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SHORT COMMUNICATION

FIRST RECORD OF FUNGUS *CRYPTOMARASMIUS* T.S. JENKINSON & DESJARDIN (PHYSALACRIACEAE: AGARICALES: BASIDIOMYCOTA) FROM INDIA

Arun Kumar Dutta & Krishnendu Acharya

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FIRST RECORD OF FUNGUS *CRYPTOMARASMIUS* T.S. JENKINSON & DESJARDIN (PHYSALACRIACEAE: AGARICALES: BASIDIOMYCOTA) FROM INDIA

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Abstract: The saprophytic fungus genus *Cryptomarasmius* has 28 reported species worldwide. We report here the first record of *Cryptomarasmius exustoides* in India, extending the distribution of *Cryptomarasmius*. A detailed taxonomic description with illustrations, and phylogenetic analysis with related species are provided.

Keywords: Fungus, Internal transcribed spacer sequence, new record, nuclear ribosomal large subunit, phylogeny, taxonomy.

The genus *Cryptomarasmius* (Agaricales, Basidiomycota) was proposed by Thomas S. Jenkinson & Dennis E. Desjardin (2014) by treating *Marasmius hygrometricus* (V. Brig.) Sacc. as the type species. Historically, all species belonging to this genus were classified as *Marasmius* under section *Hygrometrici* (Kühner), having a combination of marasmioid stature, a smaller pileus that is well-pigmented and broom cells mostly of the *Rotalis*-type or often in a combination with smooth cells, free to adnate attachment of lamellae, absence of collarium, a central and insititious stipe, usually presence of pleuro- and cheilocystidia, neither amyloid or dextrinoid nature of the pileus, lamellae or stipe trama, and medium-sized basidiospores (Singer 1976).

Phylogenetic approach based on molecular sequence data (nrDNA ITS) including the member of the sect. *Hygrometrici* was previously attempted

by Tan et al. (2009) where four included sequences of *Cryptomarasmius micraster* (Petch) T.S. Jenkinson & Desjardin (then as *Marasmius micraster* Petch) clustered together within the clade containing species belonging to the gen. *Marasmius* sects. *Marasmius* and *Sicciformes* with no statistical support. Later, in the phylogenetic analysis performed by Jenkinson et al. (2014), four previously known member of the family *Hygrometrici* (viz. *Marasmius corbariensis* (Roum.) Sacc., *M. exustoides* Desjardin & E. Horak, *M. micraster* and *M. thwaitesii* Berk. & Broome) formed a clade together with the well-known representatives of the family *Physalacriaceae* Corner with low statistical support (BPP 0.81, BS 61%). In the said phylogenetic analysis (Jenkinson et al. 2014), the four taxa were found to form a sister lineage within a clade containing other genera like *Cylindrobasidium* Jülich, *Physalacria* Peck, and *Rhodotus* Maire with moderate to weak statistical support (BPP 0.76, BS <50%). From the overall study (Jenkinson et al. 2014), based on the nuclear ribosomal large subunit sequence data (LSU), insisted Jenkinson and his group to transfer the previously accepted members of the *Marasmius* sect. *Hygrometrici* (family *Marasmiaceae*) to a new genus *Cryptomarasmius* within the family *Physalacriaceae*.

Reports on the taxa belonging to the gen.

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Cryptomarasmius are found in the literature under the name *Marasmius* (sect. *Hygrometrici*). Singer (1976) reported five species of *Cryptomarasmius* in his monographic study 'Marasmiaceae (Basidiomycetes - Tricholomataceae)' based on the collections made from several regions (viz. Argentina, Bolivia, Ecuador, Florida, Hawaii, Mexico, and Venezuela) accompanying with variable climatic conditions. Petrini et al. (1997) reported quite a number of *Cryptomarasmius* species from Papua New Guinea, New Caledonia, and New Zealand in the name of *Marasmius* under sect. *Hygrometrici*. Antonín et al. (2012) reported two species from Republic of Korea, among which *Marasmius crescentiae* (presently *Cryptomarasmius crescentiae*) was a new record from the region and *Marasmius junipericola* (presently *Cryptomarasmius junipericola*) represented a newly described species. Among the southern African region, Pegler (1982) newly described a taxon (*Marasmius parviconicus* Pegler) belonging to the sect. *Hygrometrici* based on his collection from Copperbelt Province of Zambia.

