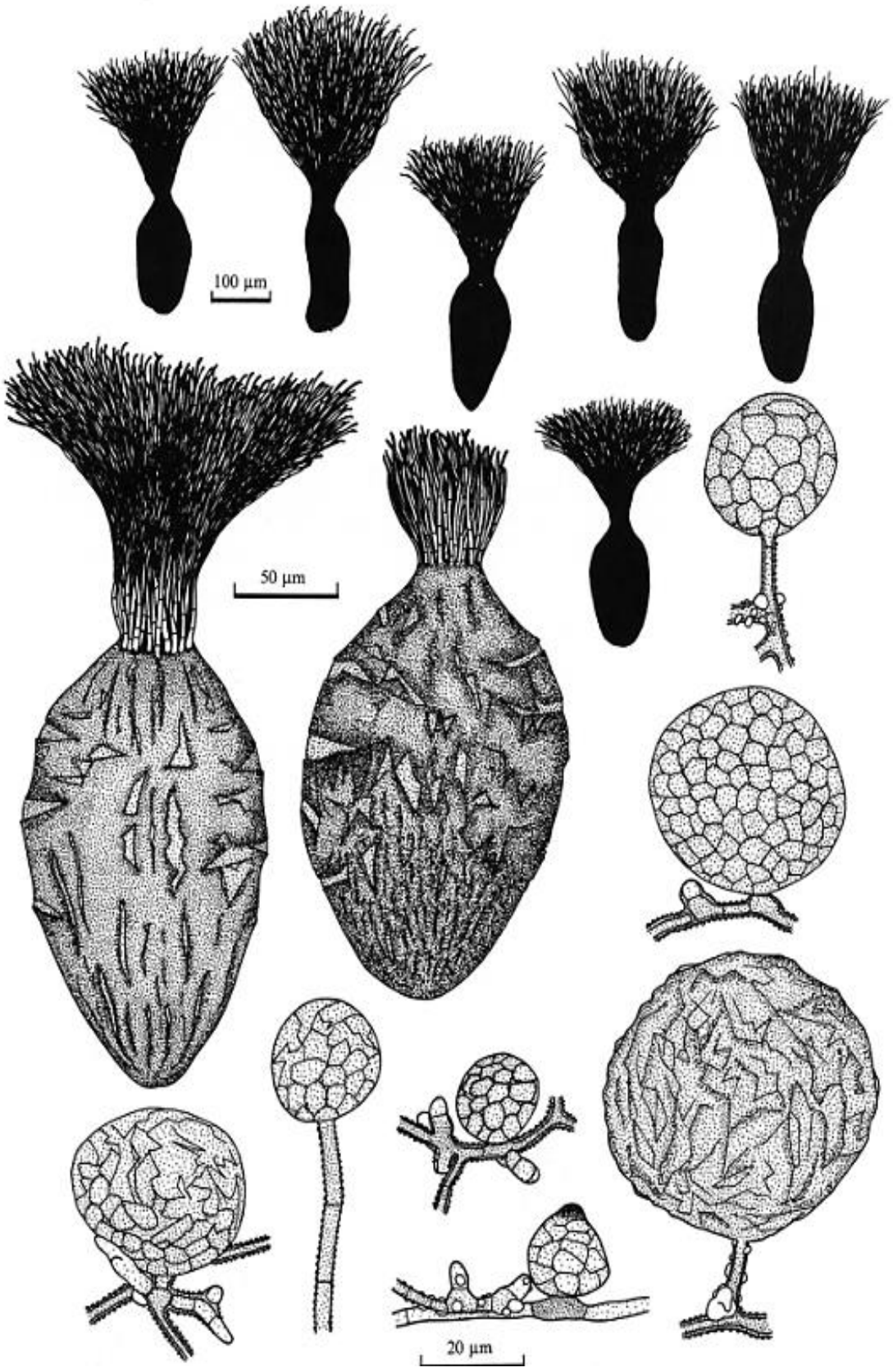


Figure 2: Lectotype illustration: Morphological characteristics and conidiogenesis of *Megacapitula villosa* (from original protologue and part of Holotype PPH17). In order to substantiate the lectotypification, the illustration is reproduced from Chen & Tzean (1993).

