Check List the journal of biodiversity data

NOTES ON GEOGRAPHIC DISTRIBUTION

Check List 11(6): 1819, 22 December 2015 doi: http://dx.doi.org/10.15560/11.6.1819
ISSN 1809-127X

Crinipellis cupreostipes (Marasmiaceae, Agaricales, Basidiomycota): a new distributional record from India

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Abstract: The present study reports *Crinipellis cupreostipes* (first described from Thailand) as a new record for Indian mycobiota. A phylogenetic analysis based on nrDNA ITS shows that *C. cupreostipes* can be readily distinguished from other morphologically similar species such as *Crinipellis nigricaulis* var. *macrospora*. A detailed taxonomic description with illustrations and an artificial key to *Crinipellis* species previously reported from India and its neighboring countries are provided.

Key words: new record; nrDNA; phylogenetic analysis; West Bengal

The genus *Crinipellis* Pat. 1889: 336 (Agaricales, Basidiomycota) comprises over 150 species and is considered to have a worldwide distribution (Kirk et al. 2001). Its cosmopolitan nature is observed through studies in Southeast Asia (Kerekes and Desjardin 2009), Singapore and the Malaysian peninsula (Corner 1996), Africa (Pegler 1968), New Zealand (Stevenson 1964), East Africa (Pegler 1977), Sri Lanka (Pegler 1986), Japan (Takahashi 2000), Republic of Korea (Antonín et al. 2009), and China (Teng 1963; Bi et al. 1993, 1997; Shao and Xiang 1997; Xia et al. 2015). The main diagnostic features include presence of thick-walled, dextrinoid, hair-like terminal cells in the pileipellis, which make the genus easy to separate from the morphologically similar genus *Marasmius* Fr. (Kerekes and Desjardin 2009).

During an investigation of macrofungi in Eastern India, a member of *Crinipellis* was discovered in Loleygaon, Darjeeling district, West Bengal, India. After thorough consultation of available literature (Singer 1943, 1955, 1986; Pegler 1977, 1983, 1986; Manjula 1983; Bi et al. 1993; Takahashi 2000; Antonín et al. 2009; Kerekes and Desjardin 2009; Antonín and Noordeloos 2010; Xia et al. 2015), the species was identified as *Crinipellis cupreostipes* Kerekes, Desjardin & Lumyong (in Kerekes and Desjardin 2009: 116) and the identification is further confirmed by molecular (nrDNA ITS sequence) data. This collection constitutes the second record of the species worldwide and expands its geographical distribution to India. We present morphological and molecular studies of the collected taxon, and an artificial key to *Crinipellis* species previously reported from India and its neighboring countries like Sri Lanka, China, etc. In addition, phylogenetically related species such as *C. nigricaulis* var. *macrospora* Antonín, Ryoo & H.D. Shin (2009: 431), *C. tabtim* Kerekes, Desjardin & Lumyong (in Kerekes and Desjardin 2009: 132), and *C. dipterocarpi* Singer (1943: 496) have also been included in the key.

Morphology — The specimen of *Crinipellis cuperostipes* was collected in August 2012 in Loleygaon, Darjeeling district, West Bengal, India. Fresh basidiomata were photographed in the field using a digital camera and extensive notes on the macromorphology were taken before drying. Colour codes and terms (mostly) follow Kornerup and Wanscher (1978). Microscopic features were obtained from reviving dried material in 5% KOH, Melzer's reagent and Congo Red. The terms used to describe lamellae spacing refer to the number of lamellae that run from the stipe to the pileus margin (L), and do not include the lamellulae whose spacing is indicated by the number of series present (l). The size of basidiospores is based on 25 measurements from each of the six collected basidiomata from a single collection. Mean basidiospore size is underlined in description and 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) under number AM116.

