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Two new bryophilous agarics from India

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ABSTRACT

Two new bryophilous agarics, *Galerina indica* and *Rickenella indica*, are described from Kerala State, India. Comprehensive descriptions, photographs, and comparisons with phenetically similar and phylogenetically related species are provided. Inferences of their phylogenetic relationships within the respective genera are provided based on the sequences of nuclear ribosomal internal transcribed spacer region. This forms the first record of the genus *Rickenella* from the entire tropics and the first report of a bryophilous *Galerina* from Kerala. Also, this is the first report of the association of the genera *Galerina* and *Rickenella* with the moss genera *Leucobryum* and *Campylopus* respectively.

1. Introduction

Fungi associated with bryophyte substrates are taxonomically diverse but their diversity remains understudied (Felix 1988; Hawksworth 2001; Davey and Currah 2006). About 400 species of obligate bryophilous fungi have been described so far, most of which are ascomycetes (Döbbeler 1997; Ptaszyńska et al. 2009). Only a few bryophilous basidiomycetes have been reported and many of them are agarics (Kost 1988). Our knowledge on bryophilous agarics is based almost entirely on studies made in temperate and far northern regions. Very little is known about the diversity of these fungi in the tropics.

During our studies on the agarics of Kerala State, India, we came across two new and remarkable bryophilous agarics that are described here along with inferences of their phylogenetic relationships based on the sequences of nuclear ribosomal internal transcribed spacer (ITS) region.

2. Materials and methods

2.1. Morphological studies

The bryophilous fungi described here were collected from a moist deciduous forest (Muthanga Wildlife Sanctuary) of Kerala State, India. Conventional morphology-based taxonomic methods as well as molecular phylogenetic methods were employed for this study. Microscopic observations were made on material stained with 1% aqueous solutions of phloxine and Congo red and mounted in 3% aqueous KOH. Melzer's reagent was used to observe whether the basidiospores and tissues were amyloid. Lactophenol-cotton blue was used for observing plage of the basidiospores. For evaluation of the range of spore-size, 20 basidiospores each from one specimen of each collection cited were measured. In the description of the basidiospores, the abbreviation Q

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represents the range of ratio of spore length to spore width calculated for each spore and Qm is the average of the Q values. Color codes used in the descriptions are from Kornerup and Wanscher (1978). The examined collections are deposited at Kew (Mycology) Herbarium and the Kew accession numbers (e.g., K(M) 190552) are indicated.

2.2. Phylogenetic analysis

2.2.1. DNA extraction, PCR and sequencing

The entire ITS region (ITS1, 5.8S rRNA gene, and ITS2) was analyzed in this study. DNA was extracted from dried specimens of the two agarics employing the procedure described by Izumitsu et al. (2012). PCR reactions were performed with primers ITS1 and ITS4. Amplification reactions were performed in a GeneAtlas™ thermal cycler (Astec, Fukuoka, Japan). Amplified PCR products were purified using column purification (GeneJet™ PCR purification kit, Thermo Fisher Scientific, Mumbai, India) as per manufacturer's guidelines and were subjected to automated DNA sequencing on ABI3730xl DNA analyzer (Applied Biosystems, Foster City, CA, USA) using the same primers used for PCR. The generated sequences were edited manually using BioEdit sequence alignment editor version 7.0.9.0 (Tom Hall, Ibis Biosciences, Carlsbad, CA, USA). The edited sequences were then used separately for BLAST search in the GenBank database (www.ncbi.nlm.nih.gov). The newly generated sequences were deposited in GenBank.

2.2.2. Sequence analysis

The newly generated ITS sequence (KJ187768; 677 bp) of the newly discovered *Galerina* species along with those retrieved from GenBank (31 ITS sequences) based on the result from a BLAST search were aligned using MUSCLE version 3.8.31 (Edgar 2004). A final set of sequences from 33 species of *Galerina* were aligned. All sequences were trimmed at the ends to a final length of 629 bp. The pre-existing sequences of *Galerina* species from GenBank were selected based on their similarity indices. *Panaeolus sphinctrinus* (Fr.) Quél. (Gulden et al. 2005) was selected as outgroup taxon for rooting purpose. Maximum parsimony (MP) analysis was conducted using MEGA version 5, with 1000 heuristic bootstrap (BS) replicates, using random step-wise addition, and holding one tree at each step. Maxtrees were set to 1000. TBR branch swapping algorithm was used to assess branch support. All characters were treated as unordered and gaps were treated as missing data. The aligned sequence data matrix has been deposited in TreeBase (http://purl.org/phylo/treebase/phylows/study/TB2:S15288).

