New Records of Hymenochaetoid Fungi from the Mangrove Forest of Muthupet, Tamil Nadu, India

Sugantha Gunaseelan, Kezhocuyi Kezo, and Malarvizhi Kaliyaperumal*

Centre for Advanced Studies in Botany, University of Madras, Guindy campus, Chennai - 600 025, Tamil Nadu, India.

 $* Corresponding \ author \ Email: malar.kaliya perumal@gmail.com$

(Submitted on October 7, 2023; Accepted on October 25, 2023)

ABSTRACT

Hymenochaetoid fungi inhabiting the mangrove trees were collected from Muthupet and delimited based on morphological and microscopical analyses. Three hymenochaetoid fungi belonging to two genera *Fulvifomes* (*F. fastuosus* and *F. mangroviensis*) and *Inonotus* (*I. rickii*), were documented.

Keywords: Hymenochaetaceae, Hymenochaetales, Phellinus s.l., Inonotus s.str., Wood decaying fungi, Pathogenicity

INTRODUCTION

Mangroves are salt-tolerant forest communities restricted to intertidal areas of tropical and subtropical latitudes (Tomlinson, 1986). India contributes about 3.3% (4992 Km²) of the total Mangrove cover worldwide (150000 Km²) (ISFR, 2021). In India, Mangroves are restricted to the eastern and western coastal lines, with the west coast having a greater diversity. Around 414 fungal species from 226 genera have been reported from Indian Mangroves. Basidiomycetes include 35 genera (Sarma and Devadatha, 2020). Basidiomycetes play a significant role in forest pathology due to their high occurrence on diseased trees (Fox, 2001; Gilbert et al., 2008). Hymenochaetoid fungi are reported to cause heart or butt rot in trees in mangrove ecosystems (Dai, 2010). Stem/butt rot of mangrove trees has been prevalently caused by *Phellinus* species (currently classified into Phellinus s.s. and Fulvifomes. Fuscoporia. Fomitiporia. Ochroporus. Phellinidium, and Porodaedalea) (Chalermpongse, 1991; Baltazar et al., 2009a,b; Fiasson and Niemelä, 1984). Inonotus rickii (Pat.) Reid, canker rot pathogen causes white rot of heartwood (Intini, 2002). Sakayaroj et al. (2012) studied the role of basidiomycetes causing heart and butt rot in mangrove trees (Xylocarpus granatum) in Thailand. Later, Hattori et al. (2014) re-examined these Hymenochaetoid fungi and classified them as Fulvifomes halophilus, F. mangrovicus, F. siamensis and F. xylocarpicola. Considering the renowned complex layers, including forests and wetland biological diversity in India, the Hymenochaetoid fungi reported so far from Mangroves are insignificant. The present study has hymