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Coprinopsis natarajanii sp. nov., a new marine basidiomycete from Puducherry mangroves, East coast of India

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ABSTRACT

Only a few basidiomycetous marine fungi are known from marine environments, while *Ascomycota* predominate the marine environments. In this paper, a novel species of *Coprinopsis* is reported from Indian mangroves based on the morphological and molecular phylogenetic analyses. The basidiomata and the pleurocystidia of the new species are smaller when compared to the other species of *Coprinopsis*.

Keywords: Basidiomycete, Mangrove fungi, Novel species, Phylogeny, Taxonomy

INTRODUCTION

Jones *et al.* (2019) listed 1,257 species of marine fungi belonging to 539 genera of which only 22 species in 17 genera belong to filamentous *Basidiomycota* whereas 805 species in 352 genera belong to *Ascomycota*. Devadatha *et al.* (2021) reported 850 mangrove fungi of which 58 species belong to *Basidiomycota*. Sarma and Devadatha (2020) listed 35 species in 28 genera from *Basidiomycota* reported from Indian mangroves including the terrestrial basidiomycetes. Marine *Basidiomycetes* are few in number when compared to their terrestrial counterparts and are an ecological group, and taxonomically diverse (*Agariomycotina, Uredinomycotina* and *Ustilaginomycotina*). Marine *Basidiomycetes* are well adapted to their habitats, with reduced basidiomata. Marine species are known only as teleomorphs with basidiospores generally released passively (Jones and Choeyklin 2008). Jones (2011) indicated that many *Basidiomycetes* are still to be discovered and described.

Studies on the macrofungal diversity in mangroves are scant and this is especially true with respect to India. Dutta *et al.* (2013) and Ghate and Sridhar (2016) have given an insight into the macrofungal diversity associated with mangroves of Sunderbans and Southwest coast of India, respectively. A number of studies on microfungal diversity from mangrove plants or habitats have been carried out in India (Borse 1988; Chinnaraj 1993; Ravikumar and Vittal 1996; Sarma and Vittal 2000, 2001; Maria and Sridhar 2002, 2003, 2004; Vittal and Sarma 2006). In a survey on litter degrading fungi from Puducherry mangroves a species of *Coprinopsis* P. Karst. was recorded. This species of *Coprinopsis*, was found to be a novel species although 27 species of *Coprinopsis* have been reported from India (Amandeep *et al.* 2014). *Coprinopsis* is characterized by cutis type pileipellis that differentiates it from its related genera like *Coprinellus* P. Karst., and *Parasola* Redhead, Vilgalys & Hopple., in *Psathyrellaceae* (Readhead *et al.* 2001). Further, the presence of pleurocystidia differentiates it from *Coprinus* Pers. that is presently placed in the family *Agaricaceae*. Amandeep *et al.* (2014) documented the diversity of *Coprinopsis* species from the coprophilous habitats in India.

Coprinopsis recorded in the present study was studied morphologically. However, due to absence of mature specimens, the description of the species lacks details of basidiospores and basidia. Since there are no reports of occurrence of *Coprinopsis* in the submerged parts of the mangroves, ITS sequence analysis was done to prove the novelty of the species.

MATERIALS AND METHODS

Morphological characterization

The dead and decaying samples of *Avicennia marina* (Forssk.) Vierh. wood was collected in large plastic bags from Pondicherry mangroves, Puducherry in India and transported to the laboratory. Morphological characters were studied using an Optika stereo zoom SZM-LED1 microscope and Nikon ECLIPSE TiU upright microscope, photographs were taken using Nikon DS-Fi2 digital camera fitted to the microscope. Microscopic examination of anatomical characters was done following Largent *et al.* (1977). Photoplates were prepared using Adobe Photoshop CS6 updated version 13.0.1 software (Adobe Systems Inc., The United States) while Nikon NIS-Elements-Imaging Software version 4.4 program was used for measurements. The specimen (air-dried wood with fungal material) was deposited at Ajrekar Mycological Herbarium (AMH), Agharkar Research Institute (ARI), Pune, India.

