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Diversity of the genus *Calocybella* Vizzini, Consiglio & Setti in Kerala State, India

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Diversity of the genus *Calocybella* Vizzini, Consiglio & Setti in Kerala State, India

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ABSTRACT

Calocybella Vizzini, Consiglio & Setti is a lyophylloid genus recently erected, based primarily on molecular phylogeny. Although the genus was originally described from Europe, several of the subsequently described species of *Calocybella* are from tropical India. Here, we present an account of all *Calocybella* species known from India including a species new to science, *C. dicholamellata* K.P.D.Latha, K.N.A.Raj & Manim., sp. nov., based on morphology coupled with molecular phylogenetic analyses. The Maximum Likelihood (ML) and Bayesian Inference (BI) analyses based on nrITS sequence dataset unequivocally placed *C. dicholamellata*, sp. nov., within the genus *Calocybella*. A comprehensive description, photographs of the basidiocarps in their natural habitat and of the microscopic structures, comparisons with phenetically and phylogenetically related species, and a phylogram to show the placement of the new species are provided. The diagnostic features and photographs of the other species of *Calocybella* from this region and a table providing a comparison of all known species of *Calocybella* also are provided.

KEY WORDS
Agaricales,
Basidiomycota,
Lyophyllaceae,
phylogeny,
tropical mycology,
new species.

RÉSUMÉ

Diversité du genre Calocybella Vizzini, Consiglio & Setti dans l'État du Kerala, en Inde.

Calocybella Vizzini, Consiglio & Setti est un genre lyophylloïde récemment établi, basé principalement sur la phylogénie moléculaire. Bien que le genre ait été décrit à l'origine en Europe, plusieurs des espèces de *Calocybella* décrites par la suite sont originaires de l'Inde tropicale. Nous présentons ici un compte rendu de toutes les espèces de *Calocybella* connues de l'Inde, y compris une espèce nouvelle pour la science, *C. dicholamellata* K.P.D.Latha, K.N.A.Raj & Manim., sp. nov., établie sur des caractères morpho-anatomiques et moléculaires. Le maximum de vraisemblance (ML) et les analyses d'inférences bayésiennes (BI) basés sur l'ensemble de données de séquence nrITS ont placé sans

MOTS CLÉS
Agaricales,
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Lyophyllaceae,
phylogénie,
mycologie tropicale,
espèce nouvelle.

équivoque *C. dicholamellata*, sp. nov., dans le genre *Calocybella*. Une description complète, des photographies des microstructures et des basidiocarpes dans leur habitat naturel, des comparaisons avec des espèces apparentées phénétiquement et phylogénétiquement, et un phylogramme positionnant cette nouvelle espèce sont fournis. Les caractéristiques diagnostiques et les photographies des autres espèces de *Calocybella* de cette région, ainsi qu'un tableau fournissant une comparaison de toutes les espèces connues de *Calocybella*, sont également fournis.

INTRODUCTION

Calocybella Vizzini, Consiglio & Setti is a lyophylloid genus originally proposed for accommodating *Rugosomyces pudicus* Bon & Contu (Vizzini *et al.* 2015; Latha *et al.* 2016). *Calocybella* remained monotypic and known only from Europe (Vizzini *et al.* 2015) until Latha *et al.* (2016) described three new species from tropical India extending the known geographical distribution of this genus. Owing to the fluidity of its morphological characters, *Calocybella* is considered as a phylogenetically circumscribed genus (Latha *et al.* 2016). The originally suggested diagnostic features of the genus such as a reddening context and basidiospores showing a *Rhodocybe* Maire – like verruculose ornamentations were found to be inconsistent necessitating a reappraisal of the morphological circumscription of the genus. Species of *Calocybella* often have collybioid or slenderly tricholomatoid basidiocarps, a cutis- or a trichoderm-type pileipellis, and clamped hyphae (Vizzini *et al.* 2015, 2017; Latha *et al.* 2016). Recently, Corriol *et al.* (2017) proposed the synonymy between *C. juncicola* (R.Heim) P.-A.Moreau, Bellanger & Corriol (basionym: *Tricholoma chrysenterum* var. *juncicola* R.Heim; synonym: *Calocybe juncicola* (R.Heim) Singer) and *C. pudica* (Bon & Contu) Vizzini, Consiglio & Setti, the first taking priority over the second.

During our continuing investigations on the agarics of Kerala State, India, we discovered a lyophylloid agaric, which on phylogenetic analysis using an nrITS sequence dataset was found to be a species of the genus *Calocybella*. It is formally described here as a new species. Additionally, the diagnostic features and photographs of the other species of *Calocybella* from this region and a table providing a comparison of all known species of *Calocybella* also are provided.

MATERIAL AND METHODS

MORPHOLOGICAL STUDIES

The basidiocarps examined were collected from the Thenmala Forest in Kollam District and Janakikad Forest in Kozhikode District of Kerala State, India. Both the localities are tropical semi-evergreen forests with a mixture of evergreen and deciduous tree species and have a humid, tropical and monsoonal climate. Traditional morphology-based taxonomic methods and molecular phylogenetic methods were