No phylogenetic analysis was carried out with the ITS sequence (KJ187769; 661 bp) of the newly discovered *Rickenella* species owing to the very limited number of *Rickenella* ITS sequences available in the GenBank.

3. Results

3.1. Taxonomy

Galerina indica K.P.D. Latha & Manim., sp. nov. Fig. 1.

MycoBank no.: MB 807699.

Diagnosis: Characterized by small, mycenoid basidiomata; a brownish orange, pellucid-striate, glabrous pileus; emarginate lamellae; ellipsoid to amygdaliform, ornamented basidiospores with smooth plage and a loose myxosporium; 2-spored basidia; versiform cheilocystidia; utriform or lageniform pleurocystidia with a somewhat subcapitate apex; cutis-type pileipellis and stipitipellis with spiral encrustations; and presence of clamp connections. Differing from *Galerina salicicola* P.D. Orton in having smaller basidiomata, 2-spored basidia, and a bryicolous habitat.

Type: India, Kerala State, Wayanad District, Muthanga Wildlife Sanctuary, on moss bed (*Leucobryum* sp., Dicranaceae, Bryopsida), in small groups, 7 September 2012, K. P. Deepna Latha (holotype, K(M)190552).

Gene sequence ex-holotype: KJ187768 (ITS).

Etymology: Specific epithet refers to India, the country from where this species was first described.

Basidiomata small, mycenoid. Pileus 2–7 mm diam., initially convex, becoming conico-convex to almost campanulate with a small umbo; surface brownish orange (6C8) on umbo and striations, golden yellow (5B7) elsewhere, weakly hygrophanous and becoming paler, eventually developing a greenish hue towards margin, pellucid-striate, glabrous to the naked eye, finely sulcate towards margin; margin slightly incurved or somewhat decurved, crenate. Lamellae 10–20, emarginate, close, grayish orange (6B4), up to 2 mm wide, with lamellulae of 3 lengths; edge entire or slightly crenate, concolorous with the sides. Stipe 7–12 × 0.5–1.5 mm, central, terete, equal or slightly tapered towards base, solid; surface brownish orange (7C5), glabrous to the naked eye, finely appressed fibrillose all over as well as finely pruinose towards apex and base under a lens; base with pale brown mycelial cords. Odor and taste not distinctive.

Basidiospores 7.5–10 × 4.5–6 (8.8 ± 0.61 × 5.2 ± 0.44) μm, Q = 1.5–2, Qm = 1.69, ellipsoid to amygdaliform, with a conspicuous plage, a golden brown wall with verruculose ornamentation and a loose wrinkled myxosporium. Basidia 14–23 × 5–7 μm, clavate, 2-spored, thin-walled, hyaline or pale yellow; sterigmata up to 4 μm long. Lamella-edge heterogeneous. Cheilocystidia 11–44 × 4.5–5 μm, versiform: globose, pyriform, utriform, lageniform or tibiiform, thin- to slightly thick-walled, pale yellow or hyaline. Pleurocystidia 30–42 × 12–16 μm, utriform or lageniform, with a somewhat subcapitate apex, thin- to slightly thick-walled, hyaline or pale yellow. Lamellar trama composed of both narrow and inflated hyphae; hyphae 4–24 μm wide, with cylindrical or subfusoid elements, thin-walled, hyaline or pale yellow. Pileus trama subregular; hyphae 4–12 μm wide, with cylindrical or subfusoid elements, inflated up to 30 μm, thin-walled, hyaline or pale yellow. Pileipellis a cutis; hyphae 4–7 μm wide, with cylindrical or subfusoid elements, thin- to slightly thick-walled, with a pale yellow wall showing spiral encrustations. Stipitipellis a cutis; hyphae 3–24 μm, with cylindrical or subfusoid elements, thin- to slightly thick-walled, with a pale yellow wall showing spiral encrustations. Clamp connections seen on all hyphae.

Habitat and distribution: On moss bed (*Leucobryum* sp., Dicranaceae, Bryopsida), India.