DNA extraction and PCR amplification

Genomic DNA was extracted using the GeneJET Plant Genomic DNA Purification kit (Thermo Fisher Scientific Inc., US) following the manufacturer's protocol, from the freshly collected specimens of *Coprinopsis* species. DNA purity was checked and estimated by Thermo Scientific™ NanoDrop 2000. In this study, the ITS sequence was amplified by PCR, following methods laid down by White *et al.* (1990). All PCR amplification reactions were performed in an Eppendorf Master cycler with a total volume of 50µL, consisting of 25µL of AMPLIQON Taq DNA Polymerase 2X Master Mix RED, 1 µL of each primer (10 µM), 2µL DNA template and the remaining volume was made up with that of HiMEDIA Molecular Biology Grade Water.

The PCR amplification products were checked on 1% agarose gel stained with ethidium bromide. The PCR products were

purified by using QIAquick PCR Purification Kit (QIAGEN, Germany) by following manufacturer's protocol. The purified PCR products were estimated by Thermo Scientific™ NanoDrop 2000 and DNA sequencing was outsourced.

Sequence alignment and molecular phylogeny

Sequences used in the phylogenetic analyses were downloaded from GenBank based on BLAST search similarity and recently published data (Gierczyk *et al.* 2017; Nguyen *et al.* 2019). Sequence alignments were performed for individual gene regions at (http://mafft.cbrc.jp/alignment/server), using iterative refinements as E-INS-i method for ITS. The unaligned portions were aligned manually using BioEdit v.7.0.5.2 (Hall 1999). The sequence alignment was converted to PHYLIP format (.phy) using ALTER (alignment transformation environment: (http://sing.eiuvigo.es/ALTER/, 2017)) for Randomized Accelerated Maximum Likelihood (RAxML) analysis.

Maximum Likelihood analyses were conducted using the RAxML-HPC2 on XSEDE (8.2.8) with GTR + G + I model (Stamatakis *et al.* 2008, Stamatakis 2014) in the CIPRES Science Gateway platform (Miller *et al.* 2010). Phylogenetic trees were visualized with FigTree v1.4.0 program (Rambaut 2012) and reorganized in Microsoft power point (2016). Sequences generated in this study were deposited in GenBank.

RESULTS

Taxonomy

Coprinopsis natarajanii Devadatha, V. Kumaresan and V.V. Sarma sp. nov.

Holotype: AMH-10164

Index Fungorum No.: IF558487; Figs. a-f

Etymology: In honour of Late Prof. K. Natarajan of CAS in Botany, University of Madras for his contributions to *Basidiomycota* of India.

Basidiomata gregarious (Fig. 1a). Pileus 2-10 mm diam. in button stage with closed pileus, 6-18 mm diam. when mature, conical to campanulate, white to pale brown at the disc, white towards the margin, pileal veil appressed fibrillose, flesh thin, membranous. Lamellae free to adnexed, crowded, up to 0.1 cm broad, white, concolorous. Stipe 10-20 × 3-5 mm, cylindrical, slightly tapering upwards, white, covered with fine erect fibrils, sericeous underneath, context hollow, white. Basidia and basidiospores not observed. Basidioles clavate, 18-21 × 9-11 µm. Pleurocystidia 50-60 µm × 10-15 µm, elongate clavate to fusoid ventricose. Cheilocystidia 40-50 µm × 6-10 µm, similar in shape to pleurocystidia. Pileus cuticle a cutis having veil in the form of hyphal chains. Context thin, composed of filamentous, radially arranged thin-walled hyphae, 4-10 µm, Hymenophoral trama subregular, composed of thin-walled, hyaline, hyphae up to 11 µm broad. Stipe cuticle hyphal, smooth, context white. Clamp connections observed. Smells mushroomy, no taste recorded.

Material examined: INDIA, Tamil Nadu, Pondicherry, Veerampattinam mangroves, on decaying wood of *Avicennia marina* (*Acanthaceae*), 20 January 2017, B. Devadatha (AMH-10164, holotype).

Notes: Morphological details and the BLAST search analysis based on ITS sequence data showed that the collected taxon belongs to the genus *Coprinopsis*. Further, phylogenetic analysis along with ITS sequence data of *Coprinopsis* species revealed that the present new taxon, *Coprinopsis natarajanii*, is clustered in the clade containing *Coprinopsis* taxa with strong support from maximum likelihood analyses (98% BS). The morophology of *C. natarajanii* is distinct from *C. phaeopunctata* (Esteve-Rav. & A. Ortega) Valade in having smaller size of pleurocystidia (50-60 µm × 10-15 µm vs 35-75 (100) × 25-50 µm). The new species described herein was recorded on decaying wood of *Avicennia marina*, a mangrove plant in marine environments, whereas *C. phaeopunctata* has been recorded on rotten wood and debris of *Abies pinsapo* (Ortega and Esteve-Raventos 2003) from terrestrial environments. Further, ITS nucleotide sequence comparison between *C. phaeopunctata* and *C. natarajanii* revealed 4.2% (28) nucleotide base pair difference which supports the establishment of a new species for our new taxon (Jeewon and Hyde, 2017). Thus, based on the differences in the molecular analysis as shown in the phylogenetic tree and the differences in the micromorphological characters, substrate and habitat, the new species, *C. natarajanii* is introduced in the genus *Coprinopsis*.