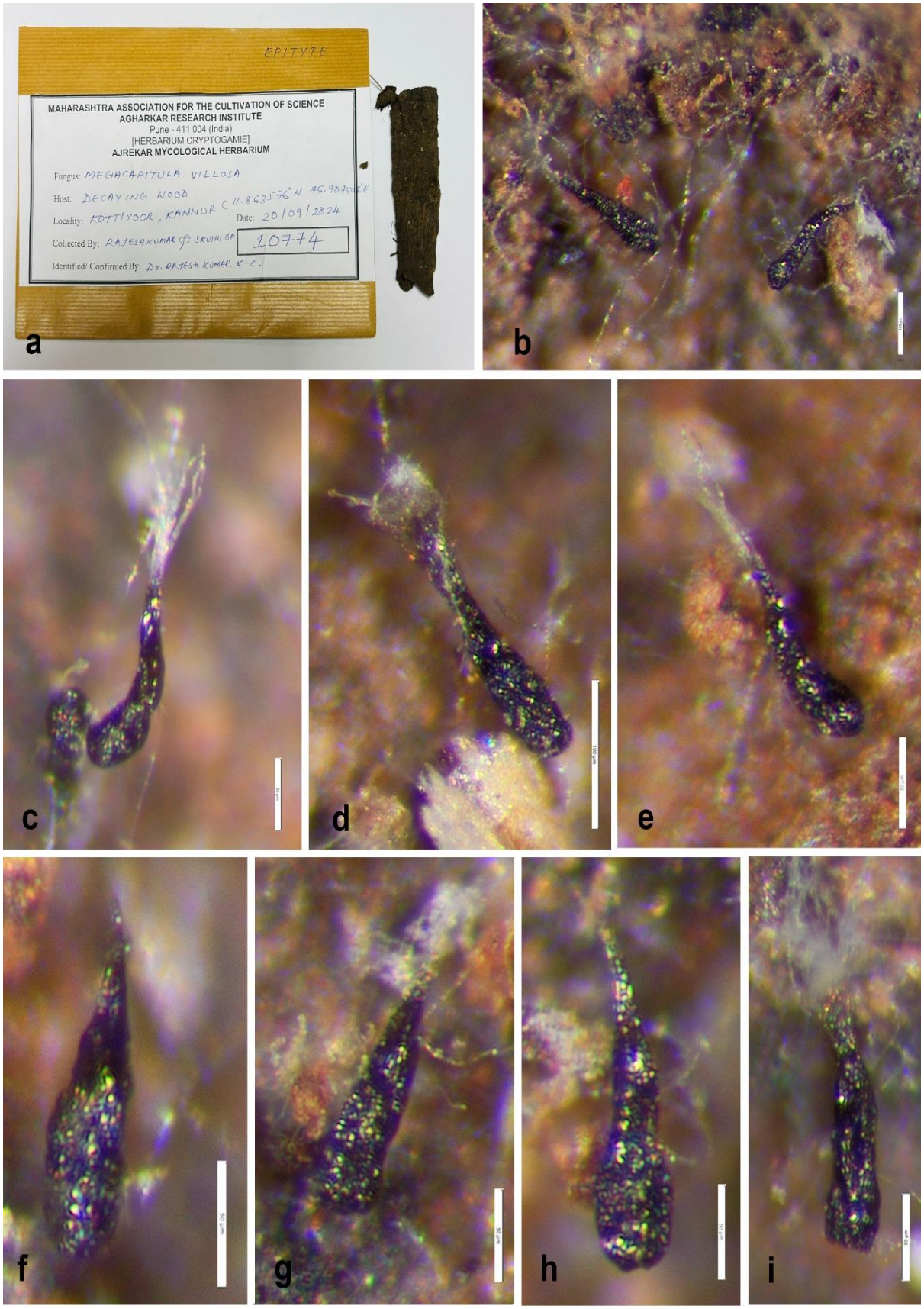


Figure 3: *Megacapitula villosa* (AMH 10774). a. Epitype. b-i. Conidia with apical branched appendages on the freshly collected specimen. Scale Bars: b = 100 µm; c = 50 µm; d = 100 µm; e-i = 50 µm.

