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# *Bactrospora mangrovei sp. nov.*, a Novel Marine Lichenized Fungus from Muthupet Mangroves of India Based on Morpho-molecular Data

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## ABSTRACT

**Muthupet mangrove forests in Tamil Nadu is relatively a smaller belt when compared to other mangrove forests on the east coast of India. On 7 mangrove hosts we have recorded more than 78 fungi. The unravelling of novel marine fungi continues with the description of *Bactrospora mangrovei*, a new marine lichenized fungus, from the Muthupet mangroves, Tamil Nadu, East coast of India which is being reported in this paper. The species *B. mangrovei* is characterized by having apothecia that are numerous, frequently non-stromatic, round to irregular, coriaceous, reddish brown to black, asci bitunicate, cylindrical with short pedicels, apically rounded and ascospores uniseriate to biseriately arranged, fasciculate, filiform, 8-10 septate, partially overlapping, hyaline, rounded at both ends.**

**Keywords:** New species, Taxonomy, Lichenized fungi, Phylogeny

## INTRODUCTION

Mangroves have gained prime importance as hosts for marine fungi as has been found in the past 4 decades of research (Hyde *et al*., 2000; Sarma and Hyde, 2001; Sarma and Devadatha, 2020; Devadatha *et al*., 2021a; b). The diversity and ecology of east coast of India has been surveyed more intensively from Godavary and Krishna deltas of Andhra Pradesh state and a dichotomous key has been provided for the marine fungi from this region (Sarma and Vittal, 1998; 2000; 2001; 2002; 2004). From Tamil Nadu, the Pichavaram mangroves have been more thoroughly sampled than Muthupet mangroves. Around 200 marine fungi have been reported from the mangroves of East coast of India (Vittal and Sarma, 2006; Devadatha *et al*., 2020; Devadatha *et al*., 2021b; c). Most of these studies focused with morphological descriptions and enumerations in the past. However, with the advent of molecular era the molecular techniques have been employed in the case of studies on marine fungi from Muthupet mangroves along the east coast of India, which were complimenting the morphological analyses. More than 22 out of 78 marine fungi reported from Muthupet mangroves, Tamil Nadu, East coast of India have been found to be new to science and these have been already reported elsewhere (Devadatha and Sarma, 2018; Devadatha *et al*., 2018a; b; c; Devadatha *et al*., 2019; 2021c). Egea and Torrente, (1993) updated the genus *Bactrospora*. The species of *Bactrospora* is found in both equatorial and temperate climates, frequently on the protected or overhanging sides of trees. *Bactrospora* is characterized by having black, sessile, round apothecia with filiform ascospores, typically without the gel that holds the paraphysoids together. There are currently 47 known species of *Bactrospora* (Index fungorum, 2023). Most of the *Bactrospora* species were described based on morphological and lack molecular data (Sobreira *et al*., 2015). *Bactrospora* is placed incertae sedis in the order Arthoniales. In this study a new species *Bactrospora mangrovei* is introduced based on morphological characteristics and molecular phylogeny.

## MATERIALS AND METHODS

### Collection of samples, isolation, and morphological studies

Dead and decomposing *Rhizophora mucronata* wood samples were collected from the Muthupet mangroves and studied under an Optika stereo zoom SZM-LED1 microscope at magnifications ranging from 7 to 45X. A sterile razor blade was used to cut the apothecia into sections, sterile seawater and 10% KOH/KI were used to prepare slides for microscopic examination. The images were taken with a Nikon DS-Fi2 digital camera and a Nikon ECLIPSE TiU upright microscope. Photo plates were created using the updated Adobe Photoshop CS6 version 13.0.1 software, and measurements were obtained using the Nikon NIS-Elements-Imaging Software version 4.4 programme (Adobe Systems inc., The United States). The type herbarium specimen was deposited at the Agharkar Research Institute (ARI), Pune, India's Ajrekar Mycological Herbarium (AMH).