employed in this study. The terminology used for describing the macro- and microscopic characters were adopted from Largent (1973); Largent *et al.* (1977) and Vellinga (1988). Thin sections from different parts of the dried specimens were stained with 1% aqueous solutions of Congo red and phloxine and mounted in 3% aqueous KOH. Cotton blue in lactophenol was used to verify whether the basidiospores are cyanophilic or acyanophilic. The basidiospores were mounted in Melzer's reagent to check for amyloid/inamyloid reactions. The iron-acetocarmine staining method (Singer 1975) was employed to examine the presence of siderophilous granules in the basidia. The colour of the microstructures was observed using preparations mounted in distilled water. For the evaluation of the range of basidiospore size, 20 basidiospores each from one specimen of each collection cited were measured. The basidiospore measurements exclude the hilar appendix. Basidiospore measurements include both the mean and the standard deviation for both the length and the width, together with the range of spore quotient (Q, the length/width ratio) and its mean value (Qm). Alphanumeric colour codes from both Kornerup & Wanscher (1978) (e.g. 5C7) and the Online Auction Colour Chart (Anonymous 2004) (e.g. OAC790) are provided for the colour names in the description. The holotype of the new species is deposited at the Central National Herbarium (CAL) of the Botanical Survey of India, Howrah, West Bengal, India and the additional collections examined are at the Ajrekar Mycological Herbarium (AMH) of the Agharkar Research Institute, Pune, India. One of the additional collections of the species is kept at the personal herbarium of the corresponding author. A Zeiss GeminiSEM 300 scanning electron microscope was used at an accelerating voltage of 3 kV for capturing SEM images and the procedure for scanning electron microscopy follows Raj *et al.* (2016).

DNA EXTRACTION, AMPLIFICATION AND SEQUENCING

The protocol described by Izumitsu *et al.* (2012) was used for extracting the genomic DNA from the dried basidiocarps of the new species. The nrITS gene was sequenced in this study. The primer pair ITS1 and ITS4 (White *et al.* 1990) was used for the amplification of the nrITS region. The protocols followed for PCR amplification and sequencing are those of Latha & Manimohan (2018). The newly generated sequences have been deposited in GenBank (<https://www.ncbi.nlm.nih.gov/genbank/>).

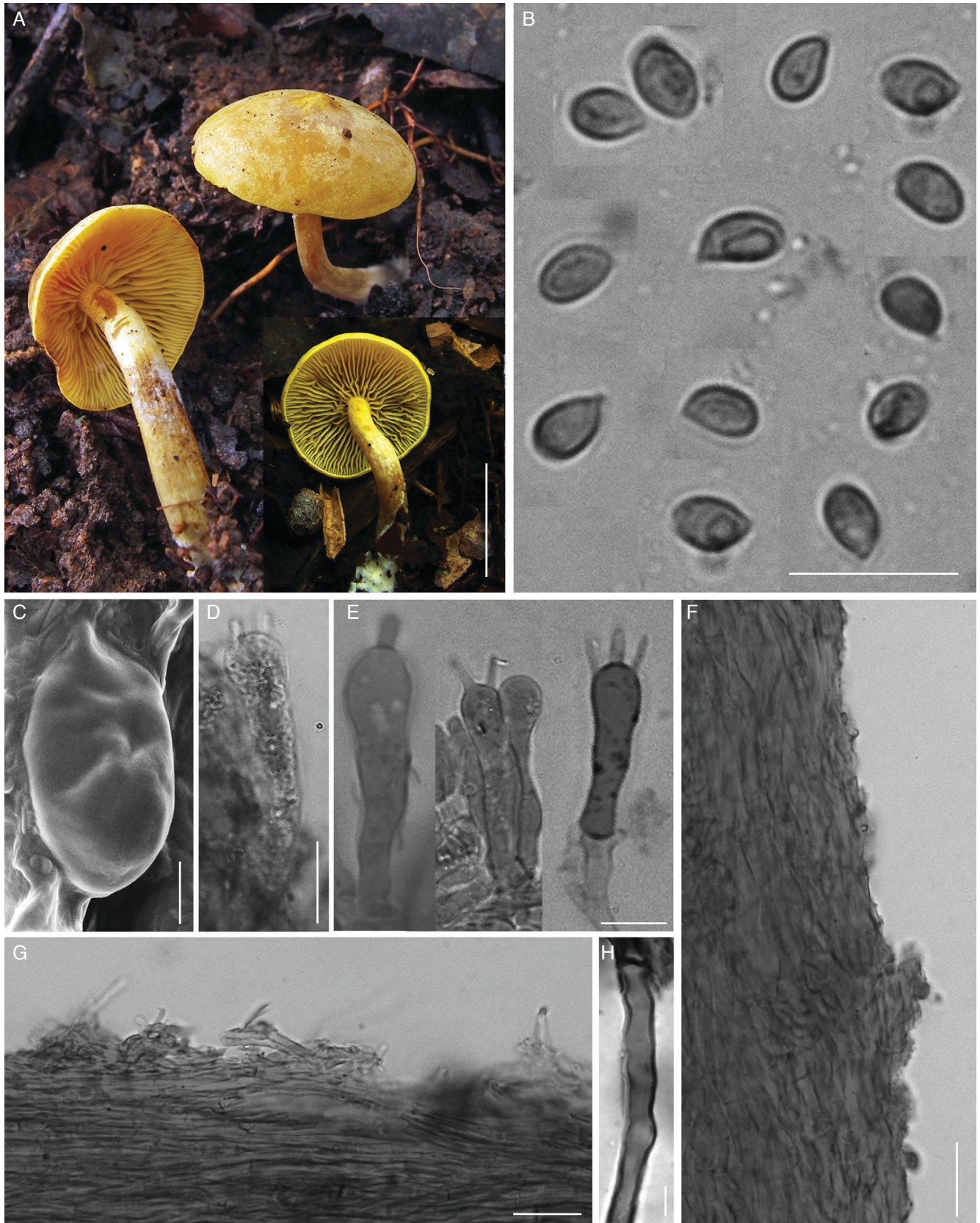


FIG. 1. — *Calocybella dicholamellata* K.P.D.Latha, K.N.A.Raj & Manim., sp. nov., DKP134 (CAL 1242, holotype): **A**, basidiocarps in natural habitat; **B**, basidiospores; **C**, basidiospore under SEM; **D**, siderophilous basidium; **E**, basidia; **F**, pileipellis; **G**, stipitipellis; **H**, hypha of pileus trama. Scale bars: A, 10 mm; B, D, E, H, 10 μ m; C, 1 μ m; F, G, 20 μ m.

