

## A new bioluminescent species of *Mycena* sect. *Exornatae* from Kerala State, India

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Recent studies on the genus *Mycena* in Kerala State, India resulted in the discovery of a new species, herein described as *M. deeptha*. It has hallmarks of *Mycena* section *Exornatae* such as gelatinous pileipellis composed of hyphae that are covered with thorn-like excrescences, non-gelatinized stipitipellis, discoid stipe base and luminescent mycelium. A combination of characters such as larger basidiomata, fimbriate pileal margin composed of cells with elongate protrusion, cheilocystidia and caulocystidia with elongate apical projections, and the presence of deterrent elements over the primordium, however, makes it unique within that section. Phylogenetic analyses based on ITS rDNA sequences using maximum parsimony and maximum likelihood methods support *M. deeptha* as a distinct species belonging to a clade containing *M. chlorophos*, a well-known luminescent species of sect. *Exornatae*. The ITS rDNA sequence of *M. deeptha* shows 99–100% similarity with those of two unidentified *Mycena* species (environmental samples) deposited in GenBank indicating that the new species may have a wider geographical distribution.

**Key words** – Agaricales – Basidiomycota – biodiversity – molecular phylogeny – Mycenaceae

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### Introduction

Several *Mycena* (Mycenaceae, Agaricales, Basidiomycota) species are renowned for their ability to emit visible light. Eighty three species of luminous fungi are currently known (Vydryakova et al. 2011), of which 35 species belong to the genus *Mycena*, distributed in 17 sections (Desjardin et al. 2008). In many species, only the mycelium is luminescent, whereas in others both the mycelium and the basidiomata emit light. In a few species, only the basidiomata have been reported as luminescent while the mycelium is non-luminescent (*M. irritans* E. Horak, *M. lamprospora* (Corner) E. Horak, *M. sublucens* Corner) (Corner 1954, Horak 1978, Desjardin

et al. 2007, 2010). In *M. polygramma* (Bull.) Gray and in *M. lamprospora* spores are luminescent (Herring 1994, Horak 1978). While some species such as *M. chlorophos* (Berk. & M.A. Curtis) Sacc. are brightly luminescent, some like *M. haematopus* (Pers.) P. Kumm., show only low-level bioluminescence, detected only by using a photometer.

During our studies on the genus *Mycena* in Kerala State, India, we discovered a species whose basidiomata were non-luminescent, but mycelium, both on its natural substratum and in culture, exhibited bioluminescence. Conventional morphology-based observations and ITS sequence-based phylogenetic analysis revealed it to be a new species, which is described,

illustrated and discussed here.

## Methods

### Morphological studies

Microscopic observations were made on material stained with 1% aqueous solution of Congo red and mounted in 3% aqueous KOH. Melzer's reagent was used to observe whether the spores and tissues were amyloid or vinoid. For evaluation of the range of spore size, twenty basidiospores from each specimen cited were measured. Mycelium of the agaric was cultured on malt extract agar by transferring basidiospores from spore prints to sterilized medium under aseptic conditions. Photographs of the bioluminescent mycelial culture were taken using a digital camera in a dark room with 40–50 minutes of exposure. Colour codes used in the description are from Kornerup & Wanscher (1978). The examined collections are deposited at Kew (Mycology) Herbarium and the Kew accession numbers (e.g., K (M) 178331) are indicated. The infrageneric taxonomy of *Mycena* followed is that of Maas Geesteranus (1992).