### DNA extraction, PCR amplification, and sequencing

Genomic DNA was isolated directly from the fruit bodies on freshly collected specimens by using DNeasy plant DNA extraction kit (QIAGEN, Germany). Polymerase chain reaction (PCR) was performed using the primers pairs: ITS1 and ITS4 for internal transcribed spacer (ITS) regions of rDNA (White *et al*., 1990) The amplifications were carried out using 50µL of Polymerase chain reaction

mixtures made up of 25µL of AMPLIQON 2X Master Mix RED, 1µL of each primer (10 µM), 5µL DNA and remaining volume of Nuclease free water. The Protocol used for PCR amplification of ITS gene region was used as in Devadatha *et al.* (2017). The amplified PCR products were purified and sent to Agri Genome in Kochi, Kerala, for sequencing.

**Sequence alignment and Phylogenetic analyses**

Phylogenetic analyses were performed using the sequences that were downloaded based on Blast search similarity and Gene Bank. The ITS gene region sequence alignments were performed online at the MAFFT server (http://mafft.cbrc.jp/alignment/ server/) (Katoh and Standley, 2013), and alignments were manually edited as needed using BioEdit. A maximum likelihood (ML) tree was constructed with RAxML-HPC2 on XSEDE (8.2.8) (Stamatakis *et al.*, 2008, Stamatakis, 2014) under the CIPRES Science Gateway platform (Miller *et al*., 2010). Above each node, maximum likelihood bootstrap values greater than 75% were provided. The phylogenetic trees were scrutinized using the FigTree v1.4.0 programme (Rambaut, 2012), and they were modernized using Microsoft Powerpoint 2016. Maximum likelihood bootstrap values higher than 75% were displayed above each node. The phylogenetic trees were examined with FigTree v1.4.0 software and modernised with Microsoft Powerpoint 2013.

## RESULTS

**Phylogenetic analyses**

The ITS gene dataset (composed of taxa with 700 nucleotide characters from *Parmeliaceae*, *Bactrospora* species (Arthoniales) and *Gypsoplaca macrophylla* AFTOL-ID 1703 as an outgroup taxon. RAxML analysis of the ITS gene dataset resulted in best scoring tree with a final ML optimization likelihood value of -7559.645033. The matrix had 520 distinct alignment patterns, with 21.67% of undetermined characters or gaps. Estimated base frequencies were as follows: A =0.220198, C =0.276020, G =0.263524 and T =0.240258; substitution rates AC =0.879814, AG =2.212563, AT =1.834435, CG =0.910391, CT =5.311602 and GT = 1.000000; proportion invariable sites I =0.244807 and gamma distribution shape parameter $\alpha = 1.456636$.

Phylogenetic analyses based on ITS nucleotide sequence dataset indicate that our taxon *B. mangrovei* shares sister group relation with *B. corticola* in a monophyletic clade with strong support from ML 100%.