DNA extraction, PCR amplification, and DNA sequencing — Genomic DNA was extracted following Dutta et al. (2014). Selective PCR amplification of nrDNA ITS

region was performed according to the modified method of Gardes and Bruns (1993) using the primer pair ITS1F (forward) and ITS4B (reverse). A hot start of 4 min. at 94°C was 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. PCR products were purified using QIAquick® Gel Extraction Kit (QIAGEN, Germany) and were used for automated DNA sequencing on ABI3730xl DNA Analyzer (Applied Biosystems, USA) using the same ITS primers. The generated sequence was edited manually using BioEdit sequence alignment editor version 7.0.9.0 (Tom Hall, Ibis Biosciences, Carlsbad, USA). The edited sequence was then used for BLAST searches in the GenBank database. The newly generated sequence was deposited in GenBank (KT346353). The newly generated ITS sequence (717 bp) and those retrieved from GenBank after BLAST search of KT346353 (17 ITS sequences) were chosen for the phylogenetic analyses. The closest hit for KT346353 was *Crinipellis cupreostipes* (GenBank FJ167641, sequence identity = 661/672 (98%), gaps = 3/672 (0%); GenBank NR_119707, sequence identity = 603/614 (98%), gaps = 4/614 (0%)]. *Marasmius crinis-equi* F. Muell. ex Kalchbr. (1880: 153) was selected as outgroup taxa for rooting purpose following Kerekes and Desjardin (2009). Altogether, twenty sequences were aligned with ClustalX (Thompson et al. 1997) using default settings, followed by additional manual adjustments. The end of the final alignment was trimmed to create a dataset of 633 bp that included 442 positions. All the sequences used for conducting the phylogenetic analysis are indicated in Figure 1.

Phylogenetic analyses — Maximum Parsimony (MP) and Maximum likelihood (ML) bootstrapping analyses were conducted using MEGA v. 6.0 (Tamura et al. 2013). For MP analysis, searches employed a heuristic search method with all characters weighted equally, gaps treated as missing data, search level 1 where the initial trees were obtained by the random addition of sequences (10 replicates), tree bisection reconnection (TBR), and collapse of zero length branches. The branch lengths were calculated using the average pathway method (Nei and Kumar 2000) and are in the units of the number of changes over the whole sequence.

ML analysis was done based on the general time reversible model (Nei and Kumar 2000) where the initial tree(s) for the heuristic search was obtained by applying the neighbour-joining method to a matrix of pairwise distances estimated using the maximum composite likelihood (MCL) approach. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+*G*, parameter = 0.2755)). The rate variation model allowed for some sites to be evolutionarily invariable ([+*I*], 0.0000% sites).

Bayesian phylogenetic analyses were carried out using Metropolis-coupled Markov chain Monte Carlo (MCMCMC) methods with MrBayes v. 3.2.2 (Ronquist et al. 2012), under a GTR+I+G model. 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

