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Pontoporeia mangrovei sp. nov, a new marine fungus from an Indian mangrove along with a new geographical and host record of *Falciformispora lignatilis*

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Abstract

Pontoporeia mangrovei, a new marine fungal species is reported from Muthupet mangroves, southeast coast of India. *Pontoporeia mangrovei* is characterized by cleistothecial, dark brown to black, carbonaceous, semi-immersed to superficial, non-ostiolate ascomata with broadly clavate, ovoid, or ellipsoidal asci. Ascospores are 1-septate, biturbinate to ellipsoidal, hyaline to purple when young and dark brown to black at maturity. The new species differs from *Pontoporeia biturbinata* in having shorter ascomata, asci and ascospores dimensions and by occurring on *Avicennia marina* and *Suaeda monoica* in mangrove environments, in contrast to a sea grass host (*Posidonia oceanica*) of the latter. *Falciformispora lignalitis* is reported from an Indian mangrove for the first time and hence the present collection expands its geographical range. *Aegiceras corniculatum* is a new host record for this fungus.

Key words – 1 new species – *Avicennia marina* – *Aegiceras corniculatum* – Pleosporales – *Suaeda monoica* – Taxonomy

Introduction

The genus *Pontoporeia* Kohlm., was established by Kohlmeyer (1963) based on *Pontoporeia biturbinata* (Durieu & Mont.) Kohlm. The new genus was characterized by broadly clavate, ovate or ellipsoidal asci, filiform septate pseudoparaphyses, irregular peridium and biturbinate to sub-ellipsoidal ascospores having 2-layered walls with a germ pore at each end and occurrence in marine environment (Kohlmeyer & Kohlmeyer 1979). *Pontoporeia* was treated as a synonym of *Zopfia* Rabenh. by Malloch & Cain (1971), which was also followed by Hawksworth & Booth (1974). *Pontoporeia biturbinata* and *Halotthia posidoniae* (Durieu & Mont.) Kohlm., are monotypic genera sharing similar morphological characters such as large ascomata enclosed within stromata or hypostroma, narrow cellular pseudoparaphyses, and single septate, thickened, dark-brown ascospores (Kohlmeyer & Kohlmeyer 1979, Suetrong et al. 2009). Both *Pontoporeia biturbinata*, and *Halotthia posidoniae* are host-specific and frequently occur on *Posidonia oceanica* in Mediterranean coasts (Kohlmeyer & Kohlmeyer 1979, Cuomo et al. 1982, 1985) and Cyprus (Jones et al. 2009, 2015).

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Molecular phylogenetic studies by Suetrong et al. (2009) revealed that the three monotypic marine genera: *Halotthia* Kohlm., *Mauritiana* Poonyth, K.D. Hyde, Aptroot & Peerally, and *Pontoporeia* formed a separate clade with moderate support in Pleosporales. However, they did not assign these three genera to any family. Later, Zhang et al. (2012) introduced the family Halotthiaceae Y. Zhang, J. Fourn. & K.D. Hyde, typified by *Halotthia*, to accommodate monotypic genera *Mauritiana*, *Phaeoseptum* Y. Zhang, J. Fourn. & K.D. Hyde and *Pontoporeia* with dark coloured spores. *Halotthia posidoniae* and *Pontoporeia biturbinata* share similar morphological characters and they also grouped together as a sister group to *Mauritiana rhizophorae* in phylogenetic studies (Zhang et al. 2012).

We are investigating the biodiversity of marine fungi in Muthupet mangroves along south east coast of India. In our recent collections and microscopic examination of mangrove specimens we found a novel taxon *Pontoporeia mangrovei* sp. nov, which belongs in Halotthiaceae and shares similar characters with *Pontoporeia biturbinata*. This new taxon is described in this paper supported by photomicrographs. A dichotomous key to the closely related genera has been provided in addition to a table that has a synopsis of important characteristics of the closely related genera considered.

Falciformispora K.D. Hyde, has been established to accommodate *F. lignatilis* K.D. Hyde, a new species colonizing unknown mangrove wood by Hyde (1992). Not many reports are available on this species after its report from mangroves. This species is reported for the first time from India and on a new host *Aegiceras corniculatum.* The fungus is illustrated with photomicrographs.

