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

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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-*tef1a-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 Boonmee et al. (2021) classified isolates. 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 WDCM- 932), Agharkar Research (NFCCI, 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 LROR 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 tefla (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 ddH₂O, 12.5 μ L 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 tefla 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-tefla-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-ITStefla-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*teflα-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 10^{th} 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 villosa GUFCC 15515 [GenBank were; M. 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 = villosa SNC122 [GenBank 1/974(0%)], М. 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 tefla 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, *tef1a*, 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-*tef1a-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-ITStef1 α -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-tef1 α 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*.

1001 – Dacampia engeliana Hafellner 72868 Dacampia hookeri Hafellner 73897 BR	GZU Dacampiaceae
97/1 92/1 1001 — Pyrenochaeta protearum CBS 131315 Quixadomyces cearensis HUEFS 23843	Dayapuyan och a sta soa s
841, Camarosporidiella mackenziei MFLUCC	14-0883 Camarosporidiellaceae
Sala Camarosporidiella mackenziei MFLUCC Sol Camarosporium palmarum CBS 758.73 [ma] Coniothyrium palmarum CBS 400.71	Coniothyriaceae
100 Leptosphaeria doliolum CBS 505	.75 Leptosphaeriaceae
917 Leptosphaeria doliolum MFLUC 1001 Libertasomyces quercus CBS 134.97 1007 June Libertasomyces quercus CBS 134.97	Libertasomycetaceae
Alternaria alternata AFTOL	D 1610
991 Pleospora herbarum CBS 191 Neocamarosporium goegapense CPC 236 100 Neocamarosporium phragmitis MFLUC	.86 Tabsportaceae
Camarosporium phragmitis MFLUC	
987 Camarosporomyces flavigenus CBS 31	4.80 Camarosporiaceae
Neophaeosphaeria agaves CPC 21264 Neophaeosphaeria phragmiticola KUMCC	16-0216 Neophaeosphaeriaceae
1000 Teanania taiwanensis NTUCC 17-005 981 Teanania taiwanensis NTUCC 17-006	Tzeananiaceae
75/98/1 100/1 Shiraia bambusicola GZAAS2 0629 98/1 Shiraia bambusicola GZAAS2 0703	Shiraiaceae
Phaeosphaeria oryzae CBS 110110	CC 14 0614 Phaeosphaeriaceae
<u>1001</u> <u>Muriphaeosphaeria galatellae MFLUC</u> 1001 <u>P</u> seudopyrenochaeta terrestris CBS 282.72	Draudoppyrou och actaogae
Pseudopyrenochaeta lycopersici CBS 306.	65 <i>Neopyrenochaetaceae</i>
100/1 Neopyreuochaeta acicola CBS 812.95	
S\$1 1001 — Pyrenochaetopsis tabarestanet Pyrenochaetopsis leptospora Cl	BS 101635 Pyrenochaetopsidaceae
Protofenestella ulmi FP5 997 Cucurbitaria berberidis MFLUCC 11-0387	Cucurbitariaceae
92/1 Dothidotthia aceris CS 2019a Neodothidotthia negundinicola CBS 1450	39 Dothidotthiaceae
Didymella calidophila CBS 683.79	
100/1 Microsphaeropsis proteae CPC 1425	Didymellaceae
1001-Microsphaeropsis olivacea CBS 233.77	Acrocalymmaceae
1001 Acrocalymma pterocarpi C233 Acrocalymma medicaginis CPC 24340 1001 Ascocytindrica marina MD6011	
Ascocylindrica marina MF416	Ascocylindricaceae
<u>100/1</u> Brevicollum hyalosporum MAFF 24340 97/1 Neohendersonia kickvii CBS 112403	
^{9/1} <u>10011</u> Halojulella avicenniae JK 5326 Halojulella avicenniae BCC 201 10011 — Lentithecium pseudoclioninum HHUF 25	A 173 Halojulellaceae
93/1 Halobyssothecium obiones MFLUCC	15-0381
Dictyocheirospora bannica KH 33 1001 Pseudodictyosporium thailandica	MELUCC 16-0029 Dictyosporiaceae
100/1 Sulcatispora acerina KT2982	Sulcatisporaceae
96.1 Magnicamarosporium diospyrie Bambusicola massarinia MFLUCO 1/2- Leucaenicola phraeana C416	C 11-0389 Bambusicolaceae
Longipedicellata aptrootii MI	FLUCC 10-0297 Longipedicellataceae
96/1 Pseudoxylomyces elegans KT 2887	Longipeutetautetau
Bimuria novae zelandiae CBS	10/./9
Flavomyces fulophazii CBS 135761	
L13 1001 Helminthosporium velutinum L13 1001 Massarina eburnea CBS	473.64
78- Fuscostagonospora sasae KT 146 1001 Fuscostagonospora cytisi MFLU	7 Fuscostagonosporaceae
1001 Splanchnonema platani CBS 222.37 Macrodiplodiopsis desmazieri CBS 1400	062 Macrodiplodiopsidaceae
100.1 Latorua caligans CBS 576.65 100.1 Latorua grootfonteinensis CBS 369.72	
100/1 Parabambusicola thysanolaena	e KUMCC 18-0147 Parabambusicolaceae
Multiseptospora thailandica MFLU	JCC 11-0183
100/1 Falciformispora senegalensis CBS	190./9
79- 98.1 Helicascus aquaticus KT 15 Morosphaeria muthu 100.1 Pleomonodictys descalsii FMR 12	716
—Pleohelicoon fagi GJ415	1 icomonouicijuuccue
2011 Corynespora torulosa CPC 15989 Corynespora cassiicola CBS 100822	Corynesporaceae
Cyclothyriella rubronotata TR9 100/1 Massariosphaeria phaeospora CBS 611.86	Cyclothyriellaceae
1001 Pleomassaria siparia CBS 279.74 1001 Prosthemium stellare CBS 126964	Pleomassariaceae
Aposphaeria corallinolutea MFLU 15-2752	Melanommataceae
Melanomma japonicum MAFF 239634	

