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A new species of *Marasmius* sect. *Globulares* from Indian Himalaya with tall basidiomata

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Abstract

A marasmioid fungus: *Marasmius indopurpureostriatus* (*Marasmiaceae*, *Basidiomycota*) is proposed here as new to science from Sikkim, a small Himalayan state in India. A comprehensive description with illustrations to aid in the identification, comparisons with morphologically similar taxa and an artificial key to the species of *Marasmius* sect. *Globulares,* previously reported from India are provided.

Key words – *Agaricomycetes* – macrofungi – new species – taxonomy

Introduction

Marasmius Fr. (*Marasmiaceae*, *Agaricomycetes*, *Basidiomycota*), as traditionally accepted by Singer (1986), is polyphyletic (Wilson & Desjardin 2005). Based on the nLSU rDNA sequences, Wilson & Desjardin (2005) restricted the genus to a monophyletic lineage containing only sections *Marasmius*, *Sicci*, *Hygrometrici*, *Globulares*, *Neosessiles*, *Scotophysini* and *Leveilleani* as defined by Singer (1976, 1986).

Marasmius sect. *Globulares* includes species, characterized by a combination of characters like a medium or large sized, variously colored pileus which often umbonate, mostly even or rarely sulcate, hygrophanous, often fleshy and putrescent; well developed, non-collariate, often broad, distant to crowded lamellae; a central, stout, not bristle like, glabrous to pubescent, non-insititious, often pallid stipe; absence of rhizomorphs; ellipsoid to clavate or subfusoid basidiospores; with or without pleurocystidia and cheilocystidia; a pileipellis composed of hymeniform layer of non-diverticulate, non-setulose cells ranging from cylindric or clavate to turbinate, vesiculose or sphaeropedunculate (*Globulares* - type elements); dextrinoidtramal hyphae and stipe tissue; presence or absence of stipe vesture; and with clamp connections (Desjardin 1989).

A thorough literature survey reveals that, at present, the genus *Marasmius* is represented by ca. 70 species and one variety from India (Atri et al. 1996, Manjula 1983, Manimohan & Leelavathy 1988, 1989, Bilgrami et al. 1991, Joseph et al. 1995, Crous et al. 2014, Dutta et al. 2014), among which only 6 taxa [*Marasmius amygdalosporus* Manim. & Leelav., *Marasmius caryotae* (Berk.) Petch, *Marasmius heliomyces* Murrill, *Marasmius hookeri* Berk and *Marasmius oreades* (Bolton) Fr., *Marasmius pegleri* Courtec. (previously reported as *Marasmius purpureus* Berk. & M.A. Curtis)] belong to the sect. *Globulares*.

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While undergoing a routine macrofungal survey of the East district of Sikkim, one (KD) of us collected some wild mushrooms from Churten (Chorten) which is a subtropical broad-leaf forested area dominated by *Schima wallichii* (DC.) Korth., *Betula alnoides* Buch.-Ham., *Castanopsis tribuloides* A. DC., *Macaranga denticulate* Müll. Arg., *Engelhardtia spicata* Blume, *Alnus nepalensis* D. Don. Thorough morphological examination of two collected materials reveals a novel taxon: *Marasmius indopurpureostriatus* sp. nov. The present paper aims to focus on the detailed macro- and micro-morphological description coupled with the illustrations for the new species.

Materials & Methods

Morphological protocols

Macro-morphological along with ecological features was recorded from the fresh young to matured basidiomata from the field or transit camp. Field photography was made with Nikon D300s. Colour codes and terms follow Methuen Handbook of Colour (Kornerup & Wanscher 1978). Micro-morphological features were obtained from dried material by mounting free-hand sections of basidiocarps in 5% KOH, cotton blue and Congo Red. Specimen was then examined with Carl Zeiss AX10 Imager A1 phase contrast microscope and Nikon Eclipse Ni-U compound microscope and photography were made with dedicated digital cameras of the respective microscopes. Q value denotes length/width ratio of the spores excluding ornamentation. Spore statistics include: X_m, the arithmetic mean of the spore length by spore width (± standard deviation) for n spores measured in a single specimen; Q, the quotient of spore length by spore width in any one spore, indicated as a range of variation in n spores measured; Q_m, the mean of Q-values in a single specimen; n, total number of spores measured; s, the number of basidiocarps. Herbarium name follows Holmgren et al. (1990).

