



## Short communication

## *Coltricia raigadensis* (Hymenochaetaceae, Basidiomycota), a new species from India

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### ABSTRACT

A new species of *Coltricia*, *C. raigadensis* is described from tropical region of Maharashtra, India. The species is recognized on the basis of morphological characteristics and phylogenetic analyses using rDNA ITS1-5.8S-ITS2, partial 28S rDNA and partial 18S rDNA sequences. *Coltricia raigadensis* is characterized by centrally stipitate basidiocarps, adpressed velutinate to tomentose pileal surface, small pores (2–4 per mm), globose to subglobose, thick walled basidiospores measuring  $5.6\text{--}7 \times 5\text{--}6.64 \mu\text{m}$ .

**Keywords:** morphology, phylogeny, polypore, taxonomy

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*Coltricia* Gray is a worldwide genus of *Hymenochaetales* (Larsen et al., 2006), typified by *C. perennis* (L.) Murrill. The majority of species are terrestrial and some have been associated with plant roots, where they are likely to be mycorrhizal (Tedersoo et al., 2007), while others have been discovered on wood. The genus *Coltriciella* Murrill is closely related to *Coltricia* by sharing similar morphological characteristics and growth habits, but they differ mainly in having either ornamented or smooth basidiospores (Ryvarden, 1991). The phylogenetic studies by Bian et al. (2022) revealed the placement of *Coltriciella* within the clade of *Coltricia* and hence *Coltriciella* was considered as a synonym under *Coltricia*. Further, based on the above conclusions, Wu et al. (2022) grouped all *Coltriciella* species under the genus *Coltricia*. So, the generic concept is broadened by basidiocarps varying from resupinate, effuse-reflexed, pendent or stipitate with a monomitic hyphal system devoid of clamp connections, poroid or lamellate hymenial surfaces, and usually pigmented, smooth or ornamented basidiospores (Corner, 1991; Dai, 2010; Ryvarden, 2004).

*Coltricia* has been substantially investigated worldwide and about 31 new taxa (adding nine new species of the originally described *Coltriciella*) were newly described in the last decade (Baltazar et al., 2010; Baltazar & Silveira, 2012; Bian & Dai, 2015, 2017, 2020; Bian et al., 2016, 2022; Dai, 2010; Dai et al., 2010; Dai & Li, 2012; Decock, 2013; Jayawardena et al., 2022; Ryvarden & Melo, 2014; Susan et al., 2018; Valenzuela et al., 2012, 2020; Vasco-Palacios, 2016; Vlasák et al., 2020; Wu et al., 2022; Zhou & Tedersoo, 2012). In India, *Coltricia* is poorly studied, so far nine species of the

genus are reported from the country (Adarsh et al., 2018; Baltazar & Silveira, 2012; Kaur et al., 2016; Kour et al., 2015; Pongen et al., 2018).

During the macrofungal surveys conducted from 2015 to 2020 in the Matheran Hills region of Maharashtra, India, amidst the monsoon periods, several new taxa of mushrooms have been collected. The region is characterized by a mixed dry deciduous forest dominated by *Memecylon umbellatum* Burm.f. In the present study, a new *Coltricia* species is described pursuant to morphological characters and molecular phylogenetic analyses.

Materials were collected after taking field photographs. Detailed macroscopic characters were studied from the fresh materials. Color notations were followed according to Kornerup and Wanscher (1978). The specimens were air dried or dried at 45–50 °C temperature in oven for 1 or 2 d. All micromorphological features were studied with assistance of a compound microscope MLX-B (Olympus, Tokyo, Japan) connected with Magnus Magcam DC-5 camera (Magnus Opto Systems India Pvt. Ltd., New Delhi, India). The thin handmade sections of dried specimens were revived in 5% (w/v) KOH, stained with 1% (w/v) phloxin in distilled water, cotton blue and Melzer's reagent. The following abbreviations were used in the text to describe the basidiospores: L for arithmetic mean of basidiospores length, W for arithmetic mean of basidiospores width and Q for quotient of length and width between samples studied, Qm for mean values of  $Q \pm$  standard deviation and n for number of basidiospores measured from given number of specimens. The studied samples were deposited at Ajrekar Mycological Herbarium (AMH), Pune, India and the paratypes were maintained at 'Matheran Mushroom Herbarium' collection (MMH) of the department of Botany, Smt. Chandibai Himathmal Mansukhani College, Thane, India.