Considerably little works had been carried out on this group from south Asian regions. Pegler (1986) reported two species (*M. micraster* and *M. thwaitesii*) from Sri Lanka. Other than that, the presence of the fungi belonging to the group is scarce in literatures. The present paper deals with the new record of *Cryptomarasmius exustoides* from India. This paper represents the first distributional record of the genus in the Indian context.

The specimen under consideration was collected during July 2014 from Kolkata, West Bengal, India. The material was photographed in the field using a digital camera and extensive notes on the basidiomata were done before drying. Colour terms follow Kornerup & Wanscher (1978). Microscopic features were obtained from free-hand sections of the dried basidiocarp tissues in 5% KOH, Melzer's reagent and Congo Red. Basidiospores size are provided as a mean value (underlined); values in parentheses indicate minimum or maximum measured values. Q value denotes length/width ratio of the basidiospores. The voucher specimen has been deposited in the Calcutta University Herbarium (CUH) with the accession number CUH AM127.

The extraction of genomic DNA as well as PCR protocol for the amplification of nrDNA regions (nrITS and nrLSU) follows Dutta et al. (2015). PCR products were purified using QIAquick® Gel Extraction Kit (QIAGEN, Germany) and used for automated DNA sequencing on ABI3730xl DNA Analyzer (Applied Biosystems, USA) using primers identical with amplification for nrDNA

regions (Vilgalys & Hester 1990; Gardes & Bruns 1993). The generated sequences were edited manually using BioEdit sequence alignment editor version 7.0.9.0 (Tom Hall, Ibis Biosciences, Carlsbad, USA).

The newly generated ITS and LSU sequences were 732 bp and 588 bp respectively. Both the sequences were then used for BLAST searches in the GenBank nucleotide database. The newly generated sequences and those retrieved from GenBank based on a BLAST search (Table 1) were chosen for conducting phylogenetic analysis. Representative taxa [viz. *Marasmius rotula* (Scop.) Fr. and *M. rotalis* Berk. & Broome] of the family *Marasmiaceae*, clearly outside the family *Physalacriaceae* (fide Jenkinson et al. 2014), were used for rooting purposes.

Datasets consisting of 23 sequences for each gene (ITS and nrLSU) were aligned using MUSCLE (Edgar 2004), with additional manual adjustments to the alignment performed in MEGA v.7.0 (Kumar et al. 2016). The ends of the data sets were trimmed to 808 bp (ITS) and 558 bp (nrLSU) respectively. Finally, a combined dataset consisting of the two regions (ITS + nrLSU) was created in MEGA v.7.0 (Kumar et al. 2016). The appropriate model of sequence evolution for phylogenetic analysis (GTR+I+G with BIC of 18916.726890) was determined using jModeltest 2.1.6 v20140903 (Darriba et al. 2012) in the CIPRES web portal (Miller et al. 2009). Maximum likelihood (ML) analysis was performed with RAxML-8.2.9 (Stamatakis 2014) on the CIPRES NSF XSEDE resource. Bayesian phylogenetic analysis was done in MrBayes v. 3.2.1 (Ronquist et al. 2012) by employing general time reversible (GTR) model with gamma-distributed substitution rates. Markov chains were run for 10⁵ generations, saving a tree every 100th generation.

Cryptomarasmius exustoides

(Desjardin & E. Horak) T.S. Jenkinson & Desjardin

Mycologia 106(1): 92 (2014)

(Image 1; Fig. 1)

Specimen examined: AKD 209/2014 (CUH AM127), 22.vii.2014, India: West Bengal, Kolkata, botanical garden of Ballygunge Science college campus, 22.61916667°N & 88.48555556°E, 14m elevation, coll. A.K. Dutta & S. Paloi.

Pileus 1–4 mm diam, convex, often with a small central depression, nonpapillate, plicate, surface dry, glabrous, initially light brown (6D5) to brown (6D7–8) overall, turns yellowish-white (1-2A2) to yellowish grey (2-3B2) or pale orange (5A3) to light orange (5A4) towards margin with light brown (6D6) to brown (6D7–8, 7F4–5) center due to heavy rain, hygrophanous. Context very thin, cream. Lamellae ≤1 mm broad, non-collariate,

Table 1. Fungal species and GenBank accession number for the sequences used in the phylogenetic analysis. Bold font represents the newly sequenced specimen.