Materials & Methods

Sample collection, Isolation and morphological studies

Decaying mangrove wood pieces of *Aegiceras corniculatum*, *Avicennia marina* and *Suaeda monoica* were collected from Muthupet mangroves (10.4 °N, 79.5 °E), Kaveri River Delta, Tiruvarur district, Tamil Nadu, southeast coast of India. Direct examination method outlined by Kohlmeyer & Kohlmeyer 1979) was followed and it has been detailed in Devadatha et al. (2017). Briefly the decaying samples were examined under a stereo zoom microscope after moisture chamber incubation and micro slides were prepared by teasing out the fungal fruiting structures and transferring on to Lactophenol with or without cotton blue. Photomicrographs were taken employing Nikon ECLIPSE TiU upright microscope with DIC objectives connected to Nikon DS-Fi2 digital camera.

Our attempts to isolate single spore cultures did not yield positive results as the spores did not germinate. The herbarium material of holotype was deposited at Ajrekar Mycological Herbarium (AMH), Agharkar Research Institute (ARI), Pune, India. Index fungorum number is provided.

Results

Taxonomic description

Pontoporeia mangrovei Devadatha, V.V Sarma, sp. nov. Fig. 1

Index Fungorum number: IF554084

Etymology – In reference to the habitat where the fungus lives

Holotype – AMH-9913

Saprobic on decaying wood of *Saueda monoica* and *Avicennia marina*. Sexual morph: *Ascomata* 300–700 µm high, 330–670 µm in diameter ($\bar{x}$= 400 × 460 µm, n= 10), solitary to gregarious, globose, dark brown to black carbonaceous, semi-immersed to superficial, with poorly developed hypostromata, fused with the host tissue, non-ostiolate, smooth. *Peridium* 25–85 µm ($\bar{x}$ = 35 µm, n=10) thick, outer layer of small irregular, dark brown, thick-walled cells, inner layer of cells with larger lumina, arranged in a *textura angularis*. *Hamathecium* composed of numerous, 1–2 µm wide, filiform, branched, septate pseudoparaphyses, anastomosing above the asci, embedded in a gelatinous matrix. *Asci* 145–200 × 55–85 µm ($\bar{x}$ = 175 × 70 µm, n=30), 8-spored, bitunicate, long pedunculate, persistent broadly clavate, ovoid or ellipsoidal, apically rounded and thickened at the apex lacking an ocular chamber. *Ascospores* 47–58 × 25–31 ($\bar{x}$ = 54 × 28 µm, n=50), 1–3 seriate, 1-

septate, constricted at the septum, thickened in the septum region with a band, initially hyaline turning purple, becoming dark brown to blackish brown at maturity, biturbinate to subellipsoidal, papillate at both ends with germ pores, lacking sheath or appendages. Ascospore walls bilayered, outer hyaline to light brown, thick exospore and an inner dark brown, thin endospore. Asexual morph: Undetermined

Known distribution – India

Material examined – India, Tamil Nadu, Tiruvarur, Muthupet mangroves (10.4°N 79.5°E), on decaying wood of *Suaeda monoica* (*Amaranthaceae*) (Holotype) and *Avicennia marina* (*Acanthaceae*) (Isotype) 24 December 2016, B. Devadatha (AMH-9913, **holotype**) and (PUHD-90, **Isotype**). Isotype is maintained at Pondicherry University, Pondicherry.

Notes – *Pontoporeia mangrovei* and *Pontoporeia biturbinata* share similar morphological features. These include cleistothecial ascomata, with a weakly developed hypostromata, a thick peridium, filiform pseudoparaphyses, long pedunculated, broadly clavate, ovoid or ellipsoidal asci, and 1-septate ascospores, constricted and thickened at the septum with a band-like appearance, dark-brown to blackish, biturbinate to sub ellipsoidal (Fig. 1). However, *Pontoporeia mangrovei* is distinct from *P. biturbinata* in having shorter ascomata, asci and ascospore dimensions and being saprobic on *Saueda monoica* and *Avicennia marina* in mangroves (Table 1). *Pontoporeia biturbinata* is host-specific and saprobic or perthophytic on living and drift rhizomes of *Posidonia oceanica* (Jones et al. 2009, 2015). The woody nature of mangrove substrata on which *P. mangrovei* thrive, are different from somewhat soft nature of the sea grass (*Posidonia oceanica*) on which *P. biturbinata* occurs. These differences warrant a new species and hence a new species (*Pontoporeia mangrovei*) has been proposed to be accommodated in *Pontoporeia.*

A Dichotomous Key to similar species of *P. mangrovei* prepared broadly based on Kohlmeyer and Volkmann-Kohlmeyer 1991