DNA extraction, PCR and cycle sequencing

Genomic DNA was extracted following Dutta et al. (2014). For amplification of the region spanning the nuclear ribosomal internal transcribed spacer region 1, 5.8S and spacer region 2 (nrITS), the primer pair ITS1-F and ITS4-B were used (Gardes & Bruns 1993). The thermal cycler protocol used in the amplification of both nrDNA regions involved a hot start of 4 min at 94°C, followed by 30 cycles consisting of 30 sec at 94°C, 30 sec at 56°C, 1 min at 72°C, and a final elongation step of 5 min at 72°C. The DNA fragments were amplified on Applied Biosystems® 2720 automated thermal cycler and purified using QIAquick® Gel Extraction Kit (QIAGEN, Germany). The purified products were then subjected to automated DNA sequencing on ABI3730xl DNA Analyzer (Applied Biosystems, USA) using primers identical with amplification for nrITS region. The newly generated sequence was edited manually using BioEdit sequence alignment editor version 7.0.9.0 (Tom Hall, Ibis Biosciences, Carlsbad, USA) and deposited in GenBank (www.ncbi.nlm.nih.gov) with accession number KT004442.

Sequence alignment and Phylogenetic analysis

The edited sequence was then used for BLAST searches in the GenBank database to determine the most closely related taxa for molecular identification. A dataset was generated from the highest scored hits most relevant for identification, as well as from samples previously used in the phylogenetic reconstruction of *Marasmius* (Wannathes et al. 2009b, Antonín et al. 2010). GenBank accession numbers for all of the acquired sequences has been indicated in Fig. 1.

Altogether thirty-seven sequences representing 25 species of *Marasmius* were aligned with ClustalX (Thompson et al. 1997) using default settings. Three species of *Crinipellis* (viz. *C. brunneipurpurea*, *C. malesiana*, and *C. dipterocarpi*) were selected for outgroup as rooting purposes following Wannathes et al. (2009b). The alignment was then imported into MEGA v. 6.0 (Tamura et al. 2013) for additional manual adjustments.

Bayesian phylogenetic analyses (BA) were carried out using MrBayes v. 3.2.2 (Ronquist et al. 2012). This program performs a Bayesian Inference (BI) of the phylogeny, using Metro-poliscoupled Markov chain Monte Carlo analyses (Geyer 1991). For a given data set, the General time reversible (GTR) model was employed with gamma-distributed substitution rates. Markov chains were run for 10^6 generations, saving a tree every 100th generation. Default settings in MrBayes were used for the incremental heating scheme for the chains (3 heated and 1 cold chain), unconstrained branch length (unconstrained: exponential (10.0)), and uninformative topology (uniform) priors. MrBayes was used to compute a 50% majority rule consensus of the remaining trees to obtain estimates of the posterior probabilities (PPs) of the groups. Bayesian posterior probabilities values over 0.50 are reported in the resulting trees (Fig. 1).

Results

Phylogenetic analyses

Based on the nrITS sequence obtained in this study and from GenBank, the phylogenetic relationships of the newly described species was inferred from MCMC analyses. ITS sequences were aligned and the ends trimmed to create a dataset of 751 base pairs. Bayesian analyses reached a standard deviation of split frequencies of 0.004 after 10^6 generations. The initial 25% trees recovered were excluded as the burn-in and the remaining trees obtained were then used to estimate the posterior probabilities of the group. The phylogenetic trees are shown in Fig. 1.

