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New and noteworthy crepidotoid agarics from India

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Abstract - Two remarkable crepidotoid agarics were collected many times from Kerala State, India. Detailed morphological and molecular analysis indicated that one is an undescribed species of *Crepidotus* while the other represents *Simocybe amara* which represents a new Asian record. Complete morphological descriptions, photographs and comparisons with phenetically similar species are provided.

***Crepidotus* / Crepidotaceae / *Simocybe* / Kerala / phylogeny / taxonomy**

INTRODUCTION

The genus *Crepidotus* (Fr.) Staude is cosmopolitan in distribution and can be keyed out from all genera of the Crepidotaceae by the combination of the following features: its lignicolous habit, pleurotoid basidiomata with a reduced stipe, pale brown to yellowish spore print, smooth or ornamented spores always lacking a germ pore, cheilocystidia always present but normally without pleurocystidia, and hyphae with or without clamps (Singer 1986).

The genus *Simocybe* Karst. is distributed from the temperate zones to the tropics and differs from *Crepidotus* by its mycenoid, collybioid basidiomata, usually with a distinct stipe, smooth basidiospores and the presence of abundant cheilo-, pileo-, and caulocystidia.

During our continuing study on crepidotoid agarics of Kerala State, India, we came across two remarkable species, *Crepidotus indicus* sp. nov. and *Simocybe amara* (Murr.) Sing. Both species are described here based on both morphology and molecular phylogeny. The genus *Simocybe* was so far known from India from a single species, *S. descendens* (Berk.) Manjula from Sikkim (Manjula 1983).

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MATERIALS AND METHODS

Morphological studies

Gross morphological descriptions are based exclusively on fresh materials collected from Kerala State, India. Microscopic characters were studied on dried material using hand cut sections of basidiomata revived in 3% solution of KOH, stained with 1% Congo red and examined under a Leica DME1000 compound microscope. The mean quotient (Q) of spore length divided by spore width was calculated from measurements of 20 basidiospores. Methods used in the examination of microscopic features are those of Largent *et al.* (1977). Colour notations refer to Kornerup & Wanscher (1978). Descriptive terms follow Vellinga (1988). Holotype is deposited at Central National Herbarium (CAL), Kolkata, India and CAL accession numbers are provided. All isotypes and additional materials examined are deposited at the Mycological Herbarium of Jawaharlal Nehru Tropical Botanic Garden and Research Institute, Trivandrum (TBGT).

DNA extraction, PCR amplification and sequencing

Genomic DNA was extracted from fresh specimens of the two species employing the procedure described by Izumitsu *et al.* (2012). The nuclear ribosomal DNA large subunit (nrLSU) region was analyzed for the newly described species. PCR reactions were performed with the primer pair LROR and LR7 for nrLSU (Vilgalys & Hester 1990). The protocols for PCR amplification and sequencing followed Latha *et al.* (2015). The newly generated sequences were deposited in GenBank.

Sequence alignment and phylogenetic analysis

The molecular phylogenetic analysis was performed using nrLSU sequences. The nrLSU sequences of the newly discovered species (*Crepidotus indicus*, 893bp) and (*Simocybe amara*, 892 bp) along with those retrieved from GenBank (Table 1) were aligned using MAFFT web tool (www.ebi.ac.uk/Tools/msa/mafft/) with default settings. The final aligned data matrix of nrLSU sequences from 31 taxa, including two species of *Inocybe* as outgroup, was then imported into BioEdit v7.2.6.1 (Hall 1999) for manual adjustment. *Inocybe phaeoleuca* and *Inocybe subpaleacea* were selected as outgroup following Ge *et al.* (2017). Ambiguously aligned sites were excluded using the web tool Gblocks (http://www.phylogeny.fr/one_task.cgi?task_type=Gblocks). Maximum likelihood (ML) and Bayesian inference (BI) analyses were carried out. For ML & BI analysis the GTR+G+I molecular evolution model was selected as the best fit model within the TOPALi v2.5 (Milne *et al.* 2004) platform. ML analysis was performed in the web platform http://iqtree.cibiv.univie.ac.at (Trifinopoulos *et al.* 2016) with 1000 bootstrap replicates. Bayesian inference (BI) analyses were carried out in TOPALi v2.5 (Milne *et al.* 2004). Four incrementally heated simultaneous Monte Carlo Markov chains (MCMC) were run for 10^6 generations, saving a tree every 100^{th} generation. The first 25% of trees were discarded as "burn-in". ML Bootstrap values ≥70% and Bayesian Posterior probability values above 0.90 were considered significant. The aligned sequence dataset has been deposited in TreeBase (http://purl.org/phylo/treebase/phylows/study/