### DNA extraction and sequencing

DNA extractions were made from freshly collected basidiomata using a commercial DNA extraction kit (Sigma GenElute™ Plant Genomic DNA Miniprep kit, Sigma-Aldrich Corp., St. Louis). PCR reactions were performed with primers ITS1 and ITS5. Amplification reactions were performed in MJMini™ thermal cycler (Bio-Rad, Hercules). Amplified PCR product was purified using column purification (GenElute™ PCR Clean-Up kit, Sigma-Aldrich Corp., St. Louis) as per manufacturer's guidelines and was subjected to automated DNA sequencing on ABI3730xl Genetic Analyzer (Applied Biosystems, USA) using primers ITS1 and ITS5. The generated sequence was edited manually using BioEdit sequence alignment editor version 7.0.9.0 (Tom Hall, Ibis Biosciences, Carlsbad, California). The edited sequence was then used for BLAST searches in the GenBank database (www.ncbi.nlm.nih.gov). The newly generated sequence was deposited in GenBank (accession number JX481737).

## Sequence alignment and phylogenetic analysis

The newly generated ITS sequence (742 bp) and those retrieved from GenBank (21 ITS sequences) were initially aligned using CLUSTAL X 2.1 and then checked in Se-Al 2.0a11 (Andrew Rambaut, University of Edinburgh, U.K.; <http://tree.bio.ed.ac.uk/software/seal>). The sequences from GenBank were selected based on their similarity indices; available sequences of representative species of related sections of *Mycena* were also added to the dataset. *Tricholoma aestuans* (Fr.) Gillet and *Suillus pictus* (Peck) A.H. Sm. & Thiers were selected as outgroup taxa for rooting purposes. Maximum Parsimony analysis was conducted using PAUP\* 4.0b10 (Swofford 2002) using heuristic search option with tree bisection-reconnection (TBR) branch swapping algorithm, with 20 replicates using random step-wise addition and holding one tree at each step. All minimal length trees were saved and maxtrees were set as unrestricted. Bootstrap analysis (Felsenstein 1985) was carried out with full heuristic search option on 1000 replicates. Bayesian analysis was done using MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003). The general time reversible model with gamma distributed rate variation option was selected for the analysis. Multiple independent analyses were done for 2 million generations, with trees saved every 100 generations. Options for incremental heating scheme, chain numbers etc. were set to MrBayes default settings. The aligned sequence data matrix has been deposited in TreeBase (<http://purl.org/phylo/treebase/phylovs/study/TB2:S13309>).