**Taxonomy**

***Bactrospora mangrovei*** Devadatha and V.V. Sarma *sp. nov*. (**Figure 2, a-v**).

Index of Fungorum number: IF900347

**Etymology:** refers to mangrove habitat of the new taxon that occurred lichenized on decaying wood of *R. mucronata*.

**Sexual morph:** Thallus epiphytic, surface glossy, whitish to pale green. Apothecia 250-700 µm high, 325-1075 µm diam. ($\bar{x}$=420×635 µm, n=10), Apothecia numerous, frequently non-stromatic, round to irregular, superficial, solitary to gregarious, coriaceous, reddish brown to black. Disc circular, concave at first, becoming flat, reddish brown, margin undulate. Receptacle surface light brown and smooth. Excipulum 25-50 µm thick, comprising an outer stratum of hyaline, thick walled, globular cells, light brown only at the outside, and an inner stratum of hyaline scleroplectenchyma. Medullary excipulum 20-35 µm composed of hyaline textura intricata. Hymenium tall light brown to hyaline 100-200 µm. Subhymenium pale brown to hyaline. Paraphysoids 1-2.5 µm ($\bar{x}$=1.9 µm, n=20) wide, hyaline, septate, filiform, apically branching, swollen at the apex, some with brown swellings of similar length to the asci. Epithecium 10-30 µm tall with pale brown to hyaline crystals of 1-3 µm diam., negative in KI. Asci 60-90 × 7.5-17 µm ($\bar{x}$=73 × 11 µm, n=30), 8-spored, bitunicate, cylindrical with short pedicels, apically rounded. Ascospores 35-62×2-4 µm ($\bar{x}$=46 × 3 µm, n=50), uniseriate to biseriately arranged, partially overlapping, fasciculate, filiform, 8-10 septate, hyaline, rounded at both ends, rarely denticulate at one end. Asexual morph: Undetermined.

**Material examined:** INDIA: Tamil Nadu, Tiruvarur District, Muthupet mangroves, on decaying wood of *R. mucronata*, 28 November 2015, B. Devadatha (AMH-9907, holotype).

*GenBank numbers*: ITS = OQ680218

**Notes:** Our taxon morphologically resembles *Vibrissea nypicola* in having reddish brown apothecial ascomata, cylindrical asci and ascospores that are hyaline and filiform. However, it is distinguishable from *V. nypicola* in having lichenized habitat, larger apothecial ascomata, shorter asci, ascospores that are 8-10 septate (Hyde *et al*., 1999). Whereas *V. nypicola* has smaller ascomata, longer asci, produces unicellular ascospores and occurs on intertidal petioles of *Nypa fruticans* (Hyde *et al*., 1999). *Vibrissea nypicola* lacks molecular data for a comparison with our new taxon. BLAST search analysis based on the ITS region shows that our taxon shares 97% similarity with *Bactrospora*. Further, phylogenetic analyses based on ITS, showed that our taxon clusters with *B. corticola* in a monophyletic clade with strong support from ML 100% (**Figure 1**). Our taxon fits well within *Bactrospora* in having lichenized habitat, ascospores that are hyaline, filiform, and septate (Sobreira *et al*., 2015).