TB2:S22083). Topology of Maximum likelihood and Bayesian phylotrees is congruent. The phylogram inferred from ML analysis (Fig.5) is displayed with MEGA 7.0.26 (Kumar *et al.* 2016).

Table 1. The rDNA nrLSU sequences used in this study

Species	*Geographic origin*	*GenBank no.*
Crepidotus amygdalosporus	USA	AF205678
Crepidotus aureus	USA	AF205685
Crepidotus cesatii	USA	AF205681
Crepidotus cf. subsphaerosporus	West Australia	AF367947
Crepidotus croceitinctus	Russia	AF367937
Crepidotus croceitinctus	Japan	AF367932
Crepidotus crocophyllus	USA	GQ893025
Crepidotus distortus	USA	AF205671
Crepidotus indicus	**India**	**MG735357**
Crepidotus martinii	Japan	AF367944
Crepidotus nephrodes	USA	AF205693
Crepidotus sinuosus	USA	AF367945
Crepidotus sp. MCA 499	USA	AF367951
Crepidotus sphaerosporus	USA	AF205682
Crepidotus variabilis	Japan	AF367949
Inocybe phaeoleuca	Hungary	KJ399958
Inocybe subpaleacea	Finland	KJ849311
Simocybe amara	**India**	**MG719983**
Simocybe amara	Japan	AF205708
Simocybe americana	Canada	AF205709
Simocybe cetunculus	USA	AF205707
Simocybe cf. serrulata	USA	GQ892980
Simocybe haustellaris	Italy	KT715791
Simocybe haustellaris	Spain	KT715793
Simocybe rhabarbarina	Italy	KT934416
Simocybe rhabarbarina	Italy	KT934414
Simocybe rhabarbarina	Italy	KT934415
Simocybe serrulata	USA	AY745706
Simocybe sp.	USA	AF205687
Simocybe sp.	USA	AF205706
Simocybe sp.	USA	GQ892979

RESULTS

Taxonomy

Crepidotus indicus A.M. Kumar & C.K. Pradeep sp.nov. **Figs 1- 2**

MycoBank: MB823917

GenBank: MG735357 (nrLSU).

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

Diagnosis: Distinguished from similar *Crepidotus* species by small to large basidiomata; white becoming grayish orange, downy wooly to floccose, hygrophanous pileus; large globose verrucose spores, flexuous-contorted, whip-like cheilocystidia; pileipellis a trichoderm with dermatocystidia and distinctive nrLSU sequence.

Holotype: INDIA. Kerala State, Thiruvananthapuram District, Palode, Plavara, 19 October 2017, ManojTBGT17161 (CAL 1647!)

Basidiomata small to large, soft, slightly fleshy to thin. *Pileus* attached dorsally at the rear end to the substratum, 6–70 mm diam., convex, dimidate, orbicular, reniform to flabelliform; surface white, off white, orange white (2A2/3A2/4A2/5A2/6A2), pale orange to grayish orange (4B2/5A3/5B3/5B4), downy wooly, floccose, denser near the base (attachment), pellucid striate, hygrophanous, moist when fresh, soon dry; margin incurved when young becoming straight, entire, rarely incised, fringed with agglutinated wooly hairs. *Lamellae* radiating from a lateral point from a rudimentary to reduced stipe, adnexed, off white to white, brownish orange to orange white (2A2/3A2/4A2/4B2/5B2/5C3/6A2), up to 5 mm wide, ventricose, close to crowded with lamellulae of 2-4 lengths; edge concolorous to the sides, entire. *Stipe* absent, rudimentary or reduced, with white floccose hairs. *Context* thin, off white, soft, up to 1 mm wide. White pubescent hairs at the base (attachment). *Odor* not distinctive. *Spore print* clay to brown (5D5/6E5).