## Results

### Taxonomy

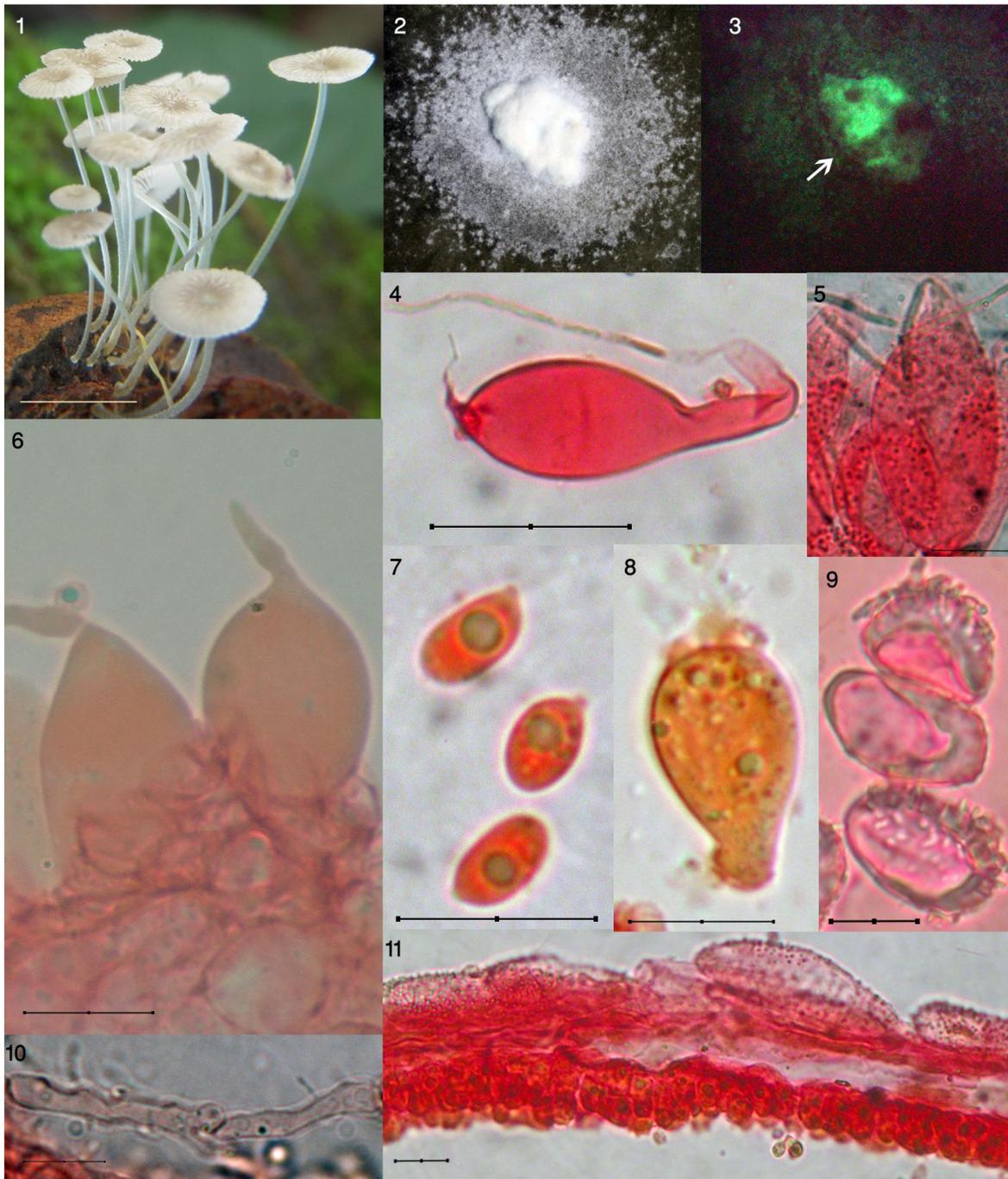
*Mycena deeptha* Aravind. & Manim. **sp. nov.**

Figs 1–11

Mycobank MB 801152

Etymology – *deeptha* (Sanskrit), luminous.

Characterised by a gelatinous pileipellis composed of hyphae that are covered with thorn-like excrescences, non-gelatinized stipitipellis, discoid stipe base and a luminescent



**Figs 1–11** – *Mycena deeptha*. **1** Basidiomata. **2** Cultured mycelium on agar medium. **3** Same mycelium in darkness showing bioluminescence. **4** Caulocystidium. **5** Pileus marginal cells. **6** Cheilocystidia. **7** Basidiospores. **8** Basidium. **9** Elements over the primordium. **10** Hypha of the pileipellis with thorn-like excrescences. **11** Radial section of the pileus showing structure of the pileipellis – Bars = 10 mm for basidiomata and 10  $\mu$ m for micro-structures.

mycelium. Differing from other known members of the section *Exornatae* such as *Mycena chlorophos*, *M. discobasis* Métrod, *M. chlorinosma* Singer and *M. margarita* (Murrill) Murrill owing to its combination of characters such as larger basidiomata, fimbriate pileal margin composed of cells with elongate

protrusion, cheilocystidia and caulocystidia with elongate apical projections, and the presence of deterrent elements over the primordium.