SEQUENCE ALIGNMENT AND PHYLOGENETIC ANALYSES

The newly generated nrITS sequence of *C. dicholamelata*, sp. nov., was incorporated into the dataset of Latha *et al.* (2016). The sequences of *C. dominicana* Angelini & Vizzini and *Gerhardtia citrinolobata* Angelini & Vizzini (Vizzini *et al.* 2017) were also added to the dataset. The final nrITS sequence dataset comprised 41 sequences with 577 nucleotide positions. *Ossicaulis lachnopus* (Fr.) Contu and *O. lignatilis* (Pers.) Redhead & Ginns were chosen as the outgroup taxa following Vizzini *et al.* (2015). The nrITS sequence dataset was aligned with MAFFT web tool (<http://align.bmr.kyushu-u.ac.jp/mafft/online/server/>) with default settings. Minor editing was made manually to the alignment using AliView v.1.23 (Larsson 2014). Maximum likelihood (ML) and Bayesian Inference (BI) analyses were executed using RAXML-HPC2 (v.8.2.10) (Stamatakis 2014) and MrBayes (v.3.2.6) (Ronquist *et al.* 2012) respectively. RAXML was run with one thousand rapid ML bootstrap replicates and a GTRGAMMA model of nucleotide substitution following Cipres RAXML instructions. In Bayesian Inference analysis, four Monte Carlo Markov Chains (MCMC) were run for ten million generations, sampling every 1000th generations, with two independent runs per analysis. GTR + GAMMA substitution model was selected as the best-fit model according to the Bayesian Information Criterion (BIC) for Bayesian Inference analysis in TOPALI v.2.5 (Milne *et al.* 2009). The first 25% of the trees were discarded as “burn-in”. All the analyses (ML and BI) were performed using online versions of the software (RAXML and MrBayes) on the XSEDE platform as implemented in the CIPRES Science Gateway web server (Miller *et al.* 2010). The aligned sequence dataset has been deposited in TreeBase (<http://purl.org/phylo/treebase/phylogs/study/TB2:S26395>). The phylograms generated from the ML and BI analyses were visualised using FigTree v.1.4.3 (Rambaut 2014).

RESULTS

TAXONOMY

Family LYOPHYLLACEAE Jülich
Genus *Calocybella* Vizzini, Consiglio & Setti

Calocybella dicholamelata K.P.D.Latha, K.N.A.Raj & Manim., sp. nov.
(Fig. 1)

MYCOBANK NUMBER. — MB 833492.

SYSTEMATIC POSITION. — Basidiomycota, Agaricomycetes, Agaricales, Lyophyllaceae, *Calocybella*.

HOLOTYPE. — **India**, Kerala State, Kollam District, Thenmala, Kattilapara, on humus-rich soil, among decaying litter, 17.VIII.2013, K. P. Deepna Latha DKP134 (holo-, CAL [CAL 1242]). GenBank no. ITS: (CAL 1242: MN708209; DKP552: MN708210).

ETYMOLOGY. — The epithet refers to the furcate lamellae of this species.

DIAGNOSIS. — Differing from *Calocybella lobitha* K.P.D.Latha & Manim. in having a brownish yellow pileus with a somewhat pruinose surface, furcate and emarginate lamellae with a decurrent tooth, a brownish orange stipe which is slightly recurved-squamulose towards the apex, slightly broader basidiospores, 4-, 2- and 1-spored basidia, slightly gelatinised hyphae of both the lamellae and pileus trama, non-gelatinised hyphae of the pileipellis that turn reddish brown in KOH, a cutis-type stipitipellis disrupted by solitary or small clusters of flaring-out hyphae towards the apex, and a distinctive nrITS sequence (MN708209).

HABITAT AND DISTRIBUTION. — In small groups, on humus-rich soil, among decaying litter. Known only from Kerala State, India.

ADDITIONAL SPECIMENS EXAMINED. — **India**, Kerala State, Kollam District, Thenmala, Kattilapara, 20.VII.2011, K. N. Anil Raj AR775 (AMH 10035); 03.XI.2015, K. P. Deepna Latha DKP411 (AMH 10036); Kozhikode District, Janakkad Forest, Maruthonkara, 13.VII.2019, K. P. Deepna Latha & K. N. Anil Raj DKP552.

DESCRIPTION

Basidiocarps

Small, collybioid.

Pileus

6–21 mm diam, convex or somewhat conico-convex with a small umbo; surface brownish yellow (5C7/OAC790), hygrophanous and becoming slightly paler, not striate, somewhat pruinose all over; margin slightly incurved when young, becoming decurved with age, entire or somewhat wavy.

Lamellae

Emarginate with a small decurrent tooth, furcate, slightly transverse, moderately crowded, greyish orange (5B5/OAC792), up to 3 mm wide, with lamellulae of 3–7 lengths; edge entire, concolourous with the sides.

Stipe

16–43 × 2–3.5 mm, central, terete, equal or slightly tapering towards the apex, fistulose; surface greyish orange (5B4/OAC793) at the apex, brownish orange (5C6/OAC776) towards the base, appressed-fibrillose all over, slightly recurved-squamulose towards the apex; base slightly enlarged, with profuse, white basal mycelium and yellowish mycelial cords.