Basidiospores (6.4–) 7–9 × (6.4–) 7–9 µm, Q=1.0µm, avL=8 µm, avW=8 µm, globose, light yellow to light brown in KOH, thick-walled, finely verrucose. *Basidia* 24–27 × 8–10.5 µm, clavate, mostly 2-spored, rarely 1, 4-spored, thin-walled, hyaline. Lamella edge sterile with crowded cheilocystidia. *Cheilocystidia* 67–92 × 5.6–6.4 µm, variable, flexuous-contorted, slender, rarely forked, whip-like, rarely 1, 2 septate, thin-walled, hyaline. *Pleurocystidia* absent. *Hymenophoral trama* regular, hyphae 4–5 µm wide, thin-walled, hyaline. *Subhymenium* pseudoparenchymatous. *Pileal trama* composed of thin-walled, hyaline hyphae, 5.6–8 µm wide. *Pileipellis* a trichoderm with erect to suberect, long, multiseptate, flexuous, whip-like elements, 91–156 × 7.8–9.1 µm, thick-walled, with yellowish brown contents. Intermingled with these trichodermial elements, some short thick-walled distinctly colored, multiseptate elements present, 20–44 × 4.8–6.4 µm, cylindric, narrowly conical or ventricose, incrusted. Pileal hyphae incrusted. *Clamp connections* present in all tissues. *Oleiferous hyphae* absent.

Habit, habitat and phenology: Scattered or in groups on dead decaying coconut palm spathe, dead Rubber wood (*Hevea brasiliensis*), dead stumps and logs of dicotyledonous trees. April-June, August- December.

Additional specimens examined: INDIA. Kerala State, Thiruvananthapuram District, Palode, Plavara: 16 Nov. 2014, Manoj TBGT15509; 02 Dec. 2014, Manoj TBGT15542; 28 April 2015, Manoj TBGT 15574; 12 May 2015, Manoj TBGT15632; 13 May 2015, Manoj TBGT15640; 18 Nov. 2015, Manoj TBGT15950; 28 June