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The specimens and sequences obtained from this study and other fungal taxa used in previous studies were enlisted in Table 1. The genomic DNA was extracted using CTAB method (Doyle & Doyle, 1987), and PCR reactions and PCR purification were carried out by Genematrix LLP (Pune, India), further the Sanger sequencing was conducted by Apical Scientific Sdn Bhd (Seri Kembangan, Selangor, Malaysia). The DNA markers ITS1-5.8S-ITS2 (nrITS), partial 28S rDNA (nrLSU) and partial 18S rDNA (nrSSU) were amplified and sequenced using the primers: ITS1/ITS4 (White et al., 1990), LR0R/LR5 (Moncalvo et al., 2000; Vilgalys & Hester, 1990), NS1/NS4 (White et al., 1990). All the sequences generated in this study were deposited to NCBI database under accession numbers OR072877, OR072932, OR053821, and OR053822. The NCBI database sequences were retrieved and used to construct the phylogenetic trees following earlier studies (Bian & Dai, 2017, 2020; Bian et al., 2016, 2022; Wu et al., 2022). The combined dataset consists of 73 nrITS, 73 nrLSU and 54 nrSSU sequences (including four new sequences of present study), representing 40 species of *Coltricia*. Based on the morphological similarities and previous molecular studies (Bian & Dai, 2020; Bian et al., 2022; Larsson et al., 2006; Wagner & Fischer, 2002), the closely allied genera *Fomitiporella chinensis* (Pilát) Y.C. Dai, X.H. Ji & Vlasák and *Inonotus griseus* L.W. Zhou were selected as outgroups. All the sequences were aligned using MUSCLE programme in the MEGA v 7.0 software (Kumar et al., 2016). Initially, the individual marker based trees were constructed and then all three markers were analysed for their combining efficacy by performing the partition homogeneity test (ILD) in PAUP v 4.0b10 (Swofford & Sullivan, 2009) to generate the combined tree. The sequences were concatenated by using TaxonDNA (Sequence Matrix) v 1.7.8 (Vaidya et al., 2010). The combined aligned data matrix of all three markers was also deposited to the treeBASE, which can be accessed using the URL: <http://purl.org/phylo/treebase/phylovs/study/TB2:S30807>. Phylogenetic analyses were performed with Maximum likelihood (ML) and Bayesian inference (BI) criteria. The ML phylogram was generated by using IQTree v 1.6.8 (Nguyen et al., 2014) and the best-fit nucleotide substitution model was selected as GTR+F+I+G4 for combined dataset, according to the ModelFinder (an inbuilt tool of IQTree, Kalyanamoorthy et al., 2017). Further, the Bayesian analysis was performed using Metropolis Coupled MCMC method in MrBayes v 3.2.6 (Ronquist et al., 2012). Two parallel chains were run for 4 million generations and standard deviation of split frequency was obtained less than 0.01 for the combined dataset. The nucleotide substitution model (GTR+I+G) was selected using jModeltest (Darriba et al., 2012). ML and Bayesian phylogenetic trees were visualized in FigTree v 1.4.2 (Rambaut, 2014). Statistical supports for the phylogram were determined in terms of bootstrap values (BS) and posterior probabilities (PP).

## Taxonomy

***Coltricia raigadensis*** P.B. Patil, S.A. Vaidya, S. Maurya & L.S. Yadav sp. nov. Figs. 1, 2.

Mycobank no.: MB 849006.