Context

Up to 3 mm wide, yellowish brown (5D8), soft, no colour reaction when treated with 5% KOH or 10% ammonia.

Odour

Indistinctive.

Taste

Indistinctive.

Spore print

Not obtained.

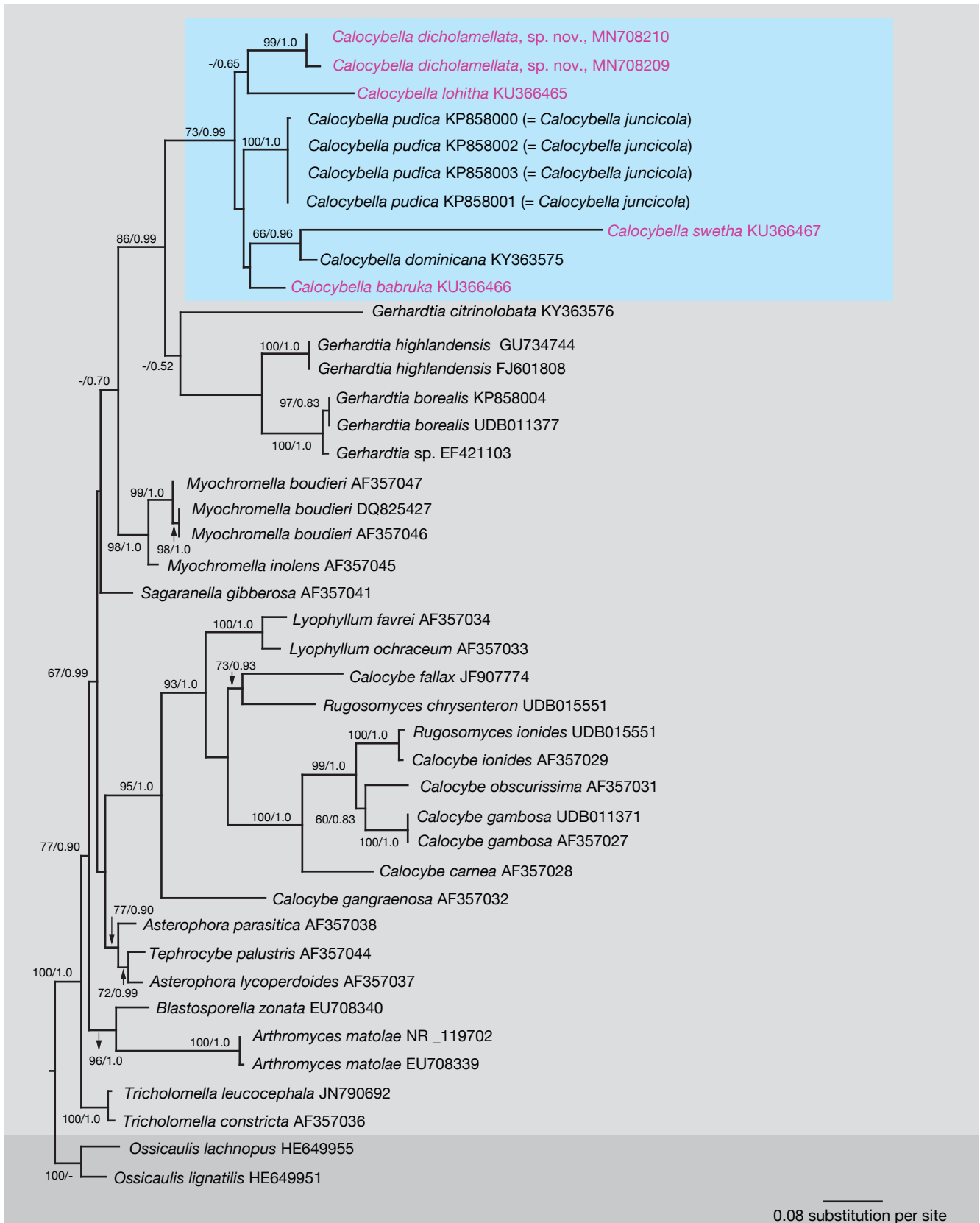


FIG. 2. — ML phylogram based on selected nrITS sequences of Lyophyllaceae showing the placement of Indian *Calocybella* Vizzini, Consiglio & Setti species within the genus. Values at the nodes indicate bootstrap and posterior probability support. MLB and BPP values $\geq 50\%$ and ≥ 0.5 respectively are shown. GenBank/UNITE number is given for each sequenced taxon. Indian *Calocybella* species are presented in coloured font.

TABLE 1. — Comparison of the key characters of species of *Calocybella* Vizzini, Consiglio & Setti.