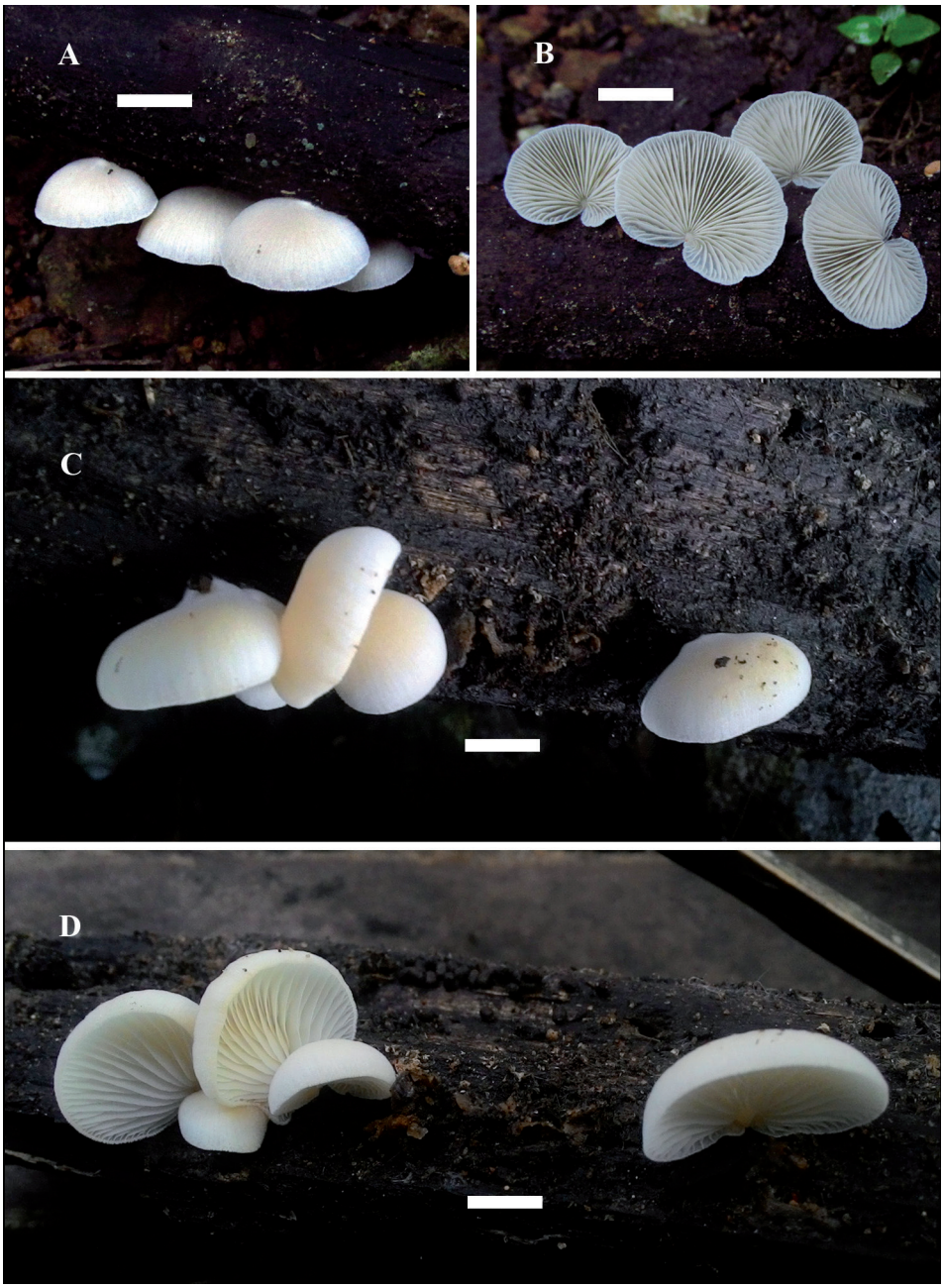

Fig 1. *Crepidotus indicus* (CAL 1647, Holotype). **A-D**. Habit *in situ.* Scale bar = 10 mm