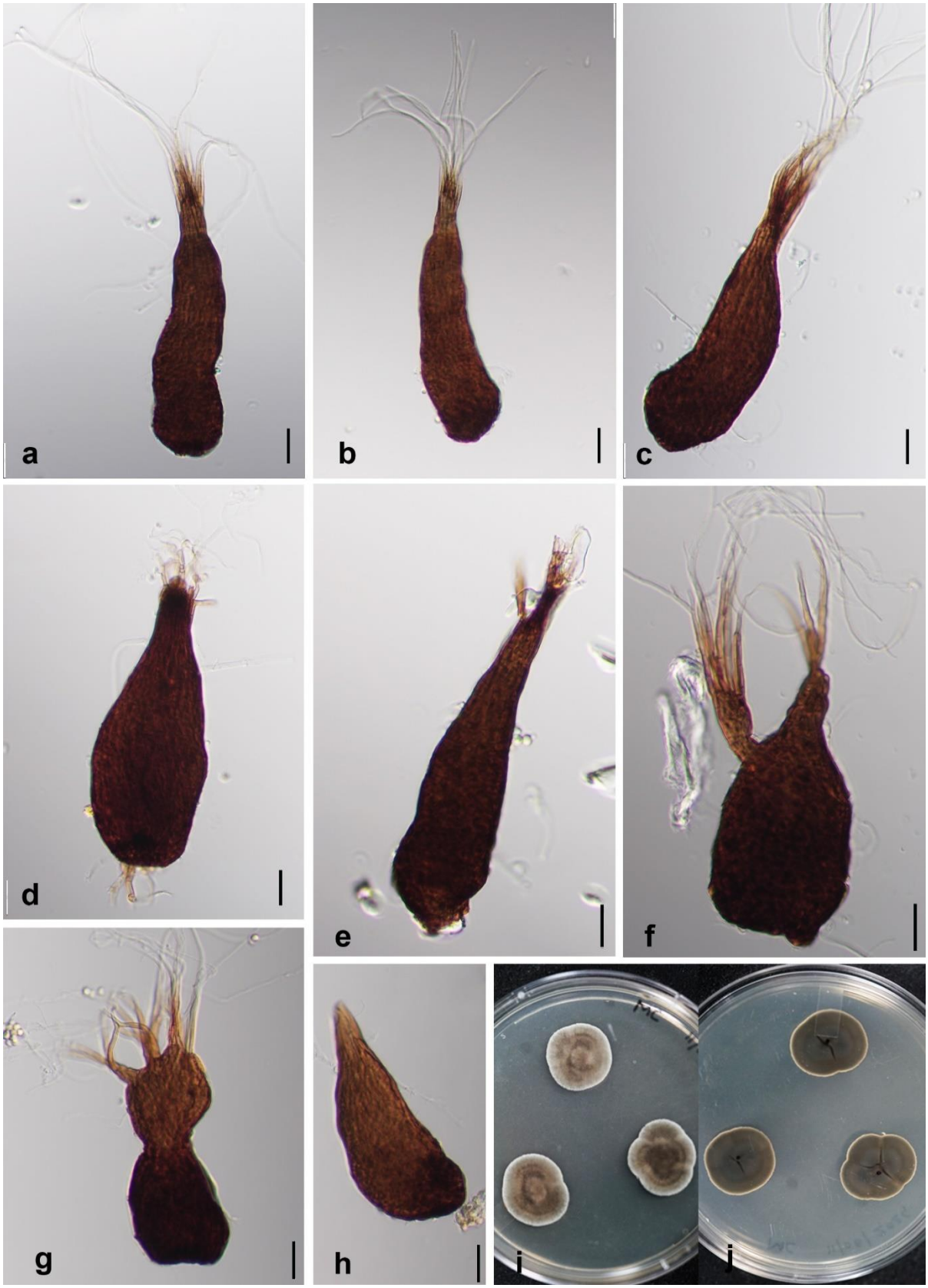


Figure 4: *Megacapitula villosa* (AMH 10774). a-e, Mature conidia; f and g, Irregular conidia with branched pedicellate appendages; h, Young conidium; i and j, Colonies obverse and reverse on MEA. Scale Bars: a-h = 20 µm.

Lectotype: TAIWAN, Taipei, 31 March 1991, J. L. Chen & S. S. Tzean (Lectotype PPH17 (illustration given in the original protologue is considered).