	<i>C. dicholamellata</i>	<i>C. babruka</i>	<i>C. lohitha</i>	<i>C. swetha</i>	<i>C. dominicana</i>	<i>C. juncicola</i> (<i>C. pudica</i>)
Habit	Collybioid	Slenderly tricholomatoid	Collybioid	Slenderly tricholomatoid	–	–
Lamellae	Emarginate with a small decurrent tooth	Emarginate with a small decurrent tooth	Sinuate	Sinuate/Emarginate with a small decurrent tooth	Adnate-emarginate	Adnate-emarginate
Context	Yellowish brown, non-reddening on bruising	Orange white, non-reddening on bruising	Dark red, no colour change on bruising	Off-white, non-reddening on bruising	Whitish, slightly reddening in the stipe on handling	Yellowish, changing to red on bruising
Basidiospores	Pip-shaped, with irregular verrucae under SEM, acyanophilous	Ellipsoid to oblong-ellipsoid, with fine verrucose ornamentations under light microscope and SEM, acyanophilous	Oblong-ellipsoid to pip-shaped, with a fine verrucose ornamentations under light microscope and SEM, acyanophilous	Ellipsoid to broadly ellipsoid or somewhat ovoid, smooth under SEM, acyanophilous	Largely ellipsoid to ellipsoid, with obtuse and irregular verrucae under light microscope and SEM, cyanophilous	Ellipsoid to elongate-ellipsoid to oblong, smooth to verrucose under light microscope and SEM, cyanophilous
Cheilocystidia	Absent	Absent	Absent	Absent	Absent	Absent
Pleurocystidia	Absent	Absent	Absent	Absent	Absent	Absent
Pileipellis	Cutis with non-gelatinised hyphae	Cutis with slightly gelatinised hyphae	Cutis with slightly gelatinised hyphae	Trichoderm with faintly gelatinised hyphae	Non-gelatinised cutis	Cutis with slightly gelatinised hyphae
Clamp connection	Present	Present	Present	Present	Present	Present
Habitat	On humus rich soil, among decaying litter	On the ground	On the ground among decaying litter	On the ground among grass or leaf litter	On deciduous forest litter	In grassy clearings with <i>Juncus</i> sp.
Distribution	Kerala State, India	Kerala State, India	Kerala State, India	Kerala State, India	The Dominican Republic	Europe (Italy, France and Spain)

Basidiospores

4-6 (7) × 3-3.5 (4) (5.15 ± 0.67 × 3.23 ± 0.33) µm, Q = 1.14-2.16, Qm = 1.60, pip-shaped, hyaline, thin- to slightly thick-walled, acyanophilous, inamyloid, surface looking smooth under the light microscope, but with irregular verrucae under the SEM.

Basidia

20-25 × 5-7 µm, clavate, with a pale yellow wall pigment, reddish brown in KOH, thin-walled, siderophilous, mostly 4-spored, at times 2-spored or single-spored; sterigmata up to 6 µm long.

Lamella-edge

Fertile.

Pleurocystidia

Absent.

Cheilocystidia

Absent.

Lamellar trama

Subregular; hyphae 3-11 µm wide, sometimes slightly gelatinised, thin- to slightly thick-walled (up to 1.5 µm thick), with a smooth wall with a pale yellow wall pigment, hyaline in KOH, inamyloid.

Subhymenium

Poorly developed.

Pileus trama

Interwoven composed of slightly gelatinised hyphae; hyphae 3-10 µm wide, thick-walled (up to 2 µm thick), with a smooth wall with a pale yellow wall pigment, pale reddish brown in KOH, inamyloid.

Pileipellis

A cutis; hyphae 2-13 µm wide, with a thin to thick (up to 1 µm thick), smooth wall with a pale yellowish brown wall pigment, reddish brown in KOH.

Stipitipellis

A cutis often disrupted by solitary or short patches of flaring out hyphae towards the apex; hyphae 2-6 µm wide, with a thin to slightly thick (≤0.5 µm thick), smooth wall with a pale yellowish brown wall pigment, reddish brown in KOH, inamyloid.

Caulocystidia

Absent.

Clamp connections

Observed on all hyphae.

NOTES

This new species is different from all the three *Calocybella* species previously described from Kerala by Latha *et al.* (2016). *Calocybella lohitha*, differs in having greyish red basidiocarps with a finely granulose pileus which is tacky when wet, sinuate and non-furcate lamellae, a longer stipe (30–50 mm) lacking squamules at the apex, slightly narrower (2–3 μm) basidiospores, consistently 4-spored basidia, hyphae of lamellar trama turning greyish violet in KOH, a duplex-type pileus trama lacking gelatinisation, slightly gelatinised hyphae of the pileipellis, and a cutis-type stipitipellis. *Calocybella babruka* K.P.D.Latha & Manim., is distinguished from *C. dicholamellata*, sp. nov., in having slenderly tricholomatoid, brownish basidiocarps, a plano-convex pileus with a granulose surface and a lobate margin, non-furcate lamellae, a shorter (up to 30 mm) and slightly compressed, stuffed stipe devoid of any squamules at the apex, ellipsoid to oblong-ellipsoid basidiospores, consistently 4-spored basidia, the occasional presence of crassobasidia, slightly gelatinised hyphae of the pileipellis lacking any discolouration in KOH, and a cutis-type stipitipellis embedded in a resinous material. *Calocybella swetha* K.N.A.Raj & Manim., has slenderly tricholomatoid, whitish basidiocarps, a non-hygrophanous pileus with a velutinous surface and a fluted or rarely lobate margin, a shorter stipe (up to 30 mm) which is slightly compressed and tapered towards the base, and a scarcely pruinose surface, ellipsoid to broadly ellipsoid, smooth basidiospores, consistently 4-spored basidia, the occasional presence of crassobasidia, a pileus trama not turning colour in KOH, and a trichoderm-type pileipellis with slightly gelatinised hyphae.

Calocybella juncicola, the only known European species, is distinguished from *C. dicholamellata*, sp. nov., by an orange to brownish orange, non-hygrophanous pileus, adnate-emarginate, orange yellow lamellae with paler edges, a pale yellow stipe which is finely pruinose and tapered towards the base, a reddening context, a mealy odour and taste, cyanophilous basidiospores and consistently 4-spored-basidia (Corriol *et al.* 2017).

