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Murinicarpus subadustus: a new record from India, its morphology and phylogeny

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Murinicarpus subadustus described from China is the only species of the genus *Murinicarpus* (*Polyporales*, *Basidiomycota*). There is no other report of this genus from any other country except China. Here, we report *M. subadustus* as a novelty to the macrofungal biota of India. This unique species is identified based on morphological features and nrDNA ITS-based phylogenetic analysis. A thorough macro- and microscopic characterisation along with field photographs, line drawings of microscopic structures and comparisons with morphologically and phylogenetically related taxa are provided.

Key words: *Agaricomycetes*, phylogeny, *Polyporaceae*, taxonomy.

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Murinicarpus subadustus, popsaný z Číny, je jediným druhem rodu *Murinicarpus* (*Polyporales*, *Basidiomycota*). Dosud nebyl zaznamenán v jiné zemi než právě v Číně. Zde je *M. subadustus* představen coby nový přírůstek mezi makromycety Indie. Identita tohoto jedinečného druhu je potvrzena na základě morfologických znaků a fylogenetické analýzy ITS nrDNA. Je podána podrobná charakteristika makro- a mikroskopických znaků, doplněná fotografiemi z terénu a kresbami mikroznaků, a provedeno srovnání s morfologicky a fylogeneticky příbuznými taxony.

INTRODUCTION

The genus *Murinicarpus* B.K. Cui et Y.C. Dai (*Polyporaceae*) was originally described from China with *M. subadustus* (Z.S. Bi et G.Y. Zheng) B.K. Cui et Y.C. Dai as the type (Cui et al. 2019). The type species was originally placed in

Wrightoporia (Zheng et Bi 1987), later in *Perenniporia* (Dai et al. 2002). Species of *Murinicarpus* are circumscribed as possessing stipitate basidiocarps with greyish pilei, dextrinoid skeletal hyphae in Melzer's reagent, thick-walled cystidia, and thick-walled, non-truncate, hyaline, ellipsoid basidiospores (Cui et al. 2019). *Murinicarpus subadustus* shares features with *Perenniporia* such as the dimitic hyphal system with dextrinoid skeletal hyphae and thick-walled, ellipsoid basidiospores, but the stipitate nature of the basidiocarps and thick-walled, apically encrusted cystidia distinctly differentiate *M. subadustus* from *Perennipora* species (Cui et al. 2019).

The state of West Bengal possesses a wide range of phytogeographical regions with various altitudinal, climatic and edaphic factors which favour abundant occurrence of various organisms including macrofungi (Saha et al. 2019). During a survey of macrofungi in the state West Bengal in the year 2017, a specimen was collected from Sukna, a subtropical broadleaved forest type of Darjeeling district, and identified as *M. subadustus*.

As there was no earlier report of *M. subadustus* from India, the present paper reports the taxon as a new record for India based on detailed morphological characterisation and phylogenetic analyses.

MATERIAL AND METHODS

Morphological protocols. The colour photographs of the specimen were taken in the field using a Nikon D300s digital camera (Tokyo, Japan). The habit and habitat of the collected specimen were carefully observed and macro-morphological data were recorded from the fresh basidiocarps in the field. For colour terms and respective codes, Methuen Handbook of Colour was followed (Kornerup et Wanscher 1978).

Micro-morphological features were obtained by mounting thin sections of dried basidiocarps in 10% KOH, and stained with Congo Red. A Carl Zeiss AXIO Imager A1 phase contrast microscope (Zeiss, Oberkochen, Germany) was used to examine the microscopic details of the specimen. Systematic identification of the collected specimen was carried out following Cui et al. (2019). The basidiospores were measured by observing thirty basidiospores. In the microscopic description, the Q value indicates the length/width ratio, while the mean Q value (Q_m) was calculated by dividing the total sum of Q values by the total number of spores measured. Hand drawings of various microscopic features were performed with the use of a camera lucida and a 0.1 mm rotring pen. The properly identified specimen was dried at a temperature of 40 °C, preserved following Pradhan et al. (2015), and deposited in the Calcutta University Herbarium (CUH), Kolkata, India.

Phylogenetic protocols.

DNA extraction, PCR and sequencing. Genomic DNA was extracted as described in Dutta et al. (2014). ITS1 and ITS4 primers were applied to amplify the entire nrITS region (White et al. 1990). The PCR reaction included a starting temperature of 94 °C for 4 min followed by 30 cycles at 94 °C for 30 s, 55 °C for 30 s, 72 °C for 1 min, and a final elongation step of 5 min at 72 °C. For purification of the amplified PCR product, a QIAquick® Gel Extraction Kit was used (QIAGEN, Hilden, Germany). DNA sequencing was done on an ABI3730xl DNA Analyzer (Applied Biosystems, Foster City, CA, USA) using the same primers as mentioned above. The quality of the generated sequencing data

was checked by editing the chromatogram in BioEdit sequence alignment editor version 7.2.5 (Hall 1999). The newly generated sequence was deposited in GenBank (www.ncbi.nlm.nih.gov).

Acquisition of sequences and preparation of datasets. The dataset was prepared by including 30 nrITS sequences, of which one was newly generated for the study, and the remaining 29 were downloaded from GenBank based on the BLAST search result and following earlier studies by Ji et al. (2017) and Wang et al. (2020). *Stereum hirsutum* (Willd.) Pers. and *Heterobasidion annosum* (Fr.) Bref. were taken as outgroup taxa following Chen et al. (2021). The GenBank accession numbers for the freshly generated sequence and the sequences retrieved from GenBank are given in Fig. 1.

Sequence alignment and phylogenetic analysis. The dataset was generated with the nrITS sequences and aligned with MAFFT v. 7.490 on XSEDE (Katoh et al. 2002) with default settings, and then manually adjusted in MEGA v. 7.0 (Kumar et al. 2016). Using the Cyber Infrastructure for Phylogenetic Research (CIPRES) web portal (https://www.phylo.org/portal2/; Miller et al. 2010),

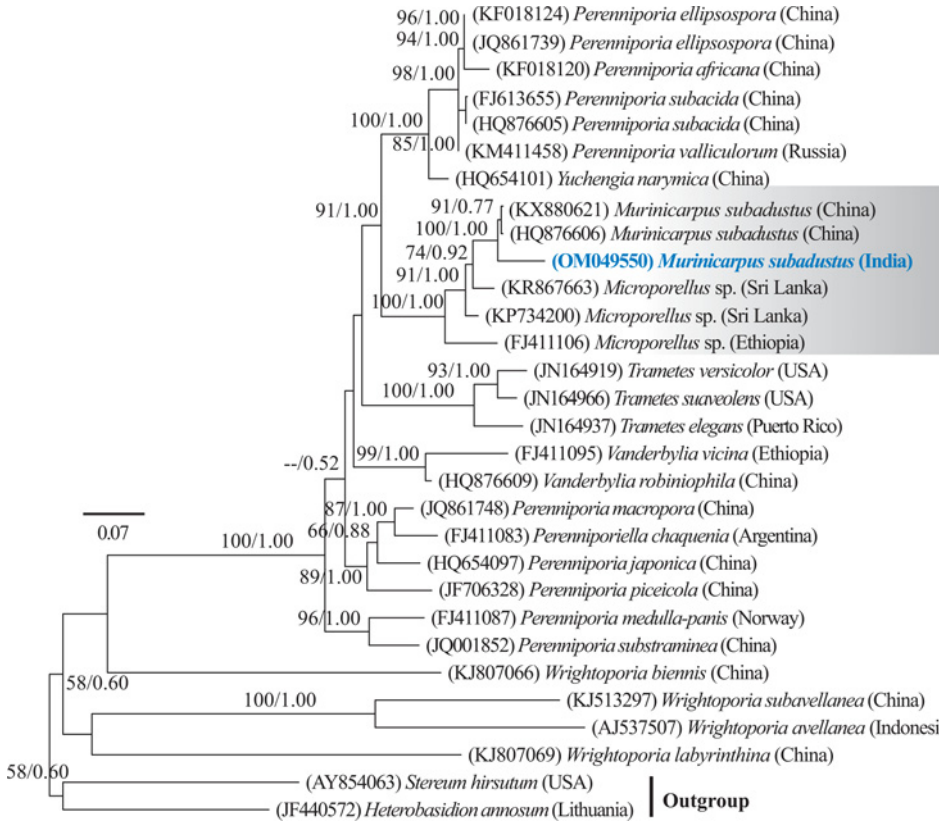

Fig. 1. Maximum likelihood (ML) tree constructed using the HKY+I+G model of nucleotide evolution (*-lnL* = 6158.10493) based on nrDNA ITS sequence data. Branches are labelled with ML bootstrap support and Bayesian posterior probabilities (PP). MLBS > 50% and PP > 0.50 values are displayed above or below the branches, respectively. The scale bar indicates the expected changes per site. The Indian collection of *Murinicarpus subadustus* is shown in blue bold font to highlight its position in the phylogenetic tree.