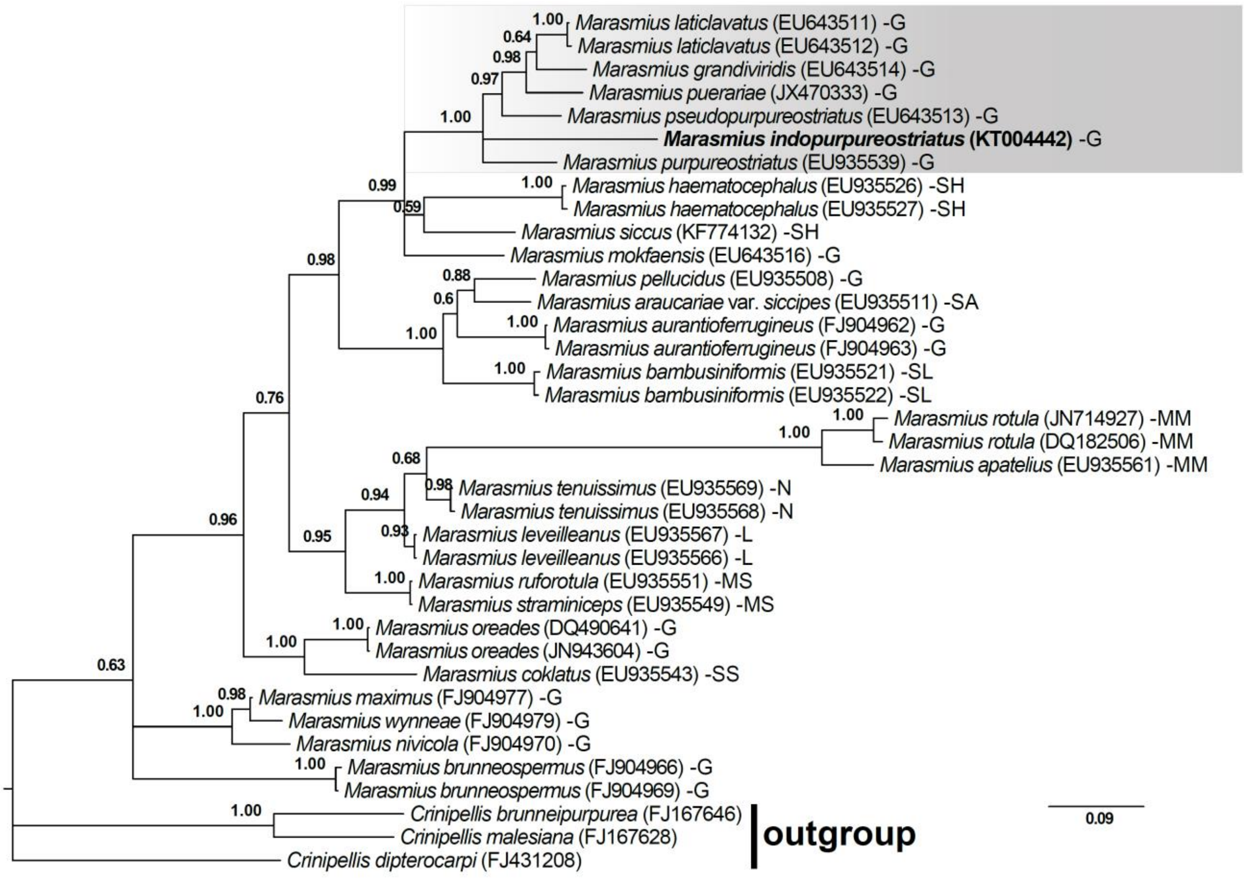

Fig. 1 – Bayesian phylogenetic analysis based on the ITS sequences for *Marasmius* species, showing mean branch lengths of a 50 % majority rule consensus tree, obtained from an MCMC analysis of one million generations. Numbers above the branches are Bayesian posterior probabilities (≥ 0.50). *Marasmius indopurpureostriatus* is placed in bold font to highlight its phylogenetic position in the tree. G – sect. *Globulares*; L – sect. *Leveilleani*; MM – sect. *Marasmius* subsect. *Marasmius*; MS – sect. *Marasmius* subsect. *Sicciformes*; N – sect. *Neosessiles*; SA – sect. *Sicci* ser. *Atrorubentes*; SH – sect. *Sicci* ser. *Haematocephali*; SL – sect. *Sicci* ser. *Leonini*; SS – sect. *Sicci* ser. *Spinulosi*.