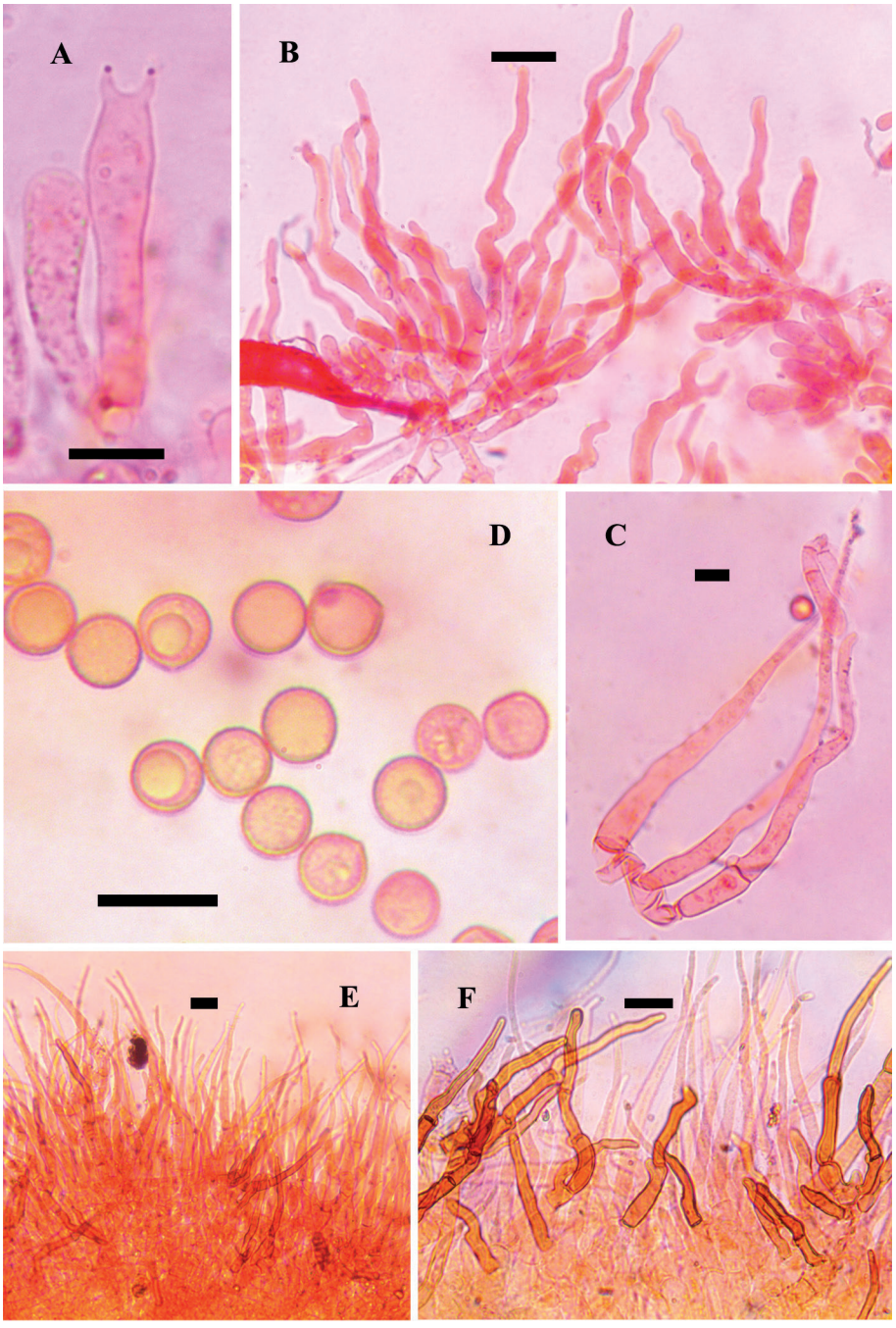

Fig 2. *Crepidotus indicus* (CAL1647). **A**. Basidium; **B-C**. Cheilocystidia; **D**. Basidiospores; **E**. Trichodermial pileipellis. **F**. Pileipellis with thick-walled elements. Scale bars = 10 µm.

2017, Manoj TBGT16856; 08 Aug. 2017, Manoj TBGT17020; 09 Sept. 2017, Manoj TBGT17074; 27 Oct. 2017, Manoj TBGT17196; 01 Nov. 2017, Manoj TBGT 17212; 28 Nov. 2017, Manoj TBGT17289; 29 November 2017, Manoj TBGT17295; 14 Dec. 2017, Manoj TBGT17314: Palode, JNTBGRI campus, 25 Aug. 2015, Manoj TBGT15808; 13 May 2015, Manoj TBGT15640; 25 Aug. 2017, Manoj TBGT17050.

Commentary: *Crepidotus indicus* belongs to section *Echinospori* subsection *Porpophorini* (Singer 1986) due to the presence of echinulate spores and abundant clamp connections. The species is distinct in the production of mostly bisterigmate basidia, flexuous-contorted, whip-like cheilocystidia, and trichodermial pileipellis with dermatocystidia.

Some of the morphologically similar species include *Crepidotus tucumanus* Horak (Horak 1964) and *C. aquosus* Murr. (Singer 1973). *Crepidotus indicus* is distinct from these in its septate versiform cheilocystidia, larger basidiospores and in the trichodermial pileipellis with distinct dermatocystidia. *Crepidotus cuneiformis* Pat. described from USA (Singer 1973) is also comparable but that species differs mainly in its smaller basidiospores (6–7.5 × 5–6.2 µm) and in the size and shape of cheilocystidia and pileipellis a cutis.

Other related species of subsection *Porpophorini* include *C. applanatus* (Pers.) P. Kumm., *C. crocophyllus* (Berk.) Sacc., *C. ehrendorferi* Hauskn. & Krisai, *C. stenocystis* Pouzar. and *C. decipiens* Sing. All these can be distinguished from *C. indicus* in having smaller spores, differently shaped cheilocystidia and pileipellis a cutis with differently shaped pileocystidia.