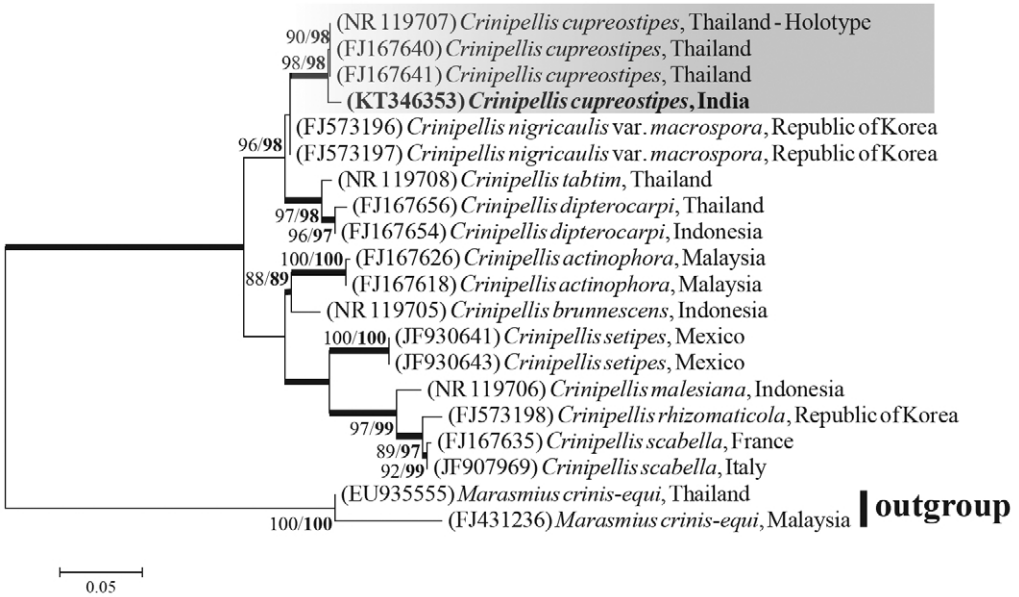


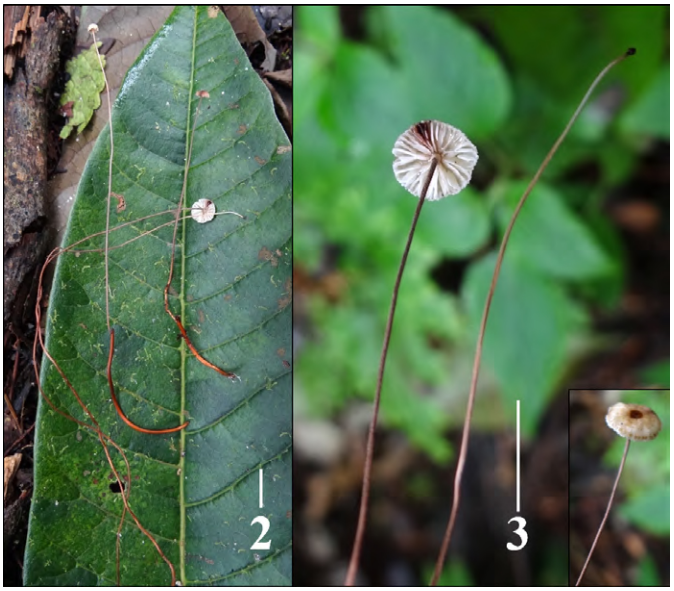
Figure 1. Maximum likelihood tree (-ln*L* = 1577.2398) generated using a GTR+I+G model of nucleotide evolution. Numbers to the left of/are MP bootstrap support, and those to the right indicate the Maximum Likelihood bootstrap (BS) support (in bold). Bayesian posterior probabilities (PP) > 0.95 are indicated as black coloured thickened lines and the scale bar represents the expected changes per site. The Indian collection of *Crinipellis cupreostipes* is placed in bold font to highlight its phylogenetic position in the tree.

obtain estimates of the posterior probabilities (PPs) of the groups. Bayesian posterior probabilities values over 0.95 are reported in the resulting trees.

Crinipellis cupreostipes Kerekes, Desjardin & Lumyong (Figures 2–9)

Pileus 2–4 mm diameter, truncately hemispherical to convex when young, becoming plano-convex with a central circular depression surrounding a papilla; papilla brown (6E6–7) to dark brown (6F7–8); disc light brown (6D5) to brown (6E6–7), becoming dark brown (6F7–8) with a creamy margin, surface radially appressed-fibrillose, fibrils light brown (6D5) to brown (6E6), becoming paler with maturity; many stipes topped with tiny, unopened, convex to conical primordial pilei with inrolled margin. *Lamellae* ca. 1 mm broad, adnexed, subdistant to close (L = 19; l = 1), white (1A1) to cream, often with dark brown (7F5–7) spots. *Stipe* 60–150 × 0.3–0.5 mm, central, cylindrical, wiry, cream towards extreme apex, otherwise dark brown (6F4–5) to greyish brown (6–7E3), pubescent; hairs of stipe white to cream; abundant rhizomorphs present. *Rhizomorphs* 0.7–1 mm broad, light brown (7D8) to brownish orange (7C8), resemble copper wire, mostly arise from decomposing leaf litter. *Context* < 1 mm thick, cream to light brown (6D5), unchanging on exposure. *Odor* and *taste* indistinctive.

Basidiospores (9–)9.6–9.9–10(–10.8) × 3.6–3.7–3.9(–4.3) µm, Q=2.2–2.6–3, fusiform, smooth, hyaline, inamyloid. *Basidia* 18–25 × 4.3–5.7(–6.5) µm, clavate, 4–spored. *Basidioles* 14.5–18(–25) × 3.5–4(–6.4) µm, clavate to cylindrical with rounded to subacute apex. *Pleurocystidia* absent. *Lamellae edge* sterile with well-developed cheilocystidia. *Cheilocystidia* composed of *Siccus*-type broom cells; main body (10–)13.5–14.5(–15) × (3–)4–5.5(–6) µm, clavate, hyaline, inamyloid, thin-walled; apical appendages (2–)3–3.6(–5.5) µm long, finger-like, hyaline, inamyloid, thin-walled. *Pileipellis* a cutis, composed of 5–5.7 µm broad, hyaline, moderately dextrinoid hyphae giving rise to terminal hairs with basal clamp-connections; terminal hairs (197–)243–275(–287) × (3.6–)4–4.3(–5.4) µm, cylindrical with bluntly rounded or tapering apices, secondary septations present, thick-walled, slightly dextrinoid, hyaline to tan when examined in water, light brown to tan in KOH. *Pileus trama* regular, hyphae 3.6–4(–5.4) µm broad, cylindrical, smooth, hyaline, thick-walled, inamyloid. *Lamellar trama* regular, hyphae 4–6 µm broad, cylindrical, smooth, hyaline, inamyloid, slightly thick-walled. *Stipe tissue* monomitic, parallel, tightly packed. *Stipitipellis* composed of repent cortical hyphae and terminal hairs; cortical hyphae 4–5.5 µm broad, cylindrical, dextrinoid, copper yellow in water, yellow to yellowish tan in KOH; medullary hyphae 6.5–7.2 µm broad, cylindrical, inamyloid, hyaline when examined in water and KOH; terminal hairs 140–175(–218) × 3.2–3.6 µm, similar with hairs in pileipellis, cylindrical, thick-walled (1–1.2 µm), dextrinoid, hyaline to brownish yellow in water and KOH, with acute to subacute at