Marasmius indopurpureostriatus K. Das, A.K. Dutta & K. Acharya, **sp. nov.** Fig. 2 A–J

MycoBank 812823

Etymology – refers to an Indian look-a-like of Japanese taxon *M. purpureostriatus*

Diagnosis – Differs from *Marasmius purpureostriatus* by its distinctly larger basidiomata, repeatedly bifurcate pattern of sulcation on pileus surface and slightly smaller basidiospores (22.3 × 3.9 µm) with a Q mean of 5.7.

Holotype – India, Sikkim, East district, Churten, 27°34′10.9″N 88°25′51.9″E, alt. 1454 m, 26 May 2014, K. Das, KD 14E-001 (CAL).

Pileus 16–110 mm diam., hemispherical, conic or campanulate when young with mostly involute margin, becoming planoconvex to plane with slightly depressed centre and uplifted margin, papilla or umbo absent; surface almost deeply sulcate or ridged in a regular and radial repeatedly bifurcate pattern towards margin; in furrows, violet brown (11F4) to dark ruby (12F3) or grayish Magenta (14D3) when young, gradually grayish ruby (12C3) to reddish grey (12B2) or paler with maturity, hygrophanous, finally discolored to brownish in heavy rain; alternatively in elevated ridges, yellowish white (3A2) when young, becoming paler to whitish with maturity; centre yellowish white (3A2). Lamellae narrowly adnexed to rounded-free, subdistant (5/10 mm) when young to distant (2/10 mm), non-intervenose, yellowish white with translucent smooth edges; lamellulae in 4 series. Stipe 75–200 × 2–6 mm, central, slender, terete, distinctly bulbous and strigose at base on surrounding thick whitish basal mycelium, up to $1/3^{rd}$ from the apex concolorous to pileus furrow i.e. grayish Magenta (14D3) when young, becoming paler up to whitish towards down the base, gradually becoming brownish orange (5C4–5) at entire stipe after maturity. Context 1.5 mm, insignificant, whitish in pileus, hollow in stipe concolorous to stipe cuticle. Odor strongly fungoid. Spore print pale yellowish white to yellowish white (2–3A2) when fresh, becoming up to pale yellow (4A3) when dry.

Basidiospores (20.8–)21.5–23.3(–24.5) × 3.6–3.9(–4.3) µm [X_m = 22.3 ± 1.5 × 3.9 ± 0.3 µm, Q = 5.3–6, Q_m = 5.7 ± 0.3, n = 50 spores, s = 5 basidiocarps], clavate to subfusoid, often curved in profile, hyaline, inamyloid, cyanophilic, thin- walled. Basidia 36–39(–43) × 9–10(–11) µm, clavate, hyaline, 4-spored; sterigmata 2.5–3.5(–5) × 1.5–2 µm diam. Basidioles 36–40 × 4–7(–11) µm, cylindrical to clavate. Pleurocystidia absent. Cheilocystidia 25–28(–33) × (9–)13–15(–17) µm, broadly clavate to pyriform, hyaline, inamyloid, thin-walled. Pileipellis a hymeniform layer of *Globulares*-type cells, (16–)17.5–19(–20) × 15–17(–18) µm, globose to subglobose, often clavate, hyaline, inamyloid, thin-walled. Pilear tramal hyphae 3.5–7 µm broad, loosely interwoven, cylindrical, smooth, hyaline, strongly dextrinoid, thin-walled, non-gelatinous. Hymenophoral tramal hyphae 6.5–7.5 µm broad, interwoven, cylindrical, smooth, hyaline, strongly dextrinoid, thin-walled, non-gelatinous. Stipitipellis hyphae 3.5–4.5 µm broad, parallel, cylindrical, smooth, inamyloid to weakly dextrinoid, thin- to thick-walled (up to 1 µm), non-gelatinous. Stipe tramal hyphae 6–7.5 µm broad, parallel, cylindrical, smooth, hyaline, dextrinoid, thin-walled, non-gelatinous. Caulocystidia absent. Clamp connections present in all tissues.

Habit and habitat–Uncommon; grows on thick bed of leaf-litter in subtropical broadleaf forests dominated by *Castanopsis*, *Betula*, *Symplocos* etc.

Known distribution –known only from India.

Additional Material examined – India, Sikkim, East district, Churten, 27°34′10.9″N 88°25′51.9″E, alt. 1454 m, 26 May 2014, K. Das, KD 14E-003 (CAL).

Notes –*Marasmius indopurpureostriatus* can easily be characterized by a small to large basidiomata; deeply sulcate pileus 16–110 mm diam.; variously coloured pileus surface i.e., violet brown to dark ruby or grayish Magenta with yellowish white disc and yellowish white to paler ridges; subdistant to distant, broad, yellowish white lamellae with 4 series of lamellulae; a large (75–200 × 2–6 mm) stipe, coloured grayish Magenta on the apex and paler to whitish at the base when young, becoming brownish orange overall at maturity, with a thick whitish basal mycelium; pale yellowish white to pale yellow spore print; clavate to subfusoid basidiospores with a mean 22.3 × 3.9 µm and mean Q = 5.7; broadly clavate to pyriform cheilocystidia.

Fig. 2 A–J – *Marasmiusindopurpureostriatus* (from Holotype KD 14E-001). **A** & **C**, Young and mature basidiomata in the field. **B**, Young basidiomata. **D,** Basidiomata discoloring in rain. **E,** Basidiomata on work-out table. **F,** Basidiole with clamped septum. **G,** Basidia. **H,** Cheilocystidia. **I,** Hymeniform pattern of pileipellis. **J,** Basidiospores. – Bars = F–G = 5 µm, H–J = 10 µm.

A provisional key to the species of *Marasmius* sect. *Globulares* in India

1. Basidiospores ≤ 10 µm long 2
1. Basidiospores > 10 µm long 5
2. Pileus smaller, stipe short (upto 2 cm long) 3
2. Pileus medium to large; stipe longer 4
3. Pileus 1–1.5 cm diam., greyish orange; basidiospores ellipsoid, 6.6–9.9 × 4.4–5.5 µm ………….. .... *M. heliomyces*
3. Pileus upto 1 cm diam., deep red with purple tone; basidiospores subglobose to ovoid, 3–4.6 × 2.3–3.8 µm .... *M. pegleri*
4. Pileus upto 5 cm diam., rust brown, often paler; stipe 2–7 cm long; basidiospores fusoid-ellipsoid, 7–10 × 4–6 µm .... *M. oreades*
4. Pileus upto 6.5 cm diam., yellowish white; stipe 3–9 cm long; basidiospores amygdaliform, 4.5–7 × 3–4 µm .... *M. amygdalosporus*
5. Basidiomes large (pileus up to 11 cm diam., stipe 7.5–20 cm × 2–6 mm); pileus surface violet brown to dark ruby or grayish magenta with yellowish white disc and yellowish white to paler ridges; basidiospores clavate to subfusoid, 21–24.5 × 3.8–4.3 µm .... *M. indopurpureostriatus*
5. Basidiomes small to medium; pileus surface differently coloured; basidiospores differently shaped 6
6. Pileus 1–5 cm diam., surface straw yellow to greyish yellow; stipe 4–8 cm long; basidiospores lanceolato-fusoid, 19–25 × 5–6.2 µm .... *M. caryotae*
6. Pileus up to 6 cm diam., surface yellowish green with yellow at the centre; stipe 15–18 cm long; basidiospores elongate, 18–25 × 3.5–4.5 µm .... *M. hookeri*

Discussion

Absence of collarium and presence of a hymeniform layer of clavate to pyriform, non-setulose cells in the pileipellis undoubtedly place the described species under the *Marasmius* sect. *Globulares*. Among species with a similarly coloured pileus, *Marasmius purpureostriatus* Hongo, described from Japan, differs by having a much smaller pileus (13–20 mm diam.) and stipe (52–103 × 0.5–1.5 mm) and larger basidiospores (19–30 × 4–7 µm) with a Q value of 4.7 (Wannathes et al. 2009b). *Marasmius pseudopurpureostriatus* Wannathes, Desjardin & Lumyong, described from Thailand, differs from the newly described species by the presence of none to one series of lamellulae, a relatively smaller stipe (62–80 × 1.5–3 mm) and slightly broader basidiospores (20–25 × 5–6.2 µm) with a Q value of 4.1 (Wannathes et al. 2009a). The African species *M. violaceoides* Antonín has a pale violaceous striae on the pileus, whitish to pale greyish orange lamellae and shorter cheilocystidia, 14–24 × 4.2–9.2 µm (Antonín 2004).