Basidiomata small, delicate. Pileus 1–9.5 mm diam., 0.5–0.75 mm high, parabolic to conic when young, becoming nearly appanate

with a centrally flattened depression or rarely umbilicate with age; surface yellowish grey (4B2) or dark blonde (5D4) at the centre, tobacco brown (5F6) in the middle, and whitish or yellowish white (4B1) towards margin when young, becoming brownish orange (5C3) at the central depression, greyish brown (5D3) in the middle and brownish grey (5C2) to marble white (5B2) towards margin with age, translucent-striate when young, becoming plicato-sulcate with age, finely pruinose all over, dry; margin initially straight, becoming plane or upturned, fimbriate. Lamellae 15–18(–23) reaching the stipe, free or narrowly adnate and then seceding to form a pseudocollarium, pale greyish or whitish, thin, 0.25–0.5 mm wide, close, with lamellulae of 2–5 lengths; edge finely hairy under a lens, concolorous with the sides. Stipe 6–30 × 0.25–0.5 mm, central, terete, almost equal when very young, tapering towards apex with age, hollow; surface translucent, white to cream (4A3), becoming yellowish or greyish yellow (4B3) or yellowish grey (4B2), entirely densely pubescent when young, becoming almost glabrous towards apex with age; base discoid, of radiating mycelium, hairy. Context not conspicuous, less than 0.5 mm wide. Odour and taste not distinctive. Basidiomata not luminescent, but the mycelium (that on the natural substratum as well as that cultured on malt extract agar) shows faint greenish luminescence.

Basidiospores 6.5–9(–11) × 3–5(–5.5) (7.64±0.386 × 3.868±0.162) µm, Q = 1.5–2.67, Q<sub>m</sub> = 1.99, ellipsoid to subcylindrical, thin-walled, hyaline, smooth, strongly amyloid, with oil guttules. Basidia 14–19 × 6.5–9.5 µm, clavate, bearing 4 sterigmata up to 3.5 µm long. Lamella-edge homogeneous. Cheilocystidia 14–30 × 5.5–10 µm, vesiculose, smooth, thin-walled, hyaline, with rostrate or acute or acuminate apex, often with an apical protrusion (4.5–15 × 1–2.5 µm) that may rarely show some branching. Pleurocystidia none. Lamellar trama regular to subregular; hyphae 3.5–17(–29) µm wide, thin-walled, hyaline, faintly vinoid in Melzer's reagent. Subhymenium ramose. Pileus trama subregular; hyphae 6–30 µm wide, thin-walled, hyaline to pale greyish, faintly vinoid in Melzer's reagent. Pileipellis an epicutis; hyphae 2–11 µm wide, thin-walled,

hyaline, with numerous lateral thorn-like protrusions (2.5–5 µm long), embedded in a gelatinous matrix, with prominent, loop-like clamp connections. Pileocystidia 23–45.5 × 10–16 µm, elongated-clavate, thin-walled, with simple, conic or cylindrical excrescences (0.5–6 × 0.5–1 µm). Detersile elements over the pileus of primordium 17–35.5 × 14–25 µm, clavate or subglobose, thick-walled (up to 3.5 µm) towards apex, hyaline, with simple, conic or cylindrical or coarse excrescences (0.5–7 × 0.5–2 µm). Pileus marginal cells 17–60 × 9.5–19 µm, clavate or lageniform, seldom with hairy protrusion (29.5–118 µm long), with simple, conic or cylindrical excrescences at the middle or towards apex (0.5–6 × 0.5–1 µm). Stipitipellis a cutis of smooth hyphae; hyphae 1.5–8 µm wide, thin-walled, hyaline. Caulocystidia 25–73.5 × 10.5–16 µm, narrowly conic or narrowly lageniform, smooth, slightly thick-walled (up to 1.5 µm), often with a long protrusion (53–92.5 µm long). Stipe basal cells 13–93.5 × 5–20 µm, clavate, vesiculose or utriform or cylindrical, thin-walled, hyaline. Stipe trama faintly vinoid in Melzer's reagent. Clamp connections seen on all hyphae.