Figures 2–3. *Crinipellis cupreostipes*. **2:** Fresh basidiomata in the field showing copper coloured rhizomorphs. **3:** Basidiomata showing lamellae, stipe and unopened primoridal pilei (inset: pileus surface view). Scale bars: Scale bars: 2–3 = 5 mm. (photographs by A.K. Dutta).

Figures 4–9. *Crinipellis cupreostipes* (CUH AM116). **4:** Basidiospores. **5:** Basidium. **6:** Basidioles. **7:** Cheilocystidia. **8:** Pileipellis hairs. **9:** Caulocystidia. Scale bars: 4–9 = 5 µm.

apex, less secondary septate. *Caulocystidia* 50–55(–81) × 3.6–4(–4.5) µm, cylindrical with obtuse apex, often irregular in outline from middle towards upper, thick-walled. *Clamp-connections* present.

Habit and Habitat — Gregarious, on dicot leaves and twigs.

Distribution — Previously, there was only one report on the occurrence of *C. cupreostipes* from Doi Inthanon National Park, Chiang Mai Province, Thailand, where it was found to grow on decomposed dicot leaves and small twigs in montane primary cloud forest containing *Quercus*, *Magnolia*, and *Ficus* species. The occurrence of this species in India, West Bengal, Darjeeling district, Loleygaon, expands its geographical distribution from Thailand to India. The Indian collection was found to grow among dead dicot leaves and twigs in the forest ecosystem predominantly covered by trees such as *Acer campbellii* Hook. f. & Thomson ex Brand., *Castanopsis indica* (Roxb. ex Lindl.) A. DC., *Eriobotrya petiolata* Hook. f., *Machilus edulis* King ex Hook. f., *Quercus lamellosa* Sm., *Quercus lineata* Blume, *Exbucklandia populnea* (R.Br. ex Griff.) R.W. Br., *Lithocarpus pachyphyllus* (Kurz) Rehder, etc.

Material examined — India: West Bengal, Darjeeling district, Loleygaon, 27°00′50.5908″N, 088°33′41.2632″ E, 1,745 m, 23 August 2012, A. K. Dutta and P. Pradhan, CUH AM116.

Diagnostic features of *Crinipellis cupreostipes* include a very long (up to 550 mm) copper coloured stipe, and abundant copper coloured rhizomorphs, many of which terminate with primordial pilei (Kerekes and Desjardin 2009). Our Indian collection mostly matches with the original description of *C. cupreostipes* (Kerekes and Desjardin 2009), except for the stipe colour, shorter stipe (up to 150 mm vs. 550 mm in the type material), somewhat shorter terminal hairs in the pilleipellis (ca. 200–290 µm vs. 260–520 µm in the type material) and the number of lamellulae. The stipe colour of the Indian collection is cream towards the extreme apex, otherwise dark brown to greyish brown and the number of lamellulae between two lamellae is one.

Each of the ML analysis iterations recovered a single tree, the likelihood values of which did not differ significantly. The tree with the highest log likelihood value has been chosen to present here (Figure 1; -ln*L* = 1577.2398). Parsimony analyses resulted in five most parsimonious trees (length = 197) among which Tree 1 with C.I. = 0.701149 and R.I. = 0.848397 has been chosen. Bayesian analyses reached a standard deviation of split frequencies of 0.003 after one million generations. Tree obtained from MP and Bayesian phylogenetic analyses did not differ significantly in topology from those recovered in the ML analyses. ML, MP Bootstrap values (BS ≥ 70%) and Bayesian posterior probabilities (PP > 0.95) have been indicated in Figure 1 (next to the branches). In all the analyses (ML, MP and Bayesian), the newly generated sequence of our collected specimen (KT346353) clustered with all sequences of *C. cupreostipes,* including the type specimen sequence, with strong support values (Figure 1; MLBS = 98%, MPBS = 98%, PP = 1.00).