Epitype: INDIA, Kannur, Kottiyoor, 11.863576°N, 75.907504°E, on decaying wood, 12 Dec 2023, Rajeshkumar KC & Sruthi OP, (Epitype AMH 10774, ex-epitype NFCCI 5894).

Culture characteristics: Colonies on MEA at 25 ± 2 ºC after 7 d, slow growing, 25–35 mm diam., initially white, when mature colonies are greyish brown and paler towards the periphery with a white thin margin. Margin regular. Colonies reverse dark brown to brownish black.

DISCUSSION

Pleosporales, the largest order of *Dothideomycetes*, has 25% of all dothideomycetous species (Kirk *et al.*, 2008). This order includes epiphytes, endophytes, parasites, hyperparasites on fungi or insects, lichenized, and saprobes of dead plant stems, leaves, or bark. *Pleosporales* families were circumscribed based on characters of ascomata, morphology of asci and their arrangement in locules, presence and type of hamathecium, shape of papilla or ostioles, morphology of ascospores and type of habitats (Luttrell, 1973). Wijayawardene *et al.* (2022) enumerated 91 families under *Pleosporales*, and an additional 41 genera were treated as *Pleosporales* genera *incertae sedis*, including *Megacapitula*, but its family status remains unresolved. *Megacapitula* exhibits some resemblances to *Chaetomium* due to its size, shape, and apical appendages of the conidia (Chen and Tzean, 1993). But the perithecial genus *Chaetomium* is unique and never produces *megacapitula*-like conidia. Likewise, *Megacapitula* resembles *Akenomyces* in having pyriform sclerotia adorned with simple, curled or sinuate, colourless hairs; nevertheless, the latter represents the asexual stage of a basidiomycete (Hornby, 1984).

This study placed *Megacapitula* as a new family-clade sister to *Phaeoseptaceae* in a well-supported clade (BS 100/PP 1.00) allied to *Teichosporaceae* and *Lophiotremataceae* (BS 94/ PP 1.00). *Sporomiaceae* and *Amorosiaceae* formed a further basal clade with strong support (BS 96 /PP 1.00) in this major clade of *Pleosporales*.

Hyde *et al.* (2018) established the family *Phaeoseptaceae*, which comprises two recognized genera, *Lignosphaeria* and *Phaeoseptum*, (*P. aquaticum* as the generic type). Members of *Phaeoseptaceae* are commonly found on decaying wood in both terrestrial and aquatic environments. *Phaeoseptum* was introduced based on collections from a woody substrate submerged in a freshwater ecosystem. Morphologically, there were no similarities between members of *Megacapitulaceae*; known solely from its conidial form, and *Phaeoseptaceae*, solely having sexual morphs.

This study, based on lecto and epi typification, warranted the establishment of a new family for the monotypic *Megacapitula villosa*, considering the unique morphology and distribution of this unusual conidial fungi. The recent Outline of fungi and fungus-like taxa (Hyde *et al.* 2024) also mentioned the necessity for fresh collection and epitypification of this species to apply this name in fungal systematics. Phylogenetic analyses using concatenated LSU-SSU-ITS-*tef1α*-*rpb2* data also supported the family proposal with high statistical support in a distant clade. *Megacapitulaceae* is morphologically not comparable with allied families of *Pleosporales* due to its unique, uncommon conidial morphology, but based on 5 gene phylogeny and interpretation, it forms a stable clade well within *Pleosporales*.

ACKNOWLEDGMENTS

K. C. Rajeshkumar would like to thank the Science and Engineering Research Board (SERB), Department of Science and Technology, Government of India, New Delhi for their financial support through CRG/2020/000668 project. KCR also thank the Kerala Forest Department for permission to conduct the survey. We also thank Dr. P. K. Dhakephalkar, the Director of MACS Agharkar Research Institute in Pune, for providing additional financial support from ARI Pune. O. P. Sruthi would like to express her gratitude to CSIR-HRDG, New Delhi, India, for providing her with financial assistance as part of the SRF fellowship (09/0670(13602)/2022-EMR-I). Sinang Hongsanan would like to thank National Natural Science Foundation of China (32400012), 2023 Pengcheng Peacock Distinguished Positions and scientific research funds for high-tech talents /high-level talents, 2024 Shenzhen University young teachers' scientific research projects (868-000001032406), Shenzhen University's Special Fund (868-0000050106), and research project of the Guangdong Provincial Key Laboratory of Plant Epigenetics (868-000003010116). Samantha C.