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

	Paradictyoarthrinium diffractum MFLUCC 13-0466 Sirodesmium olivaceum CBS 395.59	Paradictyoarthriniaceae
961	Ccultibambusa banbusae MFLUCC 13-0855	Occultibambusaceae
100/1	Nigrograna obliqua CBS 141475	Nigrogranaceae/
	Biatriospora marina CY 1228	Biatriosporaceae
861	1001 Biatriospora carollii CCF4484 1001 Halotthia posidoniae BBH 22481	Halotthiaceae
	Mauritiana rhizophorae BCC 28866	
	Lignosphaeria thailandica MFLUCC 11- 1001 Lignosphaeria thailandica MFLUCC 11- 1001 Megacapitula villosa SNC122	0376 Phaeoseptaceae
85/1	Megacapitula villosa SICC122 Megacapitula villosa NFCCI 5894	Megacapitulaceae
	Ramusculicola thailandica MFLUCC 13-0284 Magnibotryascoma mali MFLUCC 17-0933	Teichosporaceae
961	Coelodictyosporium rosarum MFLUCC 17-0776	Lophiotremataceae
901	1001 Sporormiella minima CBS 524.50	Sporormiaceae
	⁷⁰⁻ Preussia funiculata CBS 659.74 ⁷⁰⁻ Angustimassarina populi MFLUCC 13-0034	Amorosiaceae
	1001 Amorocoelophoma cassiae C259 1001 Subglobosporium tectonae 12-0393	
	Pseudocoleodictyospora tectonae 12-0385	Pseudocoleo dictyosporace ae
94/1	1001Rostriconidium pandanicola KUMCC 17-0176 Torula aquatica MFLUCC 16-1115	Torulaceae
100/1	Cycasicola leucaenae C215 Thyridariella mangroyei NFCCI 4213	Thyridariace ae
	MI Arthopyrenia salicis CBS 368.94	Arthopyreniaceae/
	1001 1001 1001 Roussoella siamensis MFLUCC 11-0149	Roussoellaceae
	1001 – Roussoella neopustulans MFLUCC 11-0609T	
	Astrosphaeriella thailandica MFLUCC 11-0191	Astrosphaeriellaceae
001	Aigialus parvus BCC 18403	Aigialaceae
-	1001 Salsuginea ramicola KT 2597 1 Salsuginea ramicola KT 2597 2	Salsugineacea
93/1	<u>971</u> Neomassaria formosana NTUCC 17-007 Neomassaria fabacearum MFLUCC 16-1875	Neomassariacea
	100.1 Massaria vomitoria WU 30606	Massariacea
	Massaria inquinans WU 30527 Delitschia winteri AFTOL ID 1599	Delitschiacea
100/1	Delitschia chaetomioides SMH 3253 2 Zopfia rhizophila CBS 207.26	Zopfiacea
	Gordonomyces mucovaginatus CBS 127273	Fusculinacea
	1001 Amniculicola immersa CBS 123083	Amniculicolacea
	Murispora galii MFLUCC 13-0819	
	1001 Wicklowia aquatica CBS 125634 Wicklowia sp MFLUCC 18-0373 1001 Lindgomyces cigarospora G619	Wicklowiaceae
	Hongkongmyces thailandica MFLUCC 16-0406	Lindgomycetaceae
100/1 100/1	Lophiotrema nucula CBS 627.86 Cryptoclypeus ryukyuensis KT 3534	Lophiotremataceae