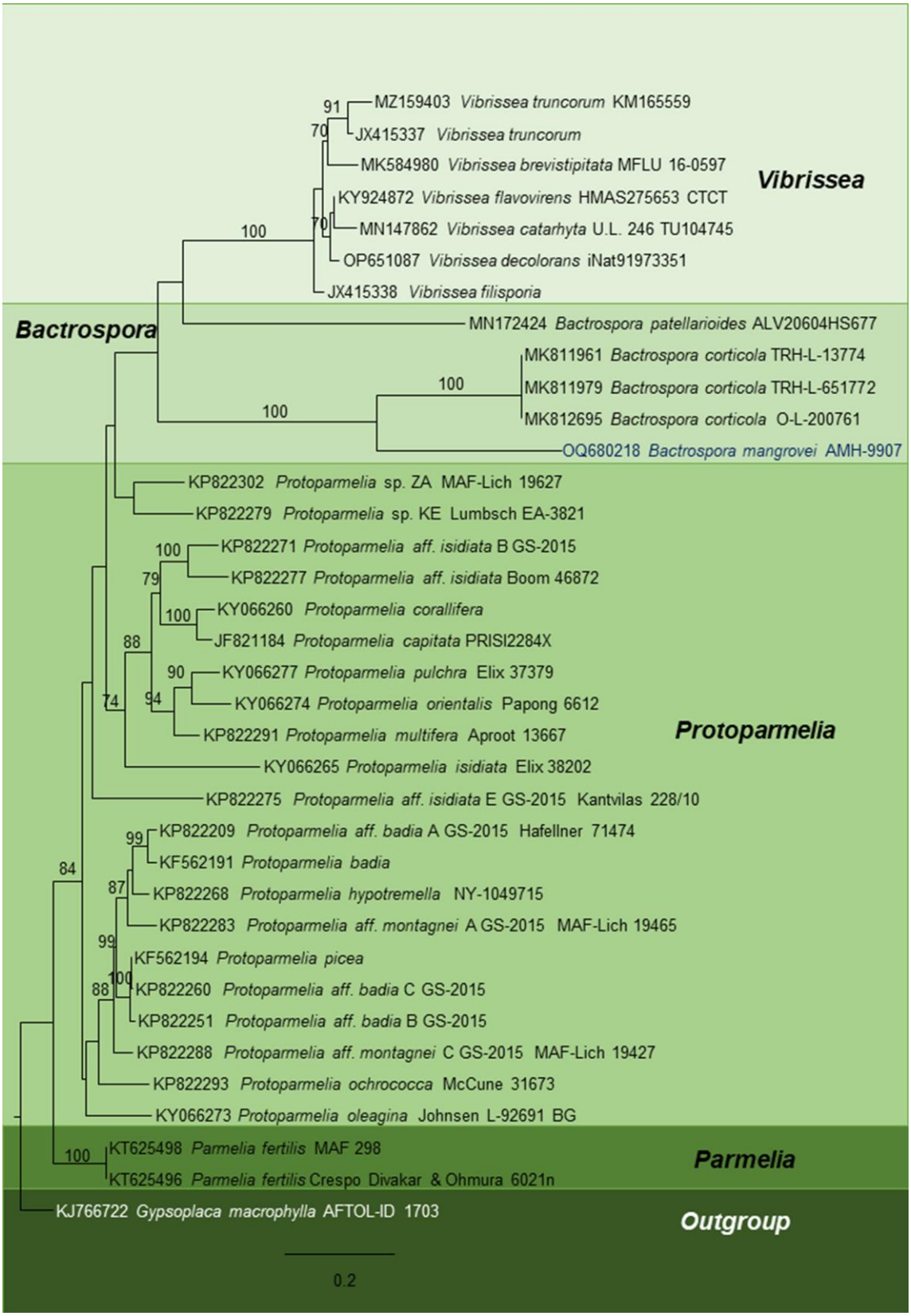


**Figure 1:** RAxML tree based on analysis of the ITS sequence data. Bootstrap support values for ML (>75%) are given above each branch. The new species is represented in blue. The tree is rooted to *Gypsoplaca macrophylla* AFTOL-ID 1703.