Diagnosis: this species is characterized by centrally stipitate basidiocarps, adpressed velutinate to tomentose pileal surface, 2–4 pores per mm, 5.3–7.6 µm wide contextual hyphae, 4.8–5.4 µm wide tramal hyphae, globose to subglobose, thick walled basidiospores measuring 5.6–7 × 5–6.64 µm.

Type: INDIA, Maharashtra, Raigad District, Matheran Hills (18°58'48.00"N, 73°16'12.00"E, 800 m a.s.l.), collected by P. B. Patil on 25 Jul 2017. (**AMH 10511**, Holotype).

DNA sequence ex-Holotype: OR072877 (nrITS).

Etymology: The species epithet “raigadensis” refers to the place

of collection.

Basidiomes annual, centrally stipitate, solitary, soft or leathery when fresh, hard, corky or brittle, light weight when dried. Pilei more or less circular, flat to infundibuliform, up to 35 mm diam, 3 mm thick at centre. Pileal surface shiny, chocolate brown (6F4) to chestnut brown (6F7) to burnt umber (6F6) when fresh, brownish black (6F8) to burnt umber (6F6) upon drying, azonate to concentrically zonate, adpressed velutinate to tomentose, hairs erected in the centre, margin entire to incised, straight to deflexed when dry, with tuft of hairs, sterile up to 3 mm. Pore surface brownish black (6F8) when fresh yellowish brown (5E8) upon drying. Pores round to angular, 2–4 per mm, dissepiments thin to fairly thick, entire to lacerate. Context dark brown, coriaceous, up to 2 mm thick. Tubes concolorous with the pore surface, brittle when dry, up to 1–2 mm long. Stipe concolorous with the upper surface of the pileus, cylindrical, rigid to pliable, velutinous to tomentose, often branched near apex, corky to leathery when dry, up to 25 to 40 mm long, 3 to 5 mm diam, mostly swollen tip up to 8 mm diam.

Hyphal system monomitic, generative hyphae with simple septa, contextual hyphae golden brown, branched at broad angles, fairly thick walled with broad lumen, 5.3–7.6 µm wide. Stipe hyphae golden brown, thick walled with a narrow lumen, distinctly narrower than those in context, parallel along the stipe, unbranched, 4–5.3 µm wide. Tramal hyphae pale yellow to buff yellow, slightly thick walled with a wide lumen, moderately branched, loosely interwoven to subparallel along the tubes, 4.8–5.4 µm diam. Cystidia and cystidioles absent. Basidia broadly clavate, 4-spored, with the basal septum, 18.5–24.5 × 5.4–7.8 µm; basidioles slightly smaller, 12.6–20.4 × 4–6.6 µm, similar in shape to basidia. Basidiospores globose to subglobose, golden brown, smooth, thick-walled, cyanophilic, inamyloid, nondextrinoid, (4.6) 5.6–7 (7.2) × (4.3) 5–6.6 µm, L = 6.3 µm, W = 5.7 µm, Q = 1.03–1.18, Qm = 1.11 ± 0.06 (n = 60/3).

Habitat and distribution: On soil, solitary to scattered in mixed dry deciduous forest. So far known only from Matheran Hills, Maharashtra, India.

Additional specimens (paratypes) examined: INDIA, Maharashtra, Raigad District, Matheran Hills (18°58'48.00"N, 73°16'12.00"E), 15 Aug 2019 (MMH 1211, OR072932 for nrITS, OR053821 for nrLSU, and OR053822 for nrSSU), 8 Sep 2019 (MMH 1212), 22 Aug 2021 (MMH 1213), Prashant B. Patil.