Name of taxa	Voucher no.	GenBank accession no.		Country
		ITS	LSU	
<i>Cryptomarasmius exustoides</i>	CUH AM127	MF189078	MF495890	India
<i>Cryptomarasmius exustoides</i>	DED6249	JN601434	JN585129	USA: Hawaii
<i>Cryptomarasmius micraster</i>	DED7647	FJ431261	JN585131	Malaysia
<i>Cryptomarasmius micraster</i>	PDD:95297	KM975400	KM975386	New Zealand
<i>Cryptomarasmius thwaitesii</i>	DED5918	JN601437	JN585132	USA: Hawaii
<i>Cryptomarasmius corbariensis</i>	Ngyuen111209	JN601433	JN585128	USA: California
<i>Cryptomarasmius crescentiae</i>	LE295992	KF774149	KF896252	Russia
<i>Cryptomarasmius crescentiae</i>	LE295993	KF774147	KF896251	Russia
<i>Cryptomarasmius aukubae</i>	–	AB512314	AB512377	Japan:Okinawa
<i>Physalacriacryptomeriae</i>	NY: Clark T Rogerson	KT201655	KT201639	USA: New York
<i>Physalacriasinensis</i>	HKAS:77294	KT201643	KT201638	China: Kunming
<i>Physalacriabambusae</i>	CBS712.83	DQ097367	DQ097349	Japan
<i>Physalacriamaipoensis</i>	2373Inderbitzin	DQ097368	AF426959	Thailand
<i>Flammulinayunnanensis</i>	HKAS 32774	DQ486704	DQ457667	China
<i>Flammulinavelutipes</i>	TENN 52002	AY854073	NG_027630	USA
<i>Rhizomarasmius pyrrocephalus</i>	TENN51091	DQ097369	DQ097351	USA
<i>Rhizomarasmius oreinus</i>	BRNM 751553	KM588669	KM588689	Italy
<i>Rhizomarasmius oreinus</i>	BRNM 751554	KM588670	KM588690	Italy
<i>Omphalotusolearius</i>	CBS 141.34	–	AF042010	Germany
<i>Omphalotusolearius</i>	CBS33285	AF525061	–	Austria
<i>Omphalotusnidiformis</i>	CBS 323.49	EU424307	EU365662	China
<i>Omphalotusilludens</i>	BR1830367	AF525047	–	–

adnexed, distant (6–7) with 0-1 series of lamellulae, white, concolorous, even. Stipe 5–14 mm long, thin (<0.5mm), central, cylindrical, wiry, equal, surface brown (7F5) to dark brown (7F6), smooth, insititious. Rhizomorphs absent. Odour and taste not distinctive.

Basidiospores (9–)10–10.2–10.5(–11) × 4–5–5.3(–6) µm, Q=1.6–1.9–2.3 µm, ellipsoid to obovoid, slightly curved in profile, smooth, hyaline, inamyloid, thin-walled. Basidia 20–22(–25) × 7–7.5(–9) µm, clavate, hyaline, thin-walled, 4-spored; sterigmata 1.5–2.5(–3.5) µm long, cylindrical. Lamellae edge sterile, with crowded cystidia. Cheilocystidia common, dimorphic: a) *Rotalis*-type broom cells with mainbody 12–13.5(–15) × 4.5–5.5(–7) µm, clavate, hyaline, thin-walled; apical setulae 0.5–2 µm long, cylindrical, pale yellow to light brownish with KOH, obtuse, thick-walled, b) fusoid, measuring 24–28(–35) × 3.5–5(–7) µm, with capitate to sub-capitate apex, hyaline, thin-walled. Pleurocystidia absent. Pileipellis a hymeniform layer, composed of *Rotalis*-type of broom cells; main-body (10–)14–17(–18) × (7–)8.5–10(–11.5) µm, cylindrical to clavate, hyaline

to pale yellow, thin- to thick-walled; apical setulae (0.7–)1–1.5(–2) µm long, cylindrical, pale yellow to light brownish with KOH, thick-walled. Pileosclerocystidia 31–37 × 5–6.5(–7.5) µm, clavate, resinous incrustations present, pale yellow to light brown. Pileus trama hyphae 3–5 µm broad, interwoven, cylindrical, hyaline, inamyloid, wall up to 0.5 µm thick. Lamellae trama hyphae 3.5–4.5 µm broad, interwoven, cylindrical, hyaline, inamyloid, thin-walled. Stipitipellis hyphae 2.5–3.5 µm broad, parallel to subparallel, cylindrical, hyaline, inamyloid, thin-walled. Stipe trama hyphae 3–4(–5.5) µm broad, more or less parallel, cylindrical, hyaline, inamyloid, smooth, thin-walled. Caulocystidia absent. Clamp-connections present in all the tissues.