Hesler & Smith's key of Subsect. *Colorantes* Hesler & A.H. Sm. (Hesler & Smith 1965) leads to two species viz., *Crepidotus contortus* Hesler & A.H. Sm. and *C. sinuosus* Hesler & A.H. Sm. *Crepidotus contortus* is quite distinct due to its small pale olive buff pileus, yellowish gray lamellae, smaller globose to subglobose basidiospores (4.5-6 µm) and cuticle a repent hyphae. The *Crepidotus sinuosus* differs from *C.indicus* by its smaller basidiomata, pale ochraceous, fibrillose to pubescent pileus, smaller basidiospores (5-7.5(8) µm) and cuticular pileipellis. Furthermore, our collection is molecularly distinct with only 95% sequence identity with *C. sinuosus.*

The nrLSU (893bp) sequences of *Crepidotus indicus* seem to be distinct. In a BLASTn search using the nrLSU sequence from *Crepidotus indicus* (893 bp), the closest hit was an unnamed *Crepidotus* species from USA, *Crepidotus* sp. (AF367951) with 99% sequence identity. The ML and Bayesian trees shows a similar topology where *C. indicus* is placed close to *Crepidotus* sp. AF367951 (Fig.5). Further information about this species, however, is unavailable for comparison.

Simocybe amara (Murr.) Sing., *Beihefte Nov. Hedw.* 44:513. 1973 **Figs 3-4**

= *Crepidotus amarus* Murrill, *Mycologia* 35:430.1943

GenBank: MG719983 (nrLSU).

Basidiomata thin. *Pileus* 3–18 mm diam., laterally attached, convex when young becoming slightly applanate in mature basidiomes, reniform, orbicular to flabelliform; surface chalky white, off white, becoming orange white to marble white (5A2/5B2) in old ones, felty, non-striate, non-hygrophanous, moist when fresh, soon dry; margin incurved to straight, wavy, slightly lobed, non-striate. *Lamellae* adnexed, white to pinkish white (7A2), up to 2 mm wide, distant with lamellulae of 2–3 lengths; edge dotted whitish. *Stipe* reduced, 1–2 × 1 mm, short, cylindrical, solid, curved, lateral; surface white, pruinose with a tomentose base.

Context thin, white to pale, soft, up to 1 mm wide. *Odor* not distinctive. *Taste* mild, bitter.

Basidiospores 6.5–8 × (5–) 5.5–6.5 µm, Q=1.25–1.42µm, avL=7.4 µm, avW=5.6 µm, broadly ellipsoid to ellipsoid, light yellow in KOH, thick-walled, smooth. *Basidia* 20–24 × 5.5–9µm, clavate, 4-spored, thin-walled, hyaline. Lamella

Fig 3. *Simocybe amara* (Murr.) Sing. **A-D**. Habit *in situ*. Scale bar = 10 mm.

edge sterile with crowded cheilocystidia. *Cheilocystidia* 20–48 × 6.4–9 µm, long, cylindric, flexuous, often with capitate or clavate apex, thin-walled, hyaline. *Pleurocystidia* absent. *Hymenophoral trama* regular, hyphae 3–6.5 µm wide, thin-