Calocybella dominicana, a species from the Dominican Republic, differs from *C. dicholamellata*, sp. nov., in having an ochraceous orange, somewhat silky pileus with a darker centre, white, close lamellae, a white-pruinose stipe with a surface reddening on handling, a mealy odour and taste, strongly warty basidiospores, consistently 4-spored basidia, regular to slightly divergent lamellar trama, pileipellis hyphae with some repent clavate terminal elements, and a gregarious habit (Vizzini *et al.* 2017). The key characters of all the known *Calocybella* species are listed in Table 1.

A comparison of the newly generated nrITS sequence of *C. dicholamellata*, sp. nov., (644 bp) with those sequences available in the GenBank suggests that the former has a distinct nrITS sequence. *Calocybella babruka* (KU366466) resulted as the closest hit with 86.99% identity in BLASTn search. The phylograms inferred from both the ML and BI analyses were identical in topology. Hence only the ML tree is shown in Fig. 2. The phylogram confirmed the placement

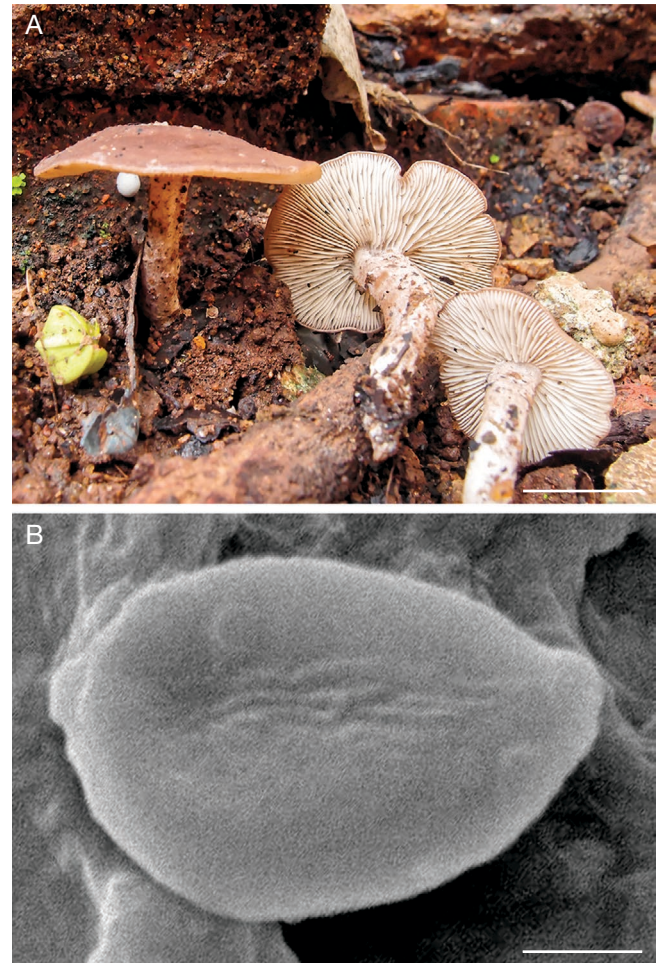


FIG. 3. — *Calocybella babruka* K.P.D.Latha & Manim. (CAL 1289): **A**, basidiocarps in natural habitat; **B**, basidiospore under SEM. Scale bars: A, 10 mm; B, 1 μm .

of *C. dicholamellata*, sp. nov., within the genus *Calocybella*. All *Calocybella* species together recovered as a monophyletic group with significant bootstrap (73% MLB) support and high BI posterior probability (0.99 BPP) value. Within this clade, the two sequences of *C. dicholamellata*, sp. nov., were found to be conspecific and is sister to a species from tropical India, *C. lohitha*.

***Calocybella babruka* K.P.D.Latha & Manim.**
(Fig. 3)

Phytotaxa 255 (2): 137 (2016).

SELECTED DESCRIPTION AND FIGURES. — Latha *et al.* 2016: 137, 138.

HOLOTYPE. — **India**. Kerala State, Malappuram District, Calicut University Campus, on the ground, 20.VI.2015, K. P. Deepna Latha DKP343 (holo-, CAL [CAL 1289]).

HABITAT AND DISTRIBUTION. — Scattered or in small groups, on the ground. Kerala State, India.

DESCRIPTION

This species is characterised by slenderly tricholomatoid basidiocarps, a brown pileus with a granulose surface



FIG. 4. — *Calocybella lohitha* K.P.D.Latha & Manim. (CAL 1288). **A**, basidiocarps in natural habitat; **B**, basidiospores under SEM. Scale bars: A, 10 mm; B, 2 μ m.

and a lobate margin, emarginate lamellae with a small decurrent tooth, a brown stipe with a whitish base, a context not discolouring on bruising, ellipsoid to oblong-ellipsoid, finely verrucose under both the light microscope and the Scanning Electron Microscope, acyanophilous basidiospores, a cutis-type pileipellis with slightly gelatinised hyphae, and a cutis-type stipitipellis composed of narrow and interwoven hyphae coated with a resinous material.

***Calocybella lohitha* K.P.D.Latha & Manim.
(Fig. 4)**

Phytotaxa 255 (2): 134 (2016)

SELECTED DESCRIPTION AND FIGURES. — Latha *et al.* 2016: 134–137.

HOLOTYPE. — **India**. Kerala State, Ernakulam District, Perumbavoor, Iringolkavu Sacred Grove, on the ground, among decaying litter, 04.VI.2013, *K. P. Deepna Latha DKP40* (holo-, CAL [CAL 1288]).

HABITAT AND DISTRIBUTION. — Scattered, on the ground, among decaying litter. Kerala State, India.