Karunarathna and Saowaluck Tibpromma are grateful to the High-Level Talent Recruitment Plan of Yunnan Province ("High-End Foreign Experts" Program), the National Natural Science Foundation of China (Grant No. 32260004) and Key Laboratory of Yunnan Provincial Department of Education of the Deep-Time Evolution on Biodiversity from the Origin of the Pearl River, Qujing Normal University, Qujing, Yunnan 655011, China, for their support. Harikrishnan K. thanks UGC, New Delhi, India, for the financial support under JRF fellowship (NTA Ref. No. 211610067166). P.A. Ansil thanks CSIR-HRDG, New Delhi, India, for the financial support under the SRF fellowship (09/670(0093)/2021-EMR-I). R. Jeewon would like to thank the DSFP of King Saud University, Riyadh, Saudi Arabia.

REFERENCES

Aljanabi, S.M. and Martinez, I. 1997. Universal and rapid salt-extraction of high-quality genomic DNA for PCR-based techniques. *Nucleic Acids Research*, **25**:4692-4693; doi: 10.1093/nar/25.22.4692.

Boonmee, S., Wanasinghe, D.N., Calabon, M.S., *et al.*, 2021. Fungal diversity notes 1387–1511: Taxonomic and phylogenetic contributions on genera and species of fungal taxa. *Fungal Diversity*, **111**:1-335; doi: 10.1007/s13225-021-00489-3.

Chethana, K.T., Manawasinghe, I.S., Hurdeal, V.G., *et al.*, 2021. What are fungal species and how to delineate them? *Fungal Diversity*, **109(1)**:1-25.

Chen, J.L. and Tzean, S.S. 1993. *Megacapitula villosa* gen. et sp. nov. from Taiwan. *Mycological Research*, **97(3)**:347-350.

Hall, T.A. 1999. BioEdit: a user- friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, **41**:95-98; doi: 10.11646/phytotaxa.314.2.6.

Hongsanan, S., Hyde, K.D., Phookamsak, R., *et al.*, 2020. Refined families of *Dothideomycetes*: *Dothideomycetidae* and *Pleosporomycetidae*. *Mycosphere*, **11(1)**:1553-2107; doi 10.5943/ mycosphere/11/1/13.

Hornby, D. 1984. *Akenomyces-costatus* sp.nov. and the validation of *Akenomyces* arnaud. *Transactions of the British Mycological Society*, **82**:653-664.

Hyde, K.D., *et al.*, 2018. Mycosphere notes 169-224. *Mycosphere*, **9(2)**:271-430.

Hyde, K.D., Maryam, T.N., Vinodhini, T., *et al.*, 2024. Outline of Fungi and fungus-like taxa. *Mycosphere*, **15(1):**5146-6239; doi: 10.5943/ mycosphere/15/1/25.

Kalyaanamoorthy, S., Minh, B.Q., Wong, T.K.F. *et al.*, 2017. ModelFinder: Fast model selection for accurate phylogenetic estimates. *Nature Methods*, **14**:587-588; doi: 10.1038/nmeth.4285.

Katoh, K., Rozewicki, J., Yamada, K.D. 2019. MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. *Briefings in Bioinformatics*, **20**: 1160-1166; doi: 10.1093/bib/bbx108.

Kirk, P.M., Cannon, P.F., Minter, D.W., *et al.*, 2008. Dictionary of the Fungi. 10th Edition. CABI Bioscience, UK, 771p.

Kornerup, A. and Wanscher, J.H. 1978. Methuen Handbook of Colour. Introduced and revised by. Don Pavey. 3rd Edition. Evry Methuen, London, 62p.

Kumar, S., Stecher, G., Tamura, K. 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular biology and evolution*, **33(7)**:1870-1874; doi: 10.1093/molbev/msw054.

Larsson, A. 2014. Aliview a fast and lightweight alignment viewer and editor for large data sets. *Bioinformatics*, **30(22)**:3267-3278; doi: 10.1093/bioinformatics/btu531.