1. Ascospores with one septum at maturity……..…….2
1′.Ascospores with one and more septa at maturity, 40-54 × 18-22µm, ascomata osiolate, papillate …….....*Coronopapilla mangrovei*
2.Ascospores verrucose to verruculose, shorter than 30 µm..……..*Caryosporella rhizophorae*
2′.Ascospores smooth, longer than 30 µm ……...3
3.Ascospores with a dark band around the septum, without distinct germ pores or apical papillae, ascomata ostiolate …….....*Halothia posidoniae*
3′.Ascospores thickened around the septum giving a band-like appearance, with distinct germ pores and apical papillae, ascomata non-ostiolate …..……...4
4.Ascospores longer than 65µm; with hyaline apical papillate cells and germ pores, on seagrasses.....……...*Pontoporeia biturbinata*
4′.Ascospores shorter than 65µm; with hyaline light brownish apical papillate cells and germ pores, on mangroves……..*Pontoporeia. mangrovei*

***Falciformispora lignalitis* K.D. Hyde**

Saprobic on dead wood of *Aegiceras corniculatum*. Sexual morph: *Ascomata* 150–300 µm high, 200–300 µm in diameter ($\bar{x}$= 215 × 240 µm, n= 10), aggregated, rarely solitary, erumpent to superficial, depressed globose to ovoid, brown to black, ostiolate. Ostiole short papillate, pale brown coloured. *Peridium* 25–40 µm ($\bar{x}$= 30 µm, n=10), comprising 4–6 layers of thick walled pale brown to reddish brown cells of *textura angularis*, internally hyaline to light brown, externally merged with the host tissue. *Hamathecium* long and composed of numerous branched septate pseudoparaphyses and anastomosing above the asci, 1.5–3 µm wide, cellular hyaline pseudoparaphyses embedded in a gelatinous matrix. *Asci* 85–125 × 20–25 µm ($\bar{x}$= 102 × 22 µm, n=30), 8-spored, bitunicate, fissitunicate, broadly clavate to fusoid, with a short thick pedicel, apically rounded and thickened at the apex with an ocular chamber. *Ascospores* 37–50 x 5–10 ($\bar{x}$= 45 × 7 µm, n=50), biseriate to triseriately arranged, overlapping, hyaline, fusoid to moderately clavate, slightly curved, smooth-

walled, 6–7 transverse septate, mostly 6-septate, surrounded by a thin gelatinous sheath longer at the base when compared to apical part, with a single scythe like appendage at the terminal end.

Fig. 1 – *Pontoporeia mangrovei* (AMH-9913, holotype). a Ascomata semi-immersed on the decaying wood of *Suaeda monoica*. b Horizontal section of ascoma. c Longitudinal section of ascoma. d Section of peridium. e Filiform, branched, septate pseudoparaphyses. f–i Immature and mature asci. j–m Ascospores. Scale bars: c = 100 µm, f,g = 50 µm, d,e,h-m = 10 µm.

Table 1 Synopsis of morphological characters of *Pontoporeia mangrovei* and other closely related species in the same genus and other related genera

Species	Host and habitat	Ascomata (µm)	Peridium (µm)	Asci (µm)	Ascospores (µm)	Source References
Pontoporeia biturbinata	Saprobic on rhizomes of *Posidonia oceanica*	805–1120 high, 805–1375	110–137	225–360 × 72–110, clavate	66–90 × 31.5–43.5, biturbinate, hyaline aplical papillae with germ pores	Kohlmeyer & Kohlmeyer 1979
Pontoporeia mangrovei	Saprobic on *Saueda monoica* and *Avicennia marina*	300–480 high, 330–670 diam	25–85	145–200 × 55–85, clavate	47–58 × 25–31, biturbinate, light brown apical papillae with germ pores	This study
Caryosporella rhizophorae	Saprobic on *Rhizophora mangle*	750–900, 830–1050 diam	90–160	200–250 × 16–18 , cylindrical	24–30 × 10–12, ellipsoidal verruculose	Kohlmeyer 1985
Coronopapilla mangrovei	Saprobic on *Rhizophora mangle*	600–850 high, 450–650 diam clypeate,	24–45	350–430 × 28–37, cylindrical,	40–54 × 18–22.5, 1–3 septate, ellipsoidal	Kohlmeyer & Kohlmeyer 1990
Halotthia posidoniae	Saprobic on rhizomes of *Posidonia oceanica*	770–1075 high, 1500–2100 diam,	230–285	275–290 × 25–35, cylindrical,	37–60.5 × 16.5–26, ellipsoidal, septum with a central porus	Kohlmeyer 1963

Material examined – India, Tamil Nadu, Tiruvarur, Muthupet mangroves (10.4°N 79.5°E), on decaying wood of *Aegiceras corniculatum.* PUHD-61, Isotype is maintained at Pondicherry University, Pondicherry.