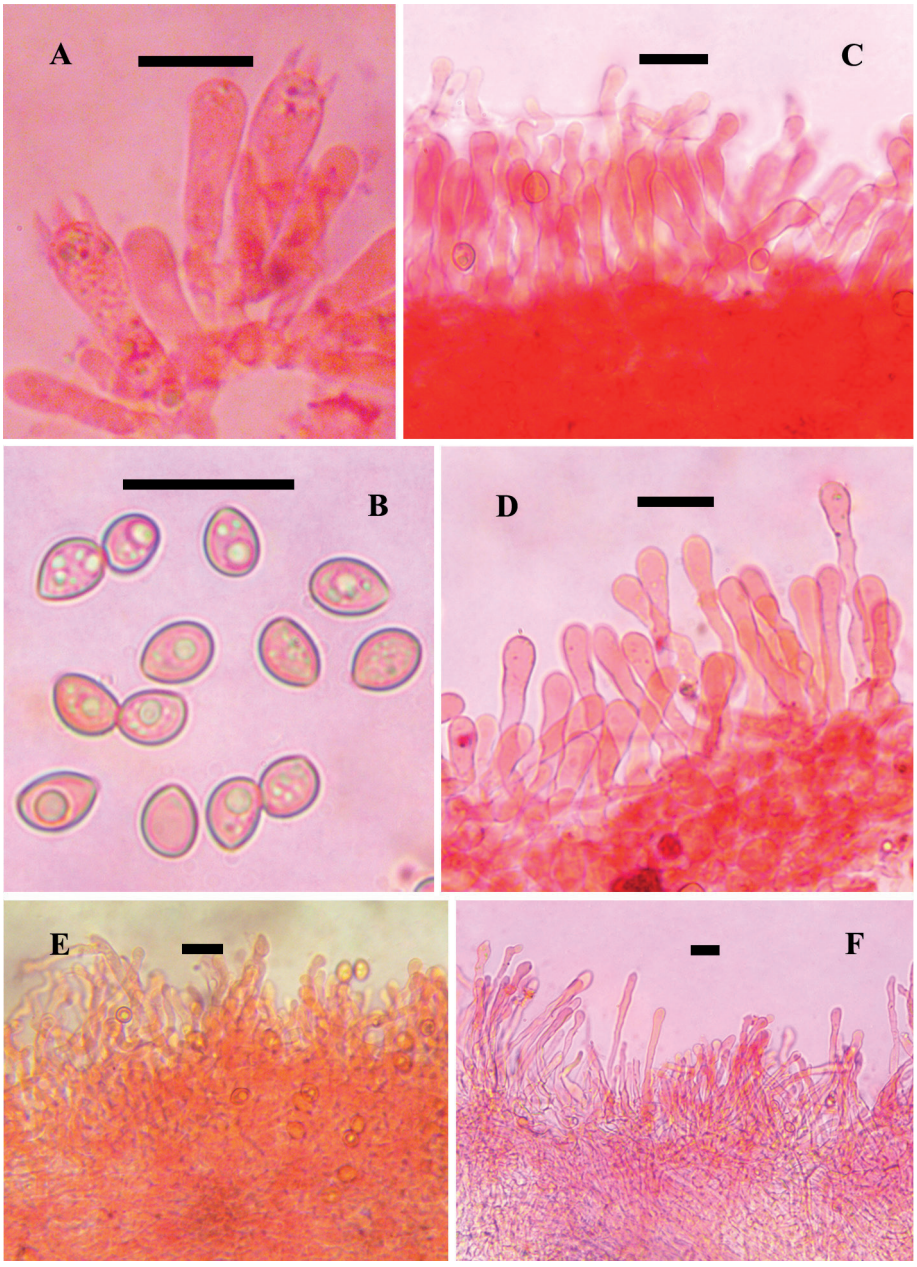

Fig 4. *Simocybe amara*. **A**. Basidia; **B**. Basidiospores; **C**. Gill edge with cheilocystidia; **D**. Cheilocystidia; **E**. Pileipellis with pileocystidia; **F**. Caulocystidia. Scale bars = 10 µm.

walled. *Subhymenium* pseudoparenchymatous. *Pileal trama* composed of thin-walled, hyaline hyphae, 5–8 µm wide. *Pileipellis* a cutis 5–6.5 µm, incrusted, disrupted by tufts of erect to suberect cystidioid elements, 32–48 × 4–5 µm, sinuous to filiform, flexuous, occasionally bifid at apex, thin-walled with pale yellowish contents. Pileal hyphae incrusted. *Caulocystidia* abundant, 30–72 × 4–6.5 µm, versiform, long cylindric, flexuous with a subclavate apex, often bifid, thick-walled, hyaline. *Clamp connections* present in all tissues. *Oleiferous hyphae* none.

Habit, habitat and phenology: Scattered on dead decaying coconut palm spathe, on dead angiosperm logs, or on bark of fallen trees. June-October.