Liu, Y.J., Whelen, S., Hall, B.D. 1999. Phylogenetic relationships among ascomycetes: evidence from an RNA polymerase II subunit. *Molecular biology and evolution*, **16**:1799-1808; doi: 10.1093/oxfordjournals.molbev.a026092.

Luttrell, E.S. 1973. Loculoascomycetes. In: *The fungi, an advanced treatise, a taxonomic review with keys: ascomycetes and fungi imperfecti*, Eds: Ainsworth, G.C., Sparrow, F.K., Sussman, A.S. Academic Press, New York, pp.135-219.

Minh, B.Q., Schmidt, H.A., Chernomor, O., *et al.*, 2020. IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era. *Molecular biology and evolution*, **37(5)**:1530-1534; doi: 10.1093/m olbev/msaa 015.

Morgulis, A., Coulouris, G., Raytselis, Y., *et al.*, 2008. Database indexing for production MegaBLAST searches. *Bioinformatics*, **24(16)**:1757-1764.

Nguyen, L.T., Schmidt, H.A., von Haeseler, A., *et al.*, 2015. IQ-TREE: a fast and efective stochastic algorithm for estimating maximum-likelihood phylogenies. *Molecular Biology and Evolution*, **32(1)**:268-274.

Nylander, J.A.A. 2004. MrModeltest v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University.

Pem, D., Jeewon, R., Chethana, K.W.T., *et al.*, 2021. Species concepts of *Dothideomycetes*: classification, phylogenetic inconsistencies and taxonomic standardization. *Fungal Diversity*, **109(1)**:283-319.

Prabhugaonkar, A. and Bhat, D.J. 2011. New record of *Megacapitula villosa* and *Paradictyoarthrinium diffractum* from India. *Mycosphere*, **2(4)**:463-467.

Rajeshkumar, K.C., Braun, U., Groenewald, J.Z., *et al.*, 2021. Phylogenetic placement and reassessment of *Asperisporium pongamiae* as *Pedrocrousiella pongamiae* gen. et comb. nov. (*Mycosphaerellaceae*). *Fungal Systematics and Evolution*, **7**:165-176; doi: 10.3114/fuse.2021.07.08.

Rajeshkumar, K.C., Varma, R.K., Sruthi, O.P., *et al.*, 2023. *Groenewaldia* (*Lentitheciaceae*), a new corticolous fungal genus from India. *Mycological Progress*, **22(6)**:43; doi: 10.1007/ s11557-023-01888-3.

Rambaut, A. 2010. FigTree v1. 3.1. Institute of Evolutionary Biology. University of Edinburgh, Edinburgh, United Kingdom. http://tree.bio.ed.ac.uk/software/figtree.

Rehner, S.A. and Buckley, E. 2005. A *Beauveria* phylogeny inferred from nuclear ITS and EF1- α sequences: evidence for cryptic diversification and links to *Cordyceps* teleomorphs. *Mycologia*, **97(1)**:84-98; doi: 10.3852/mycologia.97.1.84.

Ronquist, F., Teslenko, M., van der Mark, P., *et al.*, 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic biology*, 61(3):539-542; doi: 10.1093/sysbio/ sys029.

Senanayake, I.C., Rathnayaka, A.R., Marasinghe, D.S., *et al.*, 2020. Morphological approaches in studying fungi: collection, examination, isolation, sporulation and preservation. *Mycosphere*, **11**:2678-2754; doi: 10.5943/ mycosphere/11/1/20.

Vaidya, G., Lohman, D.J., Meier, R. 2011. SequenceMatrix: concatenation software for the fast assembly of multi-gene datasets with character set and codon information. *Cladistics*, **27(2)**:171-180.

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

Wijayawardene, N.N., Hyde, K.D., Dai, D.Q., *et al.*, 2022. Outline of *Fungi* and fungus-like taxa – 2021. *Mycosphere*, **13(1)**:53-453; doi: 10.5943/mycosphere/13/1/2.

White, T.J., Bruns, T., Lee, J., *et al.*, 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: PCR protocols: a guide to methods and applications (Eds.: Innis, M.A., Gelfand, D.H., Sninsky, J.J., *et al.*)Academic Press, New York, pp.315-322; doi: 10.1016/B978-0-12-372180-8.50042-1.