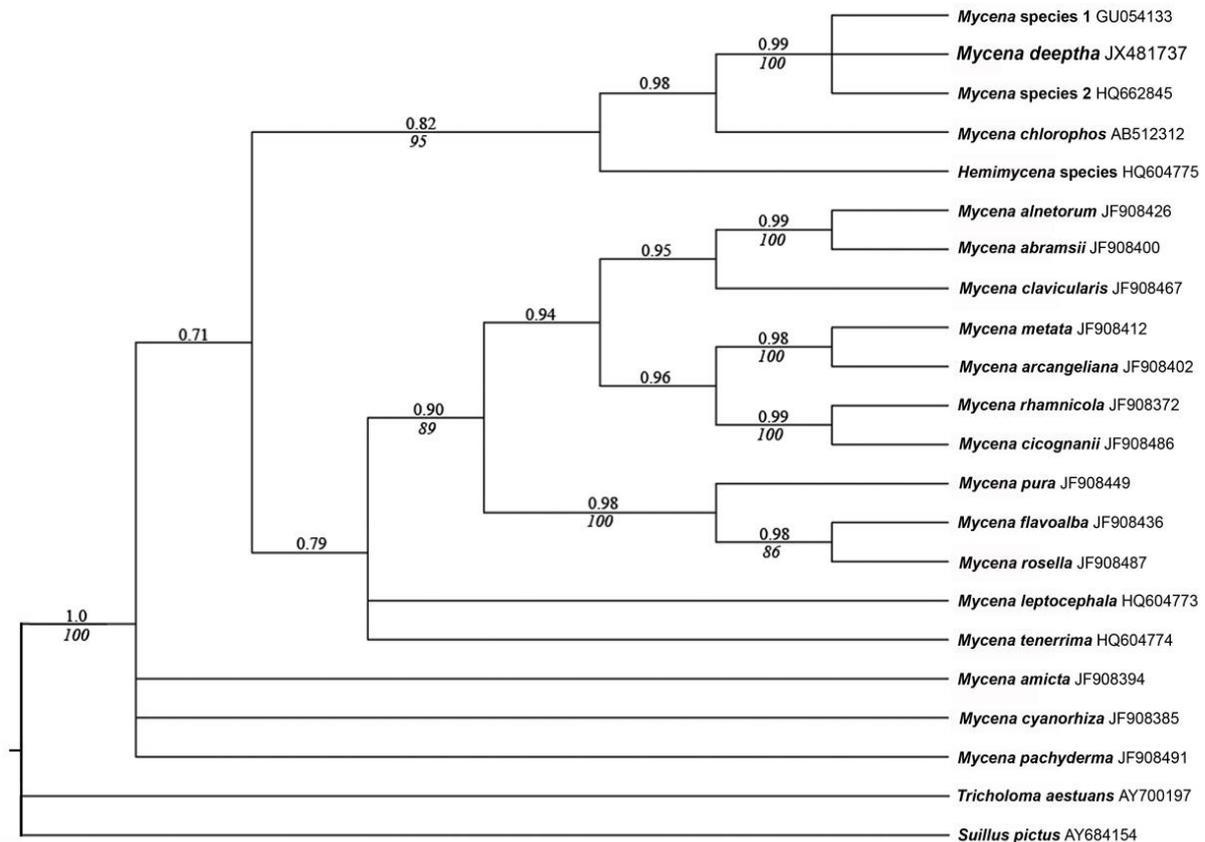
On decaying woody shells of fruits of *Vateria indica* L. and on decaying leaves of various plants, in groups or scattered, June–November.

Known distribution – widespread in Kerala State, India.

Material examined – India, Kerala State, Kozhikode District, Beypore: 22 Sept 2009, D.M. Aravindakshan DM334a (K(M) 178332); 3 Oct 2009, D.M. Aravindakshan DM334c (K(M) 178331, **Holotype**); 17 Aug 2011, D.M. Aravindakshan DM334g (K(M) 178333); Wayanad District, Thirunelli: 27 Nov 2009, D.M. Aravindakshan DM365 (K(M) 178334).