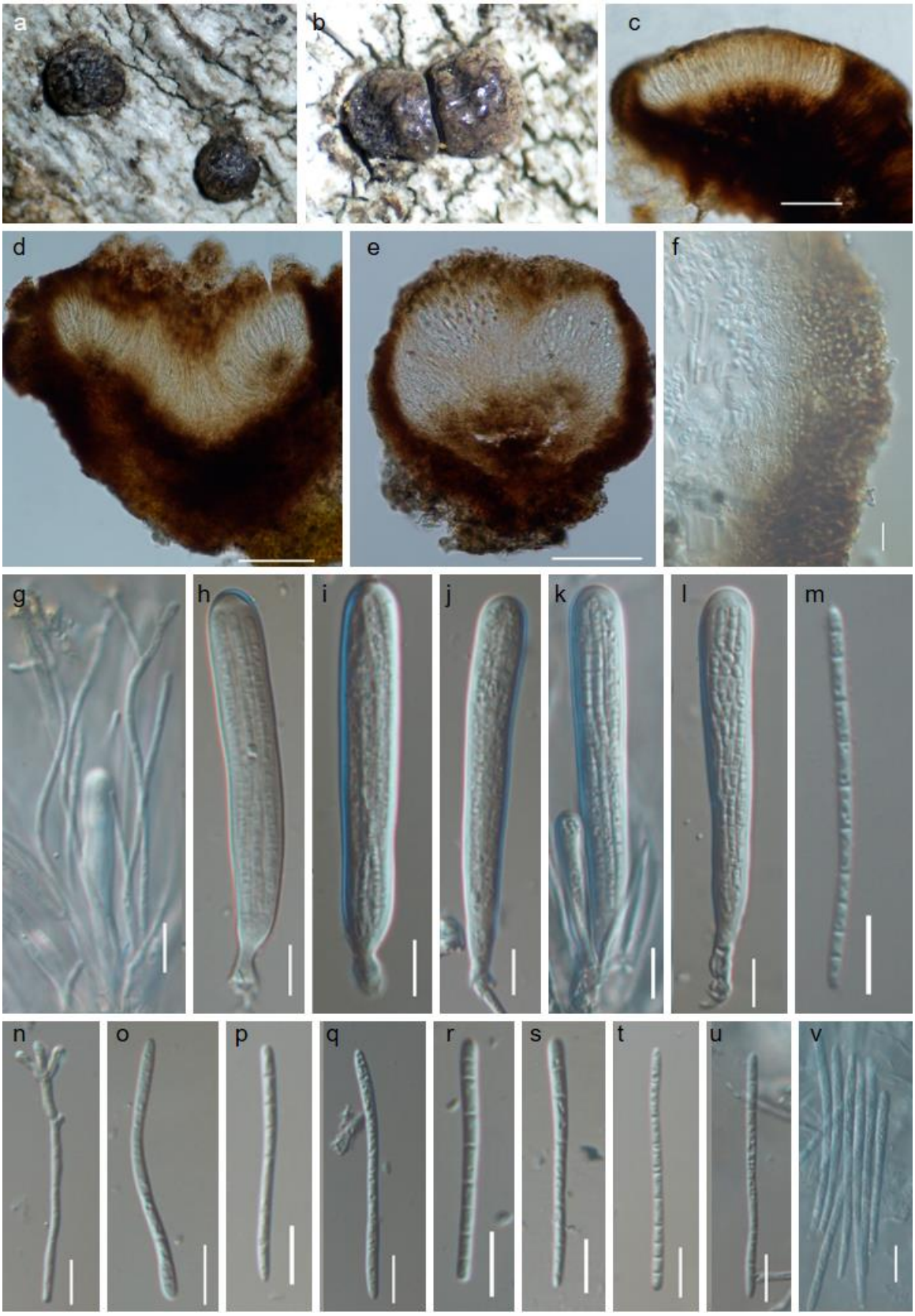


**Figure 2:** *Bactrospora mangrovei*. a-b, Apothecia superficial on decaying wood; c-e, Longitudinal sections of apothecia; f, Peridium; g,n, Paraphyses; h-l, Immature and mature asci; m,o-v, Hyaline, filiform ascospores. Scale bars: b-d=100 µm, e-q=10 µm.

However, our taxon is distinct from the species of *Bactrospora* in having shorter asci and ascospores that are also short and consistently have 8-10 septations, and occurs in the marine environment (**Figure 2**). *Bactrosproa corticola* is distinguished easily from our taxon by having ascospores that split into part spores and the cells are roundish, cubical or, rarely, cylindrical of 2-5×2-3 µm. Sobreira *et al*. (2015) provided a world key to 31 species belonging of *Bactrospora* based on morphological characters. A perusal of the key showed that the present taxon does not fit into any of the species included in the key. Though *B. thyrsodes* and *B. carolinensis* have asci and ascospore dimensions that match with the new taxon, the number of septations start from 3 and 5 in these two species, respectively. However, the new taxon has consistently 8 to 10 septa. While most of the species belonging to *Bactrospora* are known to produce black apothecia our new taxon produced reddish brown ascomata. Further, the marine habitat is entirely different as other species were reported from terrestrial environments. Hence a new species *B. mangrovei* is introduced under *Bactrospora* based on molecular sequence analysis and its distinct morphological differences in contrast to other *Bactrospora* species (Sobreira *et al*., 2015).

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