the statistically most suitable model of nucleotide substitution was determined with the jModeltest v. 2.1.6 software (Darriba et al. 2012) on XSEDE. The HKY+I+G model was chosen as the most suitable model for the alignment based on the lowest BIC (Bayesian information criterion) value of 12735.843675. Maximum likelihood bootstrapping (MLBS) analyses were carried out using RAxML-HPC v. 8.2.12 (Stamatakis 2006) with the above selected model (HKY+I+G model) with 1,000 rapid bootstrap replicates on the CIPRES NSF XSEDE resource. Bayesian inference (BI) of the phylogeny was carried out with the MrBayes v. 3.2.2 application (Ronquist et al. 2012). Markov chains were run (Geyer 1991) for 10^6 generations and trees were sampled every 100^{th} generation. The first 25% of sampled trees were discarded as burn-in and the remaining ones were used to reconstruct a majority rule consensus and calculate PPs (posterior probabilities) of the clades. Branches that received bootstrap support for maximum likelihood (MLBS) >50% and Bayesian posterior probabilities (PP) >0.50 are displayed in the resulting phylogenetic tree.

RESULTS

Molecular phylogenetic analyses

The sequencing product of the *Murinicarpus subadustus* specimen was 733 nucleotides long. Alignment of the nrITS sequences and trimming of both its ends yielded a dataset of 704 base pairs. The trees constructed based on the ML and Bayesian analyses were equal in topology. Therefore, only the phylogenetic tree constructed according to the ML analysis is displayed in Fig. 1. In this tree, the Indian collection of *M. subadustus* clusters together with two Chinese sequences of the same taxon with full statistical support values (100% BS, 1.00 PP). In the same clade, three unnamed sequences of *Microporellus* Murrill, two from Sri Lanka (GenBank KR867663 and KP734200) and the other from Ethiopia (GenBank FJ411106), appear basal to the cluster of *M. subadustus* (100% BS, 1.00 PP; Fig. 1). However, details of these three unnamed sequences of *Microporellus* species cannot be verified, as all of them lack any morphological characteristics in published literature.

Murinicarpus subadustus (Z.S. Bi et G.Y. Zheng) B.K. Cui et Y.C. Dai, in Cui, Li, Ji, Zhou, Song, Si, Yang et Dai, Fungal Diversity 97: 255, 2019 Figs. 2–3

D e s c r i p t i o n. Basidiocarp annual, laterally stipitate, pileate. Pileus 10–30 mm long, 8–20 mm wide, ca. 5 mm thick; dimidiate, surface greyish red (7B3) towards margin, brown (7E5) towards base when fresh, becoming entirely light brown (7D4) on drying, concentrically zonate, glabrous; margin entire, thin, greyish red (7B3). Context 1–2 mm thick, brownish grey (7C2) when dry. Hymenophore white (1A1) to reddish grey (7B2), poroid, pores angular, 2–3 per mm. Tube layer 1–3 mm deep, brownish grey (7C2) when dry. Stipe 15–17 mm long, 3–5 mm wide, flattened, brown (7E5) to reddish brown (8E5) when fresh, brownish grey (7C2) on drying.

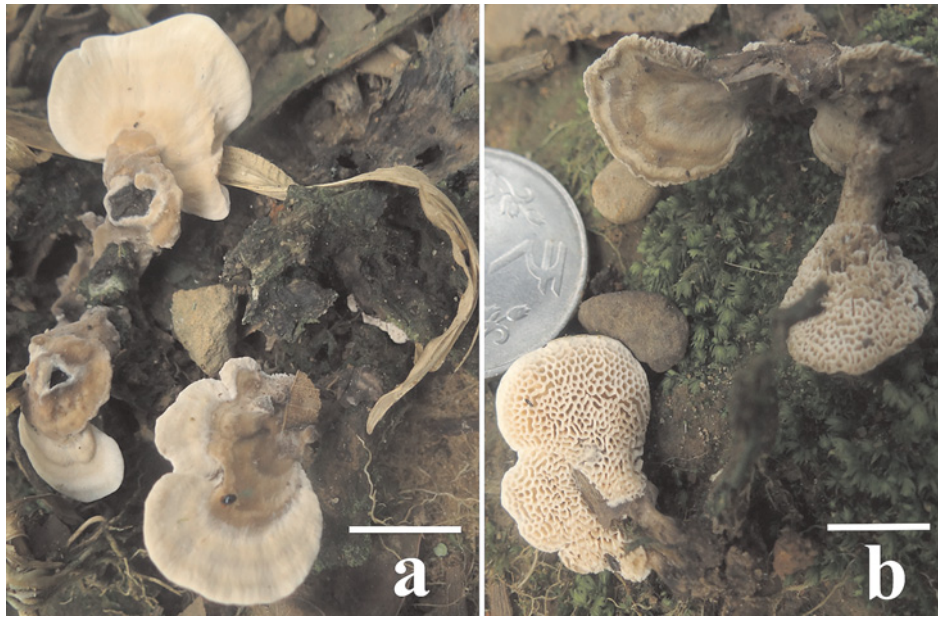

Fig. 2. *Murinicarpus subadustus* (CUH AM345): **a–b** – photographs of fresh basidiocarps in situ. Scale bars = 10 mm. Photo R. Saha.