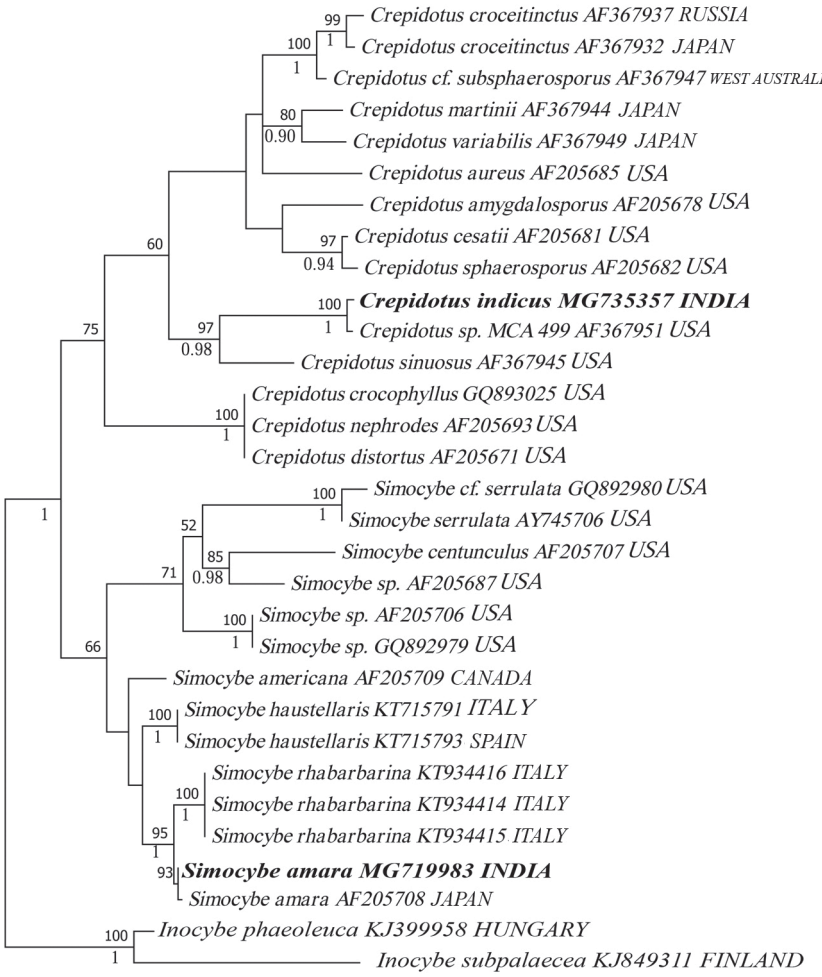

Fig 5. MLPhylogenetic analysis inferred from nrLSU sequences. Bootstrap values (ML, >50%) and Bayesian posterior probabilities (BI, >0.90) are indicated above and below branches respectively. GenBank accession numbers are given after the taxonomic names.

Specimens examined: INDIA. Kerala State, Thiruvananthapuram District, Palode, Plavara, 17 Aug. 2016, Manoj TBGT16503 (CAL1648); 18 Aug. 2014, Manoj TBGT16504; 19 Aug. 2016, Manoj TBGT16524; 21 Aug. 2016, Manoj TBGT16529; 26 Aug. 2016, Manoj TBGT16559; 27 October 2017 Manoj TBGT17197; JNTBGRI campus, 28 June 2017, Manoj TBGT16859; 9 Sept. 2017, Manoj TBGT17076; Palakkad District, Govt. college campus, Chittur, 13 June 2017, Manoj TBGT16839.

Commentary: The above description matches with the type description of *Simocybe amara* which was originally described as *Crepidotus amarus* Murr. (Murrill 1943) from Florida. Later Singer (1973) transferred *C. amarus* to *Simocybe* due to the presence of an eccentric stipe and strong bitter taste. DNA sequence data from TBGT16503(CAL 1648) support the designation of *S. amara* to our material due to the very high nrLSU similarity (99% BLAST Identity) and phylogenetic affinity to a collection under this name from Japan (GenBank: AF205708) (Moncalvo *et al.* 2002; Aime *et al.* 2005). Phylogenetic tree also supports its placement with significant ML bootstrap value (Fig.5). Indian collections feature the characteristic bitter taste (though mild) coupled with the presence of a distinct short stipe.

Simocybe rhabarbarina Poli, Musumeci & P.Alvarado, a recently described species from Italy (Poli *et al.* 2015) is also closely related both morphologically and molecularly to the Indian material. However, that species differs in its bisterigmate basidia and larger ellipsoid to ovoid basidiospores (7–10(11) × 5.5–7 µm). *Simocybe amara* is so far not known from India.

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