Habit and habitat: Solitary or in clusters on rotting bark or wood sticks.

Remarks: The characteristic features of *Cryptomarasmius exustoides* includes a small, plicate pileus coloured light brown to brown at disc with yellowish-white to pale orange or light orange margin; distant (6–7), non-collariate lamellae; an insititious stipe



Figure 1. Micro-morphological features of *Cryptomarasmium exustoides*.

a - basidiospores; b - basidium; c - cheilocystidia; d - *Rotalis*-type cells of the pileipellis; e - pileosclerocystidia (scale = 5µm).

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coloured brown to dark brown; absence of rhizomorphs; ellipsoid basidiospores measuring $9\text{--}11 \times 4\text{--}6 \mu\text{m}$; presence of dimorphic cheilocystidia i.e. fusoid with capitate to sub-capitate apex and combination with *Rotalis*-type cells; clavate pileosclerocystidia measuring $31\text{--}37 \times 5\text{--}7.5 \mu\text{m}$; and absence of pleurocystidia and caulocystidia. The features of our Indian collection match nicely with that of the type specimen description, except having a bit smaller length of the stipes (5–14 mm vs 10–25 mm; Desjardin & Horak 1997). Previously there were reports on the occurrences of the species from New Zealand (Desjardin & Horak 1997) and Hawaii (USA). The present work constitutes the first report of this taxon from India.

Among phenotypically similar taxa: *Cryptomarasmium crescentiae* (Murrill) T.S. Jenkinson & Desjardin, originally described from Cuba and later subsequently reported from South America and Papua New Guinea,

have ochraceous to ochraceous brown pileus with deep ochraceous brown center, presence of more number of lamellae (7–10), and much smaller ($6.5 \times 2.7 \mu\text{m}$), oblong basidiospores (Singer 1976). *Marasmius exustus* Berk. & M.A. Curtis, originally described from the Bonin Islands, primarily differs in having much smaller basidiospores ($6.5\text{--}8.0 \times 4\text{--}5 \mu\text{m}$), and absence of pileosclerocystidia on the pileus surface (Berkeley & Curtis 1868). *Cryptomarasmium minutus* (Peck) T.S. Jenkinson & Desjardin differs in having considerably smaller basidiospores ($6.0\text{--}9.5 \times 3\text{--}4 \mu\text{m}$) and presence of abundant fusiform to lageniform pleurocystidia and pileoleptocystidia.

Among phylogenetically related most close taxa (Fig. 2), *Cryptomarasmium micraster* (Petch) T.S. Jenkinson & Desjardin, described for the first time from Sri Lanka (Petch 1948) and later reported from Malaya and New Zealand, has rusty brown to pale soot brown pileus with darker center, subdistant (9–12), discolourous lamellae with pale rusty brown to pale brown edges, much longer stipes (up to 75mm), and presence of considerably larger *Rotalis*-type cheilocystidia ($12\text{--}30 \times 8\text{--}15 \mu\text{m}$; Desjardin & Horak 1997).

Phylogenetic analyses were performed on combined dataset (ITS plus nrLSU) of 23 sequences representing six genera viz. *Cryptomarasmium* (nine sequences), *Rhizomarasmium* (three sequences), *Flammulina* (two sequences), *Physalacria* (four sequences), *Omphalotus* (three sequences) and *Marasmius* (two sequences) of which taxa belonging to the gen. *Marasmius* was used as an outgroup for rooting purposes. The ML analysis resulted the phylogenetic tree ($-\ln L = 9264.444470$) that did not differ significantly in topology from the tree obtained from Bayesian analyses. Bayesian analyses reached a standard deviation of split frequencies of 0.005 after 1,000,00 generations, and the initial 25% trees recovered were excluded as the burn-in. Maximum likelihood bootstrap values (BS) and Bayesian posterior probabilities (PP) support the deeper nodes as well as the terminal nodes with moderate to strong support (Fig. 2).