The presently described species seems to be the largest known *Marasmius* from India. Throughout the world, among species with large basidiocarp and absence of pleurocystidia and caulocystidia: *M. grandiviridis* Wannathes, Desjardin & Lumyong differs by the presence of a pileus, coloured yellowish green with olive green plicae, one series of lamellulae and larger basidiospores, 23–30 × 4–5 µm with a mean Q value of 6.1; *M. mokfaensis* Wannathes, Desjardin & Lumyong has greyish white to brownish grey coloured lamellae with none to one series of lamellulae, faintly raphanoid to rancid odour and larger basidiospores, 27–33 × 5–6 µm (Wannathes et al. 2009a); African species *M. zenkeri* Henn. has a pale lilac lamellae, a dark reddish brown or chestnut brown stipe, and larger cheilocystidia, 14–50 × 10–15 µm (Antonín 2007); *M. brunneolus* (Beeli) Singer differs by having slightly larger basidiospores (15.5–25.5 × 3.8–5.4) with a Q value of 4.4–4.6 (Singer 1964); *M. bekolacongoli* Beeli, originally described from Congo and subsequently reported to occur in the broad leaf forest of China, differs by the combination of characters like greenish grey coloured, adnexed with faintly interveined lamellae and slightly different size (17–28 × 3.8–4.8 µm), lanceolate basidiospores (Zhishu et al. 1993); and *M. sulcatipes* Murrill is different in having gray pileus and 28–35 µm long basidiospores (Antonín & Buyck 2006).

Among other species belonging to the sect. *Globulares* with striate pileus, *M. viridis* Desjardin & E. Horak, described from Papua New Guinea, differs by forming smaller basidiomes (pileus 10–15 mm diam., stipe 30–50 × 1 mm), an olivaceous pileus with more lamellae (12–16) and cheilocystidia that are more regularly clavate (Desjardin & Horak 1997). *M. musisporus* Desjardin & E. Horak, also described from Papua New Guinea, differs by having a pileus coloured purplish lilac with yellow striae, a reddish brown stipe and distinctly larger basidiospores (30–40 × 4.5–5 µm). *M. ditopotramus*, described from Bolivia, has smaller basidiospores, 7–9 × 4.5–4.8 (Singer 1976). *M. latepileatus* Antonín & C. Sharp, described from Zimbabwe, has distinctly larger basidiospores 23–30 × 5–7.5 µm and numerous 37–60 × 9–15 µm, fusoid to clavate pleurocystidia (Antonín 2003). *Marasmius mbalmayoensis* Douanla-Meli differs by having well developed pleurocystidia (40–65 × 8–11 µm) and differently shaped, slightly broader cheilocystidia, 25–41 × 5.5–15 µm (Douanla-Meli & Langer 2008). *M. pellucidus* Berk. & Broome has a dull coloured pileus with ivory, cream to pale orange white disc, overall pruinose stipe; distinctly smaller, subfusoid to ellipsoid or amygdaliform basidiospores (6–8.5 × 2.5–4 µm) with a mean Q value of 2.2; and irregularly cylindrical to fusoid or ventricose caulocystidia, 15–85 × 4–14 µm (Wannathes et al. 2004).

The circumscription of the new species, *Marasmius indopurpureostriatus*, based on morphological characteristics is concordant with those suggested by ITS sequence similarity. In the ITS dataset represented in Fig. 1, *M. indopurpureostriatus*, form a strongly supported clade (1.00 PP) together with the taxa like *M. purpureostriatus*, *M. pseudopurpureostriatus*, *M. puerariae* R. Kirschner, *M. grandiviridis* and *M. laticlavatus* Wannathes, Desjardin & Lumyong, all of which belongs to the sect. *Globulares*. The phylogenetic analysis (Fig. 1) easily differentiates *M. indopurpureostriatus* from morphologically similar species (viz. *M. grandiviridis*, *M. mokfaensis*, *M. pellucidus*, *M. pseudopurpureostriatus* and *M. purpureostriatus*).

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