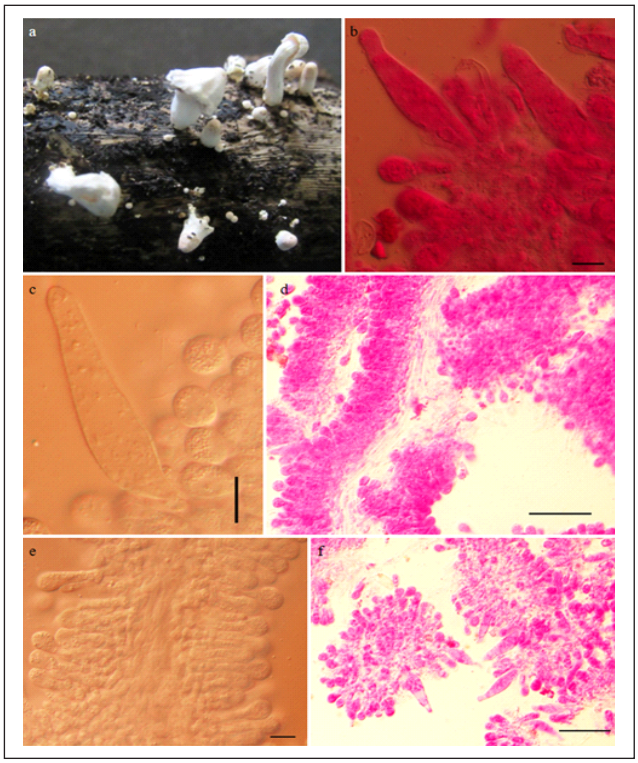

Fig. 1: *Coprinopsis natarajanii* a-b. Basidiomata on the decaying wood. b-c. Pleurocystidia. d-e. Basidioles and pseudoparaphysis in gill trama. f. Cheilocystidia. Scale bars: d,f = 50 µm. b-c,e = 10 µm

Molecular phylogeny

The ITS gene dataset composed of 69 taxa with 763 nucleotide characters from *Psathyrellaceae*, with *Lacrymaria lacrymabunda* SZMC:NL:2140 as outgroup. RAxML analysis of the ITS gene dataset yielded the best scoring tree (Fig. 1) with a final ML optimization likelihood value of -7635.776556. The matrix had 464 distinct alignment patterns, with 21.82% of undetermined characters or gaps. Estimated base frequencies were as follows: A = 0.233036, C = 0.236203, G = 0.230836, and T = 0.299925; substitution rates AC = 1.386642, AG = 2.956964, AT = 1.907401, CG = 0.514276, CT = 4.810990, and GT = 1.000000; proportion of invariable sites I = 0.320066; and gamma distribution shape parameter α = 0.592406.