001	Cryptocoryneum pseudorilstonei CBS 113641 Cryptocoryneum japonicum HHUF 30482	Cryptocoryneaceae
93/1	Aquasubmersa japonica HHUF 30469	Aquasubmersaceae
	1001 — Aquasubmersa mircensis MFLUCC 11-0401 1001 — Pseudoberkleasmium chiangmaiense MFLUCC 17-1809	Pseudoberkleasmiaceae
97/1 96/1	→ Pseudoberkleasmium pandanicola KUMCC 17-0178 —Hermatomy ces iriomotensis KT2016 1	
100/1	—Hermatoniyces tectonae KH 409 ————Pseudolophiotrema elymicola KT 1450	Hermatomycetaceae Pseudolophiotremataceae
94/1 100/1	-Anteaglonium thailandicum C 012	Anteagloniaceae
	Anteaglonium globosum ANM925 2 Hypsostroma saxicola SMH 5005	5
	Hypsostroma caimitalense GKM 1165 Paralophiostoma hysterioides PUFNI 17617	Hypsostromataceae Paralophiostomataceae
95- Mu	ritestudina chiangraiensis MFLUCC 17-2551	Testudinaceae
Pseud	uculina enalia BCC 18402 lotetraploa curviappendiculata HC 4930	Tetraplo sph aeriace ae
	olosphaeria yakushimensis KT 1906 haeria jonesii MFLUCC 15-0641	
Ligninsp	haeria jonesii GZCC 15-0080	Ligninsphaeriaceae
99/1 Longic	tula nypae MFLUCC 18-0265 orpus striataspora MFLUCC 18-0267	Striatiguttulaceae
	sphaeriella thailandensis MFLUCC 11-0144 hsphaeriella longicolla MFLUCC 11-0171	Pseudoastrosphaeriellaceae
	Asterina phenacis TH 589	Outgroup

Figure 1: Phylogram generated from maximum likelihood analysis (IQ-TREE) of *Pleosporales* (including 83 families) based on combined LSU, SSU, ITS, *tef1a*, and *rpb2* sequence data. Maximum likelihood bootstrap values (\geq 70%) and Bayesian posterior probabilities (\geq 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, 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, 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-tef1a-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, 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.

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, semimacronematous, mononematous, simple or branched, straight or flexuous, pale brown to brown, smooth, roughened or vertucose, $5-6.3 \times 3-$ 6.7 µm, Conidiogenous cells integrated, terminal, lateral or occasionally intercalary, determinate. Conidia holoblastic, solitary, ovoid, obclavate, ellipsoidal or obpyriform, muriform, $79-230 \times 47-$ 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-tefla-rpb2 from our recent collection from Kannur delineate a wellsupported clade sister to Phaeoseptaceae under named family, Pleosporales, as а new Megacapitulaceae.