DESCRIPTION

Calocybella lohitha has collybioid basidiocarps that show no discolouration on bruising, a reddish pileus with a finely granular surface, dull red, intervenose lamellae with concolourous edges, a reddish or brownish grey stipe, acyanophilous, oblong-ellipsoid to pip-shaped basidiospores with conspicuous warts under both the light microscope and the Scanning Electron Microscope, hyphae of pileus trama with pale reddish brown or maroon plasmatic pigment, a cutis-type pileipellis with slightly gelatinised hyphae, and a cutis-type stipitipellis.

***Calocybella swetha* K.N.A.Raj & Manim.
(Fig. 5)**

Phytotaxa 255 (2): 139 (2016)

SELECTED DESCRIPTION AND FIGURES. — Latha *et al.* 2016: 139–140.

HOLOTYPE. — **India**. Kerala State, Malappuram District, Calicut University Campus, on the ground, among grass or leaf litter, 21.V.2015, *K. N. Anil Raj AR1000* (holo-, CAL [CAL 1290]).

HABITAT AND DISTRIBUTION. — Scattered or in small groups, on the ground, among grass or leaf litter. Kerala State, India.

DESCRIPTION

Whitish, slenderly tricholomatoid basidiocarps not discolouring on bruising, a velutinous pileus, sinuate or emarginate lamellae with a small decurrent tooth, a fibrillose and scarcely pruinose stipe which is slightly tapered towards the base, ellipsoid to broadly ellipsoid, acyanophilous, smooth basidiospores, a trichoderm-type pileipellis, and a cutis-type stipitipellis often disrupted by flaring-out hyphae are the diagnostic features of *C. swetha*.

DISCUSSION

Although the known diversity of this genus is relatively poor worldwide, most of the described species of *Calocybella* are from the tropical regions (Latha *et al.* 2016; Vizzini *et al.* 2017). Worldwide, a total of six species of *Calocybella* are known so far (Vizzini *et al.* 2015; Latha *et al.* 2016; Vizzini *et al.* 2017; Corriol *et al.* 2017). These include the European *C. juncicola* (Corriol *et al.* 2017), the four Indian species, *C. babruka*, *C. dicholamellata*, sp. nov., *C. lohitha* and *C. swetha* (Latha *et al.* 2016), and *C. dominicana* from the Dominican Republic (Vizzini *et al.* 2017). Tropical India seems to harbour a high diversity of this genus originally described from a temperate region. As indicated earlier, *Calocybella* is primarily a phylogenetically circumscribed genus. However, based on the information available so far, the genus can be morphologically circumscribed to a certain extent as follows: collybioid to slenderly tricholomatoid basidiocarps occurring on soil, a convex pileus, somewhat adnate-emarginate lamellae, more or less ellipsoid, inamyloid basidiospores often exhibiting a fine, verrucose ornamentation visible at least under the scanning electron microscope, a hymenium devoid of any cystidia, clamped hyphae and a pileipellis that is mostly a cutis or rarely a trichoderm and often showing slight gelatinisation.

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REFERENCES

- Anonymous 2004. — *The Online Auction Color Chart*. Online Auction Color Co, Palo Alto, 12 p.
- CORRIOL G., MOREAU P.-A. & BELLANGER J. M. 2017. — *Calocybella hymenoderma* sp. nov., *Calocybella juncicola* comb. nov. et les contours taxinomiques de la section *Rugosomyces*. *Errotari* 14: 35-46.
- IZUMITSU K., HATOH K., SUMITA T., KITADE Y., MORITA A., GAFUR A., OHTA A., KAWAI M., YAMANAKA T., NEDA H., OTA Y. & TANAKA C. 2012. — Rapid and simple preparation of mushroom DNA directly from colonies and fruiting bodies for PCR. *Mycoscience* 53 (5): 396-401. <https://doi.org/10.1007/S10267-012-0182-3>
- KORNERUP A. & WANSCHER J. H. 1978. — *Methuen Handbook of Colour*. 3rd edition. London, Eyre, Methuen, 252 p.
- LARGENT D. L. 1973. — *How to Identify Mushrooms to Genus I: Macroscopic Features*. Mad River Press Inc., Eureka, 86 p.
- LARGENT D. L., JOHNSON D. & WATLING R. 1977. — *How to Identify Mushrooms to Genus III. Microscopic features*. Mad River Press, Eureka, 148 p.
- LARSSON A. 2014. — AliView: a fast and lightweight alignment viewer and editor for large data sets. *Bioinformatics* 30 (22): 3276-3278. <https://doi.org/10.1093/bioinformatics/btu531>
- LATHA K. P. D. & MANIMOHAN P. 2018. — A new species of *Hygrocybe* from Kerala State, India. *Phytotaxa* 385 (1): 13-22. <https://doi.org/10.11646/phytotaxa.385.1.2>
- LATHA K. P. D., RAJ K. N. A., THUSHARA C., SHARAFUDHEEN S. A. & MANIMOHAN P. 2016. — Three new species of *Calocybella* from India based on morphology and molecular phylogeny. *Phytotaxa* 255 (2): 133-143. <https://doi.org/10.11646/phytotaxa.255.2.2>
- MILLER M. A., PFEIFFER W. & SCHWARTZ T. 2010. — Creating the CIPRES Science Gateway for inference of large phylogenetic trees, in *Proceedings of the Gateway Computing Environments Workshop (GCE) 14 Nov. 2010*. Institute of Electrical and Electronics Engineers, New Orleans, LA: 1-8. <https://doi.org/10.1109/GCE.2010.5676129>