Notes – The genus *Falciformispora* was first established by Hyde (1992) as a monotypic genus and placed it under *Pleosporaceae* by comparing with *Setosphaeria.* Raja & Shearer (2008) collected *Falciformispora* species in freshwater in Florida and found that it is closely related to *Chaetomastia* than *Setosphaeria*, but is distinct in having hyaline ascospores. Suetrong et al. (2009) recorded it from the terrestrial oil palm (*Elaeis guineensis*) in Thailand. Later the family Trematosphaeriaceae was established to include the genera *Falciformispora, Halomassarina* and *Trematosphaeria* based on the morphology and phylogenetic characterization (Suetrong et al 2011, Hyde et al 2013). Recently we have recorded *F. lignatilis* for the first time on dead wood of *Aegiceras corniculatum* from Muthupet mangroves, south-east coast of India. Morphologically our collection is similar to the type species of *Falciformispora lignatilis* thought it has some overlapping dimensions of ascomata, asci and ascospore (Fig. 2).

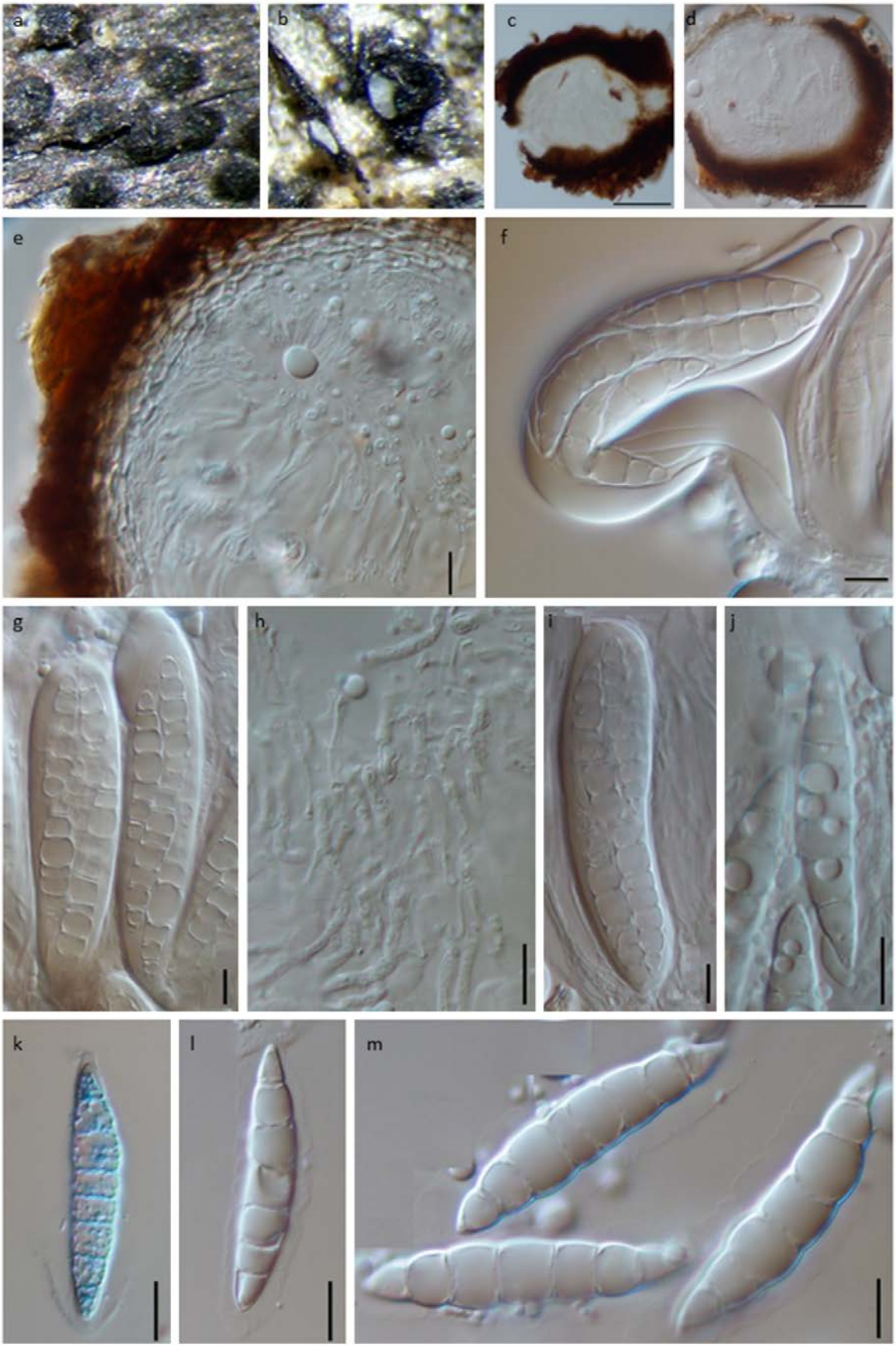

Fig. 2 – *Falciformispora lignalitis* (PUFD-61, Isotype). a Ascomata superficial on the dead wood of *Aegiceras corniculatum*. b–d Longitudinal section of ascoma. e Section of peridium. f, g, i Immature and mature asci. h Cellular pseudoparaphyses. j–k Ascospores with scythe like appendage. l–m Ascospores with thin gelatinous sheath. Scale bars: c = 100 μm, d= 50 μm, e-m = 10 μm.

Culture characteristics – Ascospores germinating on seawater agar within 24 hours, germ tubes arising from apical end of the ascospore. Colonies on malt extract agar, slow growing, reaching 30–40 mm diameter after 25 days of incubation at room temperature, olive gray at center and olivaceous black at margin, reverse deep mouse gray, moderate, granular, undulate, surface raised, irregular.

Known distribution – Mexico, Thailand, USA (Florida), India

Material examined – India, Tamil Nadu, Tiruvarur, Muthupet mangroves (10.4°N 79.5°E), on dead wood of *Aegiceras corniculatum* (L.) Blanco (*Primulaceae*), 28 November 2015, B. Devadatha (PUFD-61, Isotype at Pondicherry University; ex-type living culture, PUFD-61)

Discussion