*Coltricia raigadensis* is characterized by its centrally stipitate basidiocarps, adpressed velutinate to tomentose pileal surface, globose to subglobose, and thick walled basidiospores; these features are common with *C. albidipes* Corner ex Y.C. Dai & Hai J. Li, *C. barbata* Ryvar den & de Meijer, *C. globispora* Gomes-Silva, Ryvar den & Gibertoni, *C. hamata* (Romell) Ryvar den, *C. rigida* L.S. Bian & Y.C. Dai, and *C. velutina* Baltazar & Gibertoni. However, *C. albidipes* has pale yellowish, glabrous pileal surface and slightly smaller basidiospores (5–6 × 4.7–5.2 µm) (Dai & Li, 2012). *Coltricia barbata* is distinguishable from *C. raigadensis* by its smaller basidiomes (up to 18 mm diam) and glabrous pileal surface (Ryvar den & de Meijer, 2002). *Coltricia globispora* distinctly differs from *C. raigadensis* in having larger basidiomes (pileus up to 55 mm diam), glabrous, snuff brown pileal surface and smaller pores (7–8 per mm) (Gomes-Silva et al., 2009). *Coltricia hamata* is differs markedly by its conspicuous, dark brown setal hyphae and larger basidiospores (7.5–10 × 5.5–7 µm) (Ryvar den & Johansen, 1980). *Coltricia velutina* has smaller basidiomes (pileus up to 15.5 mm diam), smaller pores (5–7 per mm), basidia (12–15 × 7–8 µm) and basidiospores (5.5–6.5 × 4.5–5.5 µm) (Baltazar et al., 2010). *Coltricia rigida* is also having subglobose to globose basidiospores but differs from *C. raigadensis* by having laterally stipitate larger basidiocarps (up to

**Table 1.** Fungal taxa, voucher specimen numbers, substrate, localities and GenBank accession numbers for nrITS, nrLSU, and nrSSU sequences used for the present phylogenetic analyses. “-” means information not available from GenBank database. Sequences newly generated in the present study were shown in bold.

Taxon	Specimen Voucher	Substrate	Locality	GenBank accession numbers		
				nrITS	nrLSU	nrSSU
<i>Coltricia abieticola</i>	Cui 12276	Ground	China	KU360673	KU360643	KY693762
<i>C. abieticola</i>	Cui 12312	Ground	China	KU360674	KU360644	KY693763
<i>C. abieticola</i>	Cui 10265	Ground	China	KX364784	KX364803	KY693760
<i>C. abieticola</i>	Cui 10321	Ground	China	KX364785	KX364804	KY693761
<i>C. australica</i>	TU 103694	Ground	Australia	-	AM412243	-
<i>C. austrosinensis</i>	Dai 13093	Ground	China	KU360670	KU360640	KY693764
<i>C. austrosinensis</i>	Dai 13098	Ground	China	KU360671	-	KY693765
<i>C. austrosinensis</i>	Dai 13823	Ground	China	KU360672	KU360642	KY693766
<i>C. barbata</i>	AMV 1866	Ground	Colombia	KT724137	-	-
<i>C. barbata</i>	AMV 1925	Ground	Colombia	KT724136	KT724149	-
<i>C. baoshanensis</i>	Cui 8147	Rotten wood of <i>Castanopsis</i>	China	KX364799	KX364819	-
<i>C. baoshanensis</i>	Dai 13075	Rotten wood of <i>Castanopsis</i>	China	KX364800	KX364820	KY693812
<i>C. cinnamomea</i>	Cui 12549	Ground	China	KY693728	KY693742	KY693769
<i>C. cinnamomea</i>	Cui 12584	Ground	China	KY693729	KY693743	KY693770
<i>C. confluens</i>	TAA 181460	Ground	Estonia	AM412241	-	-
<i>C. confluens</i>	TF 072287	Ground	USA	MN121008	-	-
<i>C. crassa</i>	Cui 10255	Ground	China	KU360678	KU360647	KY693777
<i>C. crassa</i>	Dai 15163	Ground	China	KU360679	KU360648	KY693778
<i>C. dependens</i>	Dai 10944	Rotten wood	China	KY693737	KY693757	KY693813
<i>C. dependens</i>	Cui 9210	Rotten wood	China	KY693738	KY693758	KY693814
<i>C. fimbriata</i>	Dai 22300	Ground	China	OL691607	OL691616	OL691612
<i>C. folicola</i>	Dai 16090	Ground	China	KX364786	-	-
<i>C. globosa</i>	Cui 7545	Ground	China	KJ540930	KJ000226	KY693815
<i>C. globosa</i>	Dai 18420	Ground	Vietnam	MT174245	-	-
<i>C. hamata</i>	3947	Ground	Brazil	MZ484545	MZ437402	-
<i>C. hamata</i>	4054	Ground	Brazil	MZ484546	-	-
<i>C. hamata</i>	AMV 1897	Ground	Colombia	KT724146	KT724150	-
<i>C. hamata</i>	AMV 2076	Ground	Colombia	KT724142	KT724151	-
<i>C. hirtipes</i>	Dai 16647	Ground	Thailand	KY693734	KY693750	-
<i>C. hirtipes</i>	Dai 16651	Ground	Thailand	-	KY693751	-
<i>C. kinabaluensis</i>	Dai 13957	Fallen wood	Thailand	KX364787	KX364806	KY693780
<i>C. kinabaluensis</i>	Dai 13958	Fallen wood	Thailand	KX364788	KX364807	KY693781
<i>C. lateralis</i>	Cui 12563	Ground	China	KX364789	KX364808	KY693782
<i>C. lateralis</i>	Dai 13564	Ground	China	KX364790	KX364809	-
<i>C. lenis</i>	Dai 22367	Ground	China	OL691608	OL691617	OL691613
<i>C. lenis</i>	Dai 22373	Ground	China	-	OL691618	OL691614
<i>C. lenis</i>	Dai 22374	Ground	China	OL691609	OL691619	OL691615
<i>C. macropora</i>	Cui 9019	Ground	China	KU360680	KJ000220	KY693783
<i>C. macropora</i>	Cui 9039	Ground	China	KU360681	KJ000221	KY693784
<i>C. minima</i>	Dai 15206	Ground	China	KU360682	KU360649	KY693785
<i>C. minima</i>	Dai 15222	Ground	China	KU360683	KU360650	KY693786
<i>C. minor</i>	Dai 16088	Rotten wood	China	KU360684	KU360651	KY693787
<i>C. montagnei</i>	Cui 10169	Ground	China	KU360685	KU360652	KY693788
<i>C. montagnei</i>	Dai 12137	Ground	China	-	KX364810	KY693789
<i>C. navispora</i>	MCA 3921	Fallen wood	Guyana	KC155387	KC155386	-
<i>C. navispora</i>	TH 9529	Fallen wood	Guyana	KT339262	-	-
<i>C. oblectabilis</i>	AMV 2255	Ground	Colombia	KT354690	-	-
<i>C. oblectabilis</i>	TH 9187	Ground	Guyana	KC155387	-	-
<i>C. perennis</i>	Cui 10318	Ground	China	KU360686	KJ000224	KY693790
<i>C. perennis</i>	Cui 10319	Ground	China	KU360687	KU360653	KY693791
<i>C. perennis</i>	JV 0809/66	Ground	USA	KX364791	KX364811	KY693792
<i>C. pseudodependens</i>	Cui 8138	Rotten wood	China	KJ540931	KJ000227	KY693816
<i>C. pseudodependens</i>	Cui 12582	Rotten wood	China	KX364801	KX364821	KY693817
<i>C. pusilla</i>	Dai 15168	Rotten wood	China	KU360701	KU360667	KY693818
<i>C. pusilla</i>	MN 26.7.95	Rotten wood	Japan	-	AY059060	-
<i>C. raigadensis</i>	<b>AMH 10511T</b>	<b>Ground</b>	<b>India</b>	<b>OR072877</b>	-	-
<i>C. raigadensis</i>	<b>MMH 1211</b>	<b>Ground</b>	<b>India</b>	<b>OR072932</b>	<b>OR053821</b>	<b>OR053822</b>
<i>C. rigida</i>	Dai 13622a	Ground	China	KX364793	KX364813	KY693796
<i>C. rigida</i>	Dai 16322	Ground	China	KX364794	KX364814	KY693797
<i>C. sinoperennis</i>	Dai 11625	Ground	China	KY693735	KY693753	KY693804
<i>C. sinoperennis</i>	Dai 13095	Ground	China	KY693736	KY693754	KY693805
<i>C. sonorensis</i>	RV 13144	Ground	Mexico	-	HQ439179	-
<i>C. strigosipes</i>	Dai 15145	Ground	China	KX364795	KX364815	KY693798
<i>C. strigosipes</i>	Dai 15586	Ground	China	KU360692	KU360658	KY693799
<i>C. subcinnamomea</i>	Dai 17016	Ground	China	KY693740	KY693755	KY693810
<i>C. subcinnamomea</i>	Dai 17022	Ground	China	-	KY693756	KY693811
<i>C. subglobosa</i>	Dai 15158	Rotten wood	China	KU360702	KU360669	KY693820
<i>C. subglobosa</i>	Yuan 6253	Rotten wood	China	-	KX364822	KY693821
<i>C. subverrucata</i>	Dai 12919	Ground	China	MT174242	MT174235	MT174233
<i>C. subverrucata</i>	Dai 15600	Ground	China	MT174243	MT174236	MT174234
<i>C. tenuihypha</i>	Dai 22684	Ground	China	OL691610	OL691620	-
<i>C. tenuihypha</i>	Dai 22690	Ground	China	OL691611	OL691621	-
<i>C. tibetica</i>	Cui 12208	Dead tree of <i>Picea</i>	China	MZ484551	MZ437407	-
<i>C. velutina</i>	Dai 16980	Ground	China	-	KY693752	-
<i>C. verrucata</i>	Dai 15120	Ground	China	KU360694	KU360660	KY693801
<i>C. verrucata</i>	Dai 15125	Ground	China	KU360695	KU360661	KY693802
<i>C. weii</i>	Cui 11011	Ground	China	KU360698	KU360664	KY693806
<i>C. weii</i>	Cui 12624	Ground	China	KX364796	KX364816	KY693807
<i>C. weii</i>	Dai 13422	Ground	China	KX364797	KX364817	KY693808
<i>C. wenshanensis</i>	Dai 15585	Ground	China	KX364798	KX364818	KY693809
<i>Fomitiporella chinensis</i>	Cui 11230	Rotten wood of <i>Quercus</i>	China	KX181309	KY693759	-
<i>Inonotus griseus</i>	Dai 13436	Rotten wood	China	KX364802	KX364823	-



**Fig. 1.** – Macromorphologies of *Coltricia raigadensis* (AMH 10511, holotype). A, B: Basidiomata in the natural habitat. C: Basidiomata with scale bar. D: Basidiomata showing abhyemial surface with close-up view in inset image.

70 mm wide and 23 mm thick at base), smaller pores (7–8 per mm) and basidia (11–14 × 6–8  $\mu\text{m}$ ; Bian & Dai, 2017). Another species *C. lateralis*, phylogenetically close to *C. raigadensis* but primarily differs in its laterally stipitate smaller basidiocarps (pileus up to 15 mm diam), larger basidia (23–29 × 7–8  $\mu\text{m}$ ) and broadly ellipsoid larger basidiospores (7–8 × 5–6  $\mu\text{m}$ ) (Bian & Dai, 2017).

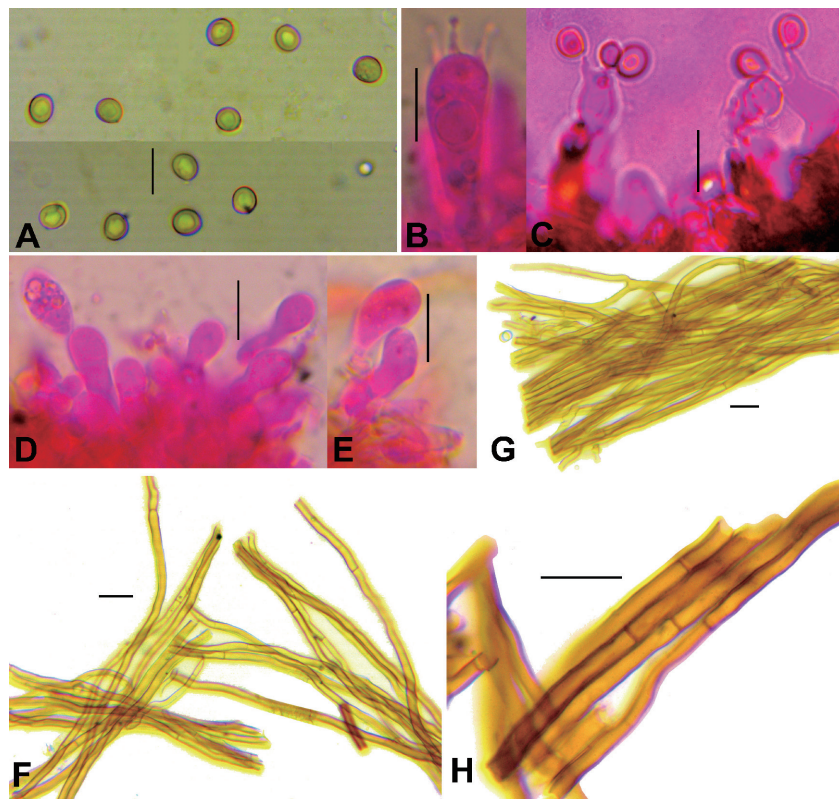
The combined dataset alignment contained 3459 characters, which includes the aligned sequence dataset composed of 1094 bp from nrITS, 1357 bp from nrLSU, and 1008 bp from nrSSU for the analyses. The exhaustive ILD test analysis with 1000 bootstrap showed congruence with the p value 0.85 at the significance level of 0.05. So, the dataset was combined for the further analysis. Based on combined analysis using ML and Bayesian methods we obtained similar tree topologies. The above morphological results were strongly supported by the molecular data and our phylogenetic study revealed monophyletic origin of *C. raigadensis* and appeared as sister to *C. hamata* (Romell) Ryvardeen with moderate bootstrap and posterior probability supports (0.86/ 85) (Fig. 3). Moreover, it falls in the clade shared by *C. lateralis* L.S. Bian & Y.C. Dai, *C. velutina* and *C. rigida* (Fig. 3). Based on the above morphological and molecular dataset, here we propose the new species *C. raigadensis*, under the genus *Coltricia*.

### Disclosure

The authors declare no conflicts of interest.

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**Fig. 2.** – Microscopic characters of *Coltricia raigadensis* (AMH 10511, holotype). A: Basidiospores. B, C: Basidia. D, E: Basidioles. F: Hyphae from trama. G: Hyphae from context. H: Hyphae from stipe. Bars: A–E 10  $\mu\text{m}$ ; F–H 20  $\mu\text{m}$ .



**Fig. 3.** – Maximum likelihood phylogram of *Coltricia* generated from the combined dataset of nrITS, nrLSU, and nrSSU sequences. Bootstrap values (BS) > 70 and Bayesian posterior probability (PP) > 0.8 are given at the internodes. *Fomitiporella chinensis* and *Inonotus griseus* are selected as outgroups. Scale bar represents a phylogenetic distance of 0.08 nucleotide substitutions per site.

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