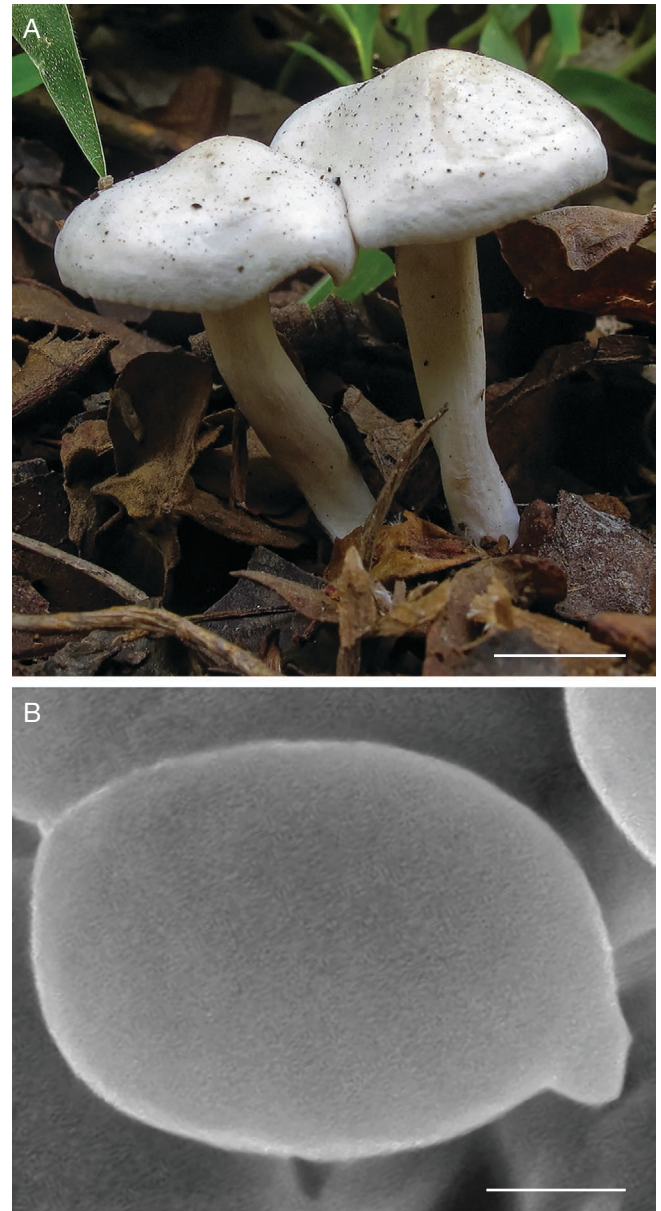


FIG. 5. — *Calocybella swetha* K.N.A.Raj & Manim. (CAL 1290): **A**, basidiocarps in natural habitat; **B**, basidiospore under SEM. Scale bars: A, 10 mm; B, 1 µm.

- MILNE I., LINDNER D., BAYER M., HUSMEIER D., MCGUIRE G., MARSHALL D. F. & WRIGHT F. 2009. — TOPALi v2: a rich graphical interface for evolutionary analyses of multiple alignments on HPC clusters and multi-core desktops. *Bioinformatics* 25 (1): 126-127. <https://doi.org/10.1093/bioinformatics/btn575>
- RAJ K. N. A., LATHA K. P. D., IYYAPPAN R. & MANIMOHAN P. 2016. — *Rhodophana squamulosa*—a new species of Entolomataceae from India. *Mycoscience* 57 (2): 90-95. <https://doi.org/10.1016/j.myc.2015.10.001>
- RAMBAUT A. 2014. — FigTree 1.4.2 software. Institute of Evolutionary Biology, University of Edinburgh. <http://tree.bio.ed.ac.uk/software/figtree/>
- RONQUIST F., TESLENKO M., VAN DER MARK P., AYRES D. L., DARLING A., HÖHNA S., LARGET B., LIU L., SUCHARD M. A. & HUELSENBECK J. P. 2012. — MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systems Biology* 61 (3): 539-542. <https://doi.org/10.1093/sysbio/sys029>
- SINGER R. 1975. — *The Agaricales in modern taxonomy*. 3rd edition, Cramer, Vaduz, 912 p.
- STAMATAKIS A. 2014. — RAxML Version 8: A tool for Phylogenetic Analysis and Post-Analysis of Large Phylogenies. *Bioinformatics* 30 (9): 1312-1313. <https://doi.org/10.1093/bioinformatics/btu033>
- VELLINGA E. 1988. — Glossary, in BAS C., KUYPER T. H. W., NOORDELOOS M. E., VELLINGA E. C. (eds), *Flora Agaricina Neerlandica*. Volume 1. A. A. Balkema, Rotterdam: 54-64.
- VIZZINI A., CONSIGLIO G., SETTI L. & ERCOLE E. 2015. — *Calocybella*, a new genus for *Rugosomyces pudicus* (Agaricales, Lyophyllaceae) and emendation of the genus *Gerhardtia*. *IMA Fungus* 6 (1): 1-11. <https://doi.org/10.5598/imafungus.2015.06.01.01>
- VIZZINI A., ANGELINI C. & ERCOLE E. 2017. — Is the species diversity in the lyophylloid genera *Calocybella* and *Gerhardtia* (Agaricales, Basidiomycota) underestimated? Two new species from the Dominican Republic. *Phytotaxa* 291 (4): 241-252. <https://doi.org/10.11646/phytotaxa.291.4.1>
- WHITE T. J., BRUNS T., LEE S. & TAYLOR J. 1990. — Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenies, in INNIS M. A., GELFAND D. H., SNINSKY J. J., WHITE T. J. (eds), *PCR protocols, a guide to methods and applications*. Academic, San Diego: 315-322. <https://doi.org/10.1016/b978-0-12-372180-8.50042-1>

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