Studies on salt marsh plants like *Spartina alterniflora*, *Juncus roemerianus* and *Phragmites australis* have resulted in a rich diversity of marine fungi akin to many mangrove plants (Jones 1963, Poon & Hyde 1998, Fell & Hunter 1979, Wong & Hyde 2002, Kohlmeyer & Kohlmeyer 2002, Barata 2002, Ryckegem & Verbeken). To our knowledge *Suaeda monoica*, coming under the category of mangrove associated plants, has so far not been explored for marine fungi. With this in mind we collected and examined the decaying samples of *Suaeda monoica* which resulted in a novel taxon *Pontoporeia mangrovei*. Further, this fungus has also been recorded from the typical mangrove plant *Avicennia marina*. The monotypic genus *Pontoporeia* typified with *P. biturbinata* had been reported to be frequent on *Posidonia oceanica* (Kohlmeyer & Kohlmeyer 1979, Jones et al. 2009). In our recent collections of decaying mangrove specimens and their examination, we found a morphologically similar taxon described here as *P. mangrovei* which colonizes decaying twigs of *Suaeda monoica* and *Avicennia marina*. *Pontoporeia mangrovei* fits well into the genus *Pontoporeia* as it has ascospores that are biturbinate or sub-ellipsoidal, papillate with germ pores, 1-septate, dark brown to blackish brown (Fig. 1). *P. mangrovei* could be easily distinguished from *P. biturbinata* based on the shorter ascomata, asci and ascospore dimensions and also by occurring on *Suaeda monoica* and *Avicennia marina* in a mangrove habitat. A synopsis of important characteristics of closely related taxa of *P. mangrovei* is presented in Table 1. As could be seen from the table and the dichotomous key proposed, *P. mangrovei* could be easily delineated based on the non-ostiolate ascomata, with ascospores that are uniseptate having a constriction and thickening at the septum, apical papillate structures and pores. Our attempts to isolate single spore pure cultures failed as the spores did not germinate. After several field collections we also found this taxon on *Avicennia marina* with a very few ascomata that turned out to be insufficient for isolations and molecular studies. Hence we have proposed the new species *P. mangrovei* based on the morphological characteristics alone.

So far there are no reports of *Falciformispora lignatilis* from India. In the present study we have recorded this species (Fig. 2) for the first time from India and hence the present collection extends the geographical range of this fungus. Further, the occurrence of this taxon on decaying twigs of *Aegiceras corniculatum* extends its host range also.

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