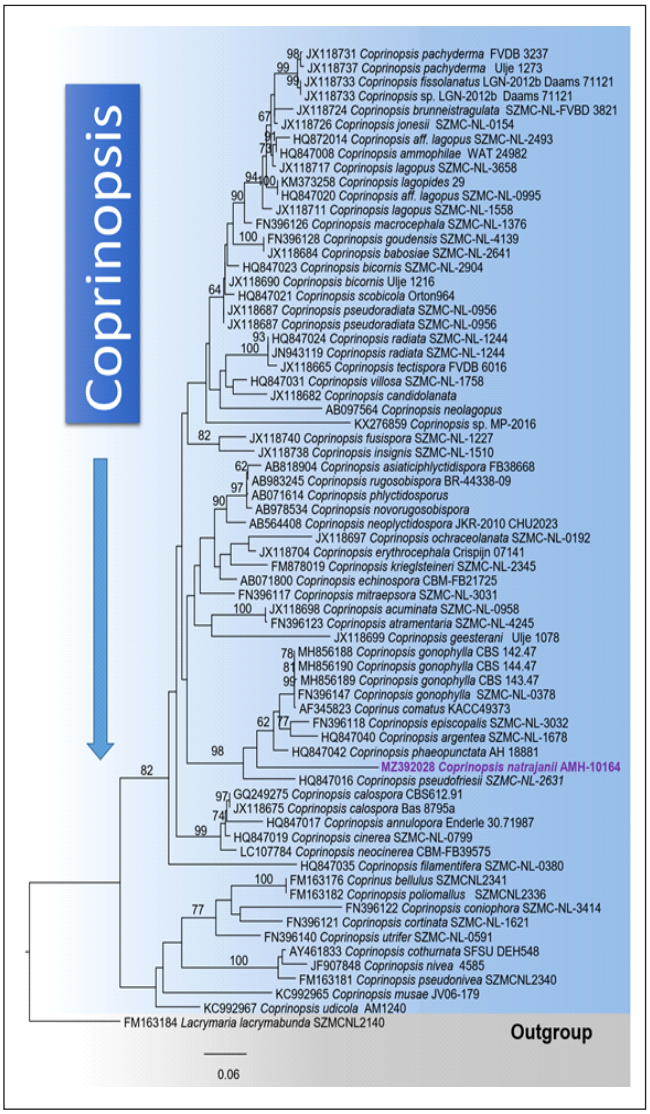

Fig. 2: Phylogram generated through maximum likelihood analyses based on nrITS nucleotide sequence data for *Coprinopsis natarajanii* and other species of *Coprinopsis* with *Lacrymaria lacrymabunda* as the outgroup taxon. Maximum likelihood bootstrap values ≥60% are given at the nodes. The new isolate is in purple colour

Phylogenetic analyses based on ITS nucleotide sequence dataset indicate that our new species C*oprinopsis natarajanii* grouped within the *Coprinopsis*. Our taxon *C. natarajanii* forms a distinct lineage basal to *Coprinopsis phaeopunctata* although, the position is unsupported. The tree topology is supported by lower bootstrap values (62% ML; Fig. 2) indicating that *C. natarajanii* is a new taxon.

DISCUSSION

Only a few filamentous *Basidiomycetes* are considered as marine fungi reported from marine environments. In our ongoing investigations on the marine fungal diversity in mangroves of Pondicherry, East coast of India, we have encountered a small basidiomycete which fits in *Coprinopsis*. Further characterisation of the taxon based on morphological and molecular phylogenetic analyses, based on single-locus (ITS) maximum likelihood tree revealed that it is a novel species within the genus *Coprinopsis*. Hence *C. natarajanii* is described herein as a new species.

Coprinopsis species are normally recorded from dung habitats (Amandeep *et al.,* 2014) and very few from plant litter. A few species of *Coprinopsis* have earlier been reported from mangroves including *Coprinopsis atramentaria* (Bull.) Redhead, Vilgalys & Moncalvo and *C. lagopus* (Fr.) Redhead, Vilgalys & Moncalvo, but not as marine fungi. *Coprinopsis lagopus* has been recorded from Sundarban mangroves (Dutta *et al.* 2013), which is known to possess larger fruit-bodies (Pileus with 30-90 mm diam.) and broader pleuro- and cheilocystidia compared to *C. natarajanii.* Li *et al.* (2016), while studying the endophyte diversity of mangrove fungi from southern China, isolated *Coprinopsis atramentaria* from the twigs of *Kandelia candel*. The identification of this species, however, was based on ITS sequence analysis. *Coprinopsis atramentaria* has earlier been described to consist larger fruit-bodies (Pileus with 30-60 mm diam) and longer and broader pleuro- and cheilocystidia (Orton and Watling 1979).

Molecular studies revealed, the genus *Coprinus* is subdivided into *Coprinus sensu stricto* (*Agaricaceae*), *Coprinellus*, *Coprinopsis* and *Parasola* in the family *Psathyrellaceae* and among these the generic name *Coprinopsis* P. Karst. has not been in active use for 100 years (Redhead *et al.* 2001). It is characterized by pileipellis, which is not a hymeniderm, not a cystoderm but a cutis. At present there are about 150 species belonging to this genus. Most of them are known to colonize dung materials. Very few are known from other substrata.

Our new species, as already mentioned in the notes section, differs morphologically and also on molecular basis. Hence the new species *C. natarajanii* in the genus *Coprinopsis* is reported in this paper.

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