Among species with the presence of rhizomorphs and longer stipe, *C. nigricaulis* var. *macrospora*, described from Republic of Korea, differs by having pubescent lamellae edge, brown-black coloured rhizomorphs, and much longer stipe hairs (up to 400 µm long; Antonín et al. 2009). *Crinipellis actinophora* (Berk. & Broome 1875: 39) Singer (1955: 397), described from Sri Lanka, differs from the present specimen by the presence of longer pileus hairs and grey to dark brown coloured rhizomorphs (Singer 1955). The Japanese species *C. nigricaulis* Har. Takah. (2000: 178) has a pileus coloured black at the papilla, smaller stipe (25–50 µm long), much longer pileus hairs (up to 1500 µm), broader basidiospores (8.0–11 × 4.5–6.0 µm) and larger cheilocystidia, 17–30 × 6–11 µm (Takahashi 2000).

Throughout the world, among species with small basidiocarp and similar size of the basidiospores: *C. dipterocarpi* differs by shorter stipe (up to 50 mm long), mostly absent rhizomorphs, if present then reddish brown to black coloured, much longer pileipellis terminal hairs (400–537 µm long; Kerekes and Desjardin 2009); *C. tabtim* has a pileus coloured violet brown to dark ruby that fades to almost cream-coloured in age, lamellae with 2–3 series of lamellulae, much smaller stipe (15–30 mm long; Kerekes and Desjardin 2009).

An artificial key to the species of *Crinipellis*, either morphologically or phylogenetically related to the newly reported *C. cupreostipes* from India and its adjoining countries, such as Sri Lanka and China, is provided here.

1a Stipe short (≤ 50 mm long) 2
1b Stipe very long (always > 50 mm long) 10
2a Rhizomorphs uncommon to mostly absent 3
2b Rhizomorphs always present 9
3a Pileus center dark violet brown to dark ruby ..... *C. tabtim*
3b Pileus center differently coloured (shades of violet not present) 5
5a Lamellae dark pinkish brown ..... *C. omotricha* (Berk.) D.A. Reid
5b Lamellae differently coloured 6
6b Pleurocystidia present, clavate shaped ..... *C. scabellus* (Alb. & Schwein) Murrill
6a Pleurocystidia absent 7
7a Pileus small (1-10 mm); cheilocystidia of the *Siccus*-type ..... *C. dipterocarpi*
7b Pileus larger; cheilocystidia differently shaped 8

8a On rotten bark or wood of deciduous trees, basidiospores (5.5–)6–8 µm long
..... *C. floccosa* T.H. Li, Y.W. Xia & W.Q. Deng

8b On herbaceous debris (mostly grass remnants), basidiospores ca. 7.5–12.5 µm long
..... *C. subtomentosa* (Peck) Singer

9a Rhizomorphs grey, greyish brown to dark brown; pileus hairs up to 750 µm long ..... *C. actinophora*

9b Rhizomorphs blackish; pileus hairs up to 1500 µm long ..... *C. nigricaulis*

10a Rhizomorphs light-brown to brownish orange (resemble copper wire); stipe hairs up to 175(–220) µm long ..... *C. cupreostipes*

10b Rhizomorphs brown-black coloured; stipe hairs up to 400 µm long ..... *C. nigricaulis* var. *macrospora*

ACKNOWLEDGEMENTS

This paper was made through financial support by the Department of Environment, Government of West Bengal, India. The first author (AKD) gratefully acknowledges Prof. Dennis E. Desjardin (San Francisco State University, San Francisco, United States) for his valuable suggestions during the identification of the specimen.

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Author contributions: AKD collected and identified the species, prepared the macro- and microscopic data, drew the microscopic plate, made the phylogenetic analysis, wrote the text, PP collected the species, wrote the text, AR wrote the text, and KA identified the species and wrote the text.

Received: 13 August 2015
Accepted: 1 December 2015
Academic editor: Matias J. Cafaro