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

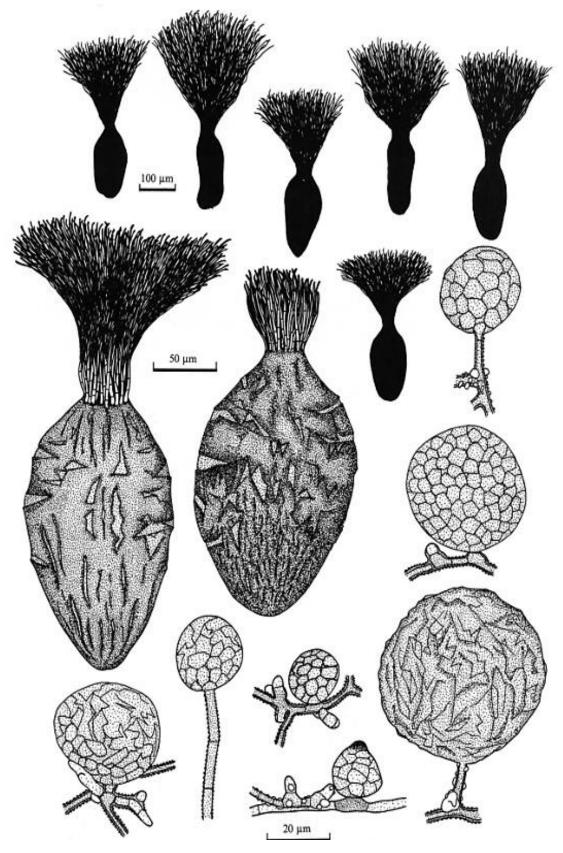


Figure 2: Lectotype illustration: Morphological characteristics and conidiogenesis of *Megacapitula villosa* (from original protologue and part of Holotype PPH17). In order to substantiate the lectotypification, the illustration is reproduced from Chen & Tzean (1993).

MAHARASHTRA ASSOCIATION FOR THE CULTIVATION OF SCIENCE AGHARKAR RESEARCH INSTITUTE Pune - 411 004 (India) Pune - 411 004 (India) [HERBARIUM CRYPTOGAMIE] AJREKAR MYCOLOGICAL HERBARIUM MEGACAPITULA VILLOSA 76 N 45.9075 ECAYING NOOD , KANNUR 20/09/2024 10774 AK C SRUTHI ON Collected By Identified/ Confirmed By: 27, RAJESH a C e

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

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

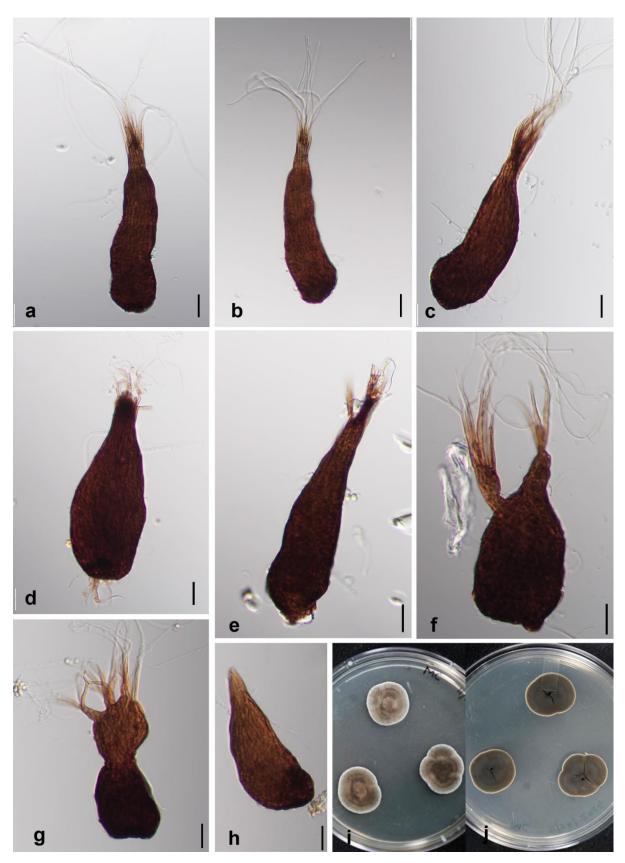


Figure 4: *Megacapitula villosa* (AMH 10774). a-e, Mature conidia; f and g, Irregular conidia with branched pedicellate appendages; h, Young conidium; i and j, Colonies obverse and reverse on MEA. Scale Bars: $a-h = 20 \mu m$.

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 °C 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 2008). This order includes epiphytes, al., 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 familyclade 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-tefla-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.

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