The phylogenetic analyses clustered all of the members belonging to the family Physalacriaceae within a strongly supported clade (100% BS, 1.00 PP) where nine sequences representing six species of the genus *Cryptomarasmium* was found to monophyletic with strong support values (97% BS, 1.00 PP). In the resulted phylogenetic analysis, the acquired sequence of *Cryptomarasmium aukubae* was found to cluster with the deposited two Russian sequences of *Cryptomarasmium crescentiae* with full support values (100% BS, 1.00



Image 1. *Cryptomarasmium exustoides*. a - Natural habitat of the collection place; b - Fresh basidiomes growing in cluster on rotting bark; c - Basidiomes growing on dead wood sticks; d - Basidiomes showing cap surface and lamellae side (scale = 5mm). © A.K. Dutta

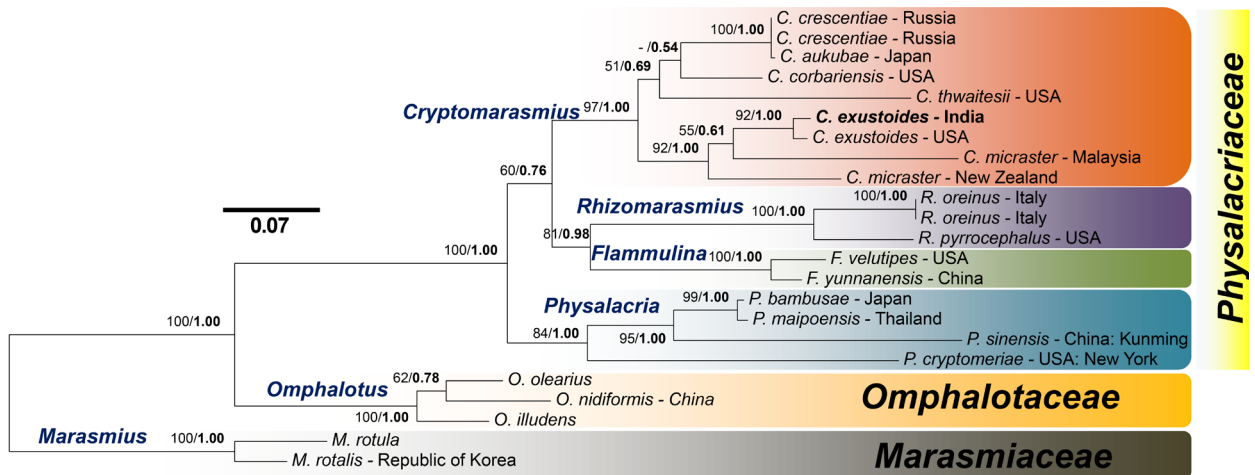


Figure 2. Maximum likelihood (ML) tree generated using combined datasets of nrITS and nrLSU sequences by employing GTR+I+G model of nucleotide evolution ($-lnL = 9264.444470$). Numbers to the left of / are ML bootstrap support, and those to the right indicate the Bayesian posterior probabilities (PP). ML >50% and PP > 0.50 values are indicated below or above the branches and the scale bar represents the expected changes per site. The Indian collection of *Cryptomarasmium exustoides* is placed in bold font to highlight its phylogenetic position in the tree.

PP) that could be attributed to the misidentification for the deposited sequence of *Cryptomarasmium aukubae* in GenBank database. The Indian collection of

Cryptomarasmium exustoides cluster with the sequence of the same taxon earlier reported from USA with significant support values (92% BS, 1.00 PP).

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Breeding behaviour of the Coromandel Marsh Dart Damselfly (Zygoptera: Coenagrionidae: *Ceriagrion coromandelianum* (Fabricius)) in central India

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Short Communications

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First record of fungus *Cryptomarasmius* T.S. Jenkinson & Desjardin (Physalacriaceae: Agaricales: Basidiomycota) from India

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-- A. Sabeena, V.B. Hosagoudar & V. Divaharan, Pp. 11470–11479

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-- Licha Jeri, Nazir Ahmad Bhat & Yogendra Kumar, Pp. 11480–11483

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-- Sampa Ghosh & Debjyoti Bhattacharyya, Pp. 11484–11492

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A preliminary but incomplete checklist of Gujarat spiders

-- R.V. Vyas & B.M. Parasharya, Pp. 11493–11494

Miscellaneous

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