### Molecular analysis

The ITS sequence-based molecular phylogenetic analyses generated trees with good support for most of the clades (Fig. 12). Topologies were similar in both maximum parsimony (MP) and maximum likelihood (ML) analyses. In Bayesian analysis, two independent runs reached a standard deviation of split frequencies of 0.01 after 40000 generations. Log-likelihood scores reached stationarity after 10000 generations. In both



**Fig 12** – Bayesian consensus tree obtained from Bayesian MCMC analysis using nuclear ITS sequence data. Values above the branches indicate Bayesian posterior probabilities greater than 0.70 and values below branches indicate Maximum Parsimony bootstrap values greater than 70%. GenBank accession numbers are given after the taxonomic names.

MP and ML analyses, *Mycena deeptha* settled close to two unidentified *Mycena* species (GenBank accession numbers GU054133 and HQ662845, designated here as unidentified *Mycena* sp. 1 and unidentified *Mycena* sp. 2, respectively). This close relationship correlates with the result of the GenBank associated BLAST search. In BLAST search, the two unidentified species showed 99 and 100 percent sequence similarities, respectively. *Mycena chlorophos* another luminescent mushroom of the section *Exornatae* Maas Geest. settled close to the three *Mycena* species (*Mycena deeptha*, unidentified *Mycena* species 1 and 2). *Mycena deeptha* and the two unidentified *Mycena* species along with *M. chlorophos* always formed a distinct clade. This clade, although with weak bootstrap value, has good posterior probability support and can be identified as the section *Exornatae* clade.

## Discussion

Characters such as a gelatinous pileipellis composed of hyphae that are covered with thorn-like excrescences, non-gelatinized stipitipellis, discoid stipe base and the luminescent mycelium of this species are hallmarks of section *Exornatae*. However, the combination of characters such as larger basidiomata, fimbriate pileal margin composed of cells with elongate protrusion, cheilocystidia and caulocystidia with elongate apical projections, and the presence of deterrent elements over the primordium makes it unique and distinguishes it from other known members of that section (*M. chlorophos*, *M. discobasis*, *M. chlorinosma* and *M. margarita*).

Morphologically and microscopically, the present species is very similar to *M. rhenana* Maas Geest. & Winterh. of sect. *Basipedes* (Fr.) Quel. that according to Desjardin et al. (2007) is best placed in sect.

*Exornatae*. But that species has somewhat smaller basidiomata, numerous lamellae (20–26), and marginal cells, caulocystidia, and stipe basal cells of a different type. In addition, it has no cheilocystidia and its pileipellis is not gelatinized. Also, that species is not known to be luminescent. *Mycena cecidiophila* A.P. Berg, Berg-Block, Noordel. & Uljé placed under the monotypic section *Cecidiophilae* A.P. Berg, Berg-Block, Noordel. & Uljé (van den Berg et al. 2000) is also found phenetically very similar to the present species. Remarkably, Noten & Vannieuwerburgh (2009) and Aronsen (www.mycena.no/rhenana.htm) after re-examining the type of *M. cecidiophila* confirmed that it was conspecific with *M. rhenana* and they argued for the deletion of section *Cecidiophilae*.

Morphological characters such as larger basidiomata, fimbriate pileal margin composed of cells with elongate protrusion, cheilocystidia and caulocystidia with elongate apical projections, and the presence of deterrent elements over the primordium when combined with results of the molecular analyses shows that *Mycena deeptha* can be placed in *Mycena* section *Exornatae* where it differs considerably from other related species including *M. chlorophos*. Remarkably, the ITS rDNA sequence of *M. deeptha* shows 99–100% similarity with those of two unidentified *Mycena* species deposited in GenBank indicating that the new species may have a wider geographical distribution. Both the latter are environmental samples, the first (GenBank Acc. No. GU054133) from a soil sample collected from Korea and the second (GenBank Acc. No. HQ662845) from air filter samples with no locality details.

### Acknowledgements

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