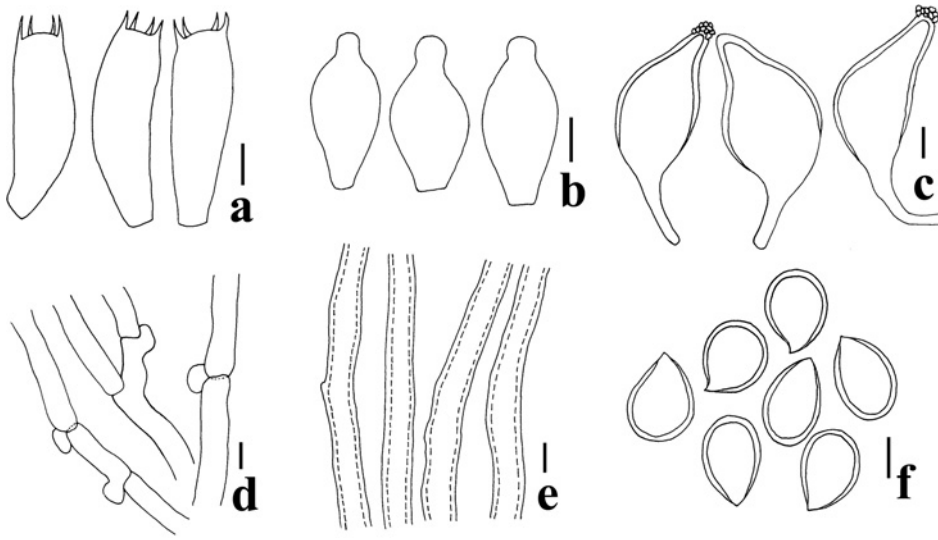

Fig. 3. Microscopic features of *Murinicarpus subadustus* (CUH AM345): **a** – basidia; **b** – cystidioles; **c** – cystidia; **d** – generative hyphae; **e** – skeletal hyphae; **f –** basidiospores. Scale bars = 5 µm. Drawings by R. Saha.

Hyphal system dimitic. Generative hyphae 2.7–6.7 µm wide, hyaline, branched, thin-walled, clamp-connections present. Skeletal hyphae 3.3–7.4 µm wide, thick-walled with narrow lumen, hyaline in KOH, cyanophilous in cotton blue, strongly dextrinoid with Melzer's reagent. Cystidia 26.7–47.3 × 11.6–16.7 µm, fusoid, apically encrusted, hyaline, thick-walled. Cystidioles 16.6–20.7 × 6.6–8.4 µm, fusoid, hyaline, thin-walled. Dendrohyphidia absent. Basidia 16.6–23.3 × 6.6–10 µm, clavate, hyaline, thin-walled, 4-spored. Basidiospores 6.6–6.9–7.6(9.0) × 3.3–4.0–6.0 µm, Q = 1.4–2.0, Q_m = 1.78, drop-shaped to ellipsoid, hyaline, smooth, thick-walled, inamyloid, cyanophilous.

Habit and habitat. Solitary to gregarious, on rotten wood.

Specimen examined

India. West Bengal, Darjeeling District, Sukna, 26°47'18.6" N, 88°21'45.0" E, 1527 m a.s.l., on rotten wood, 17 September 2017, leg. et det. R. Saha et K. Acharya, CUH AM345 (RSKA-35/2017).

DISCUSSION

Murinicarpus subadustus was previously only reported from China. The present collection morphologically and phylogenetically matches with the earlier reported Chinese specimens (Cui et al. 2019). Hence, this is the first report of this taxon from India.

Among morphologically and phylogenetically related taxa (Fig. 1), *Perenniporia ellipsospora* Ryvarden et Gilb. differs from *M. subadustus* by the presence of resupinate basidiocarps, absence of cystidia and cystidioles (Cui et al. 2019); *Perenniporia africana* Ipulet et Ryvarden possesses resupinate basidiocarps and lacks cystidia (Cui et al. 2019); *Perenniporia subacida* (Peck) Donk has resupinate basidiocarps and a trimitic type of hyphal system (Cui et al. 2019); and *Yuchengia narymica* (Pilát) B.K. Cui differs from *M. subadustus* in the presence of resupinate basidiocarps and smaller basidiospores measuring (4.2)4.5–5.8(6.2) × (2.6)2.8–3.9(4.1) µm, and in the absence of cystidia (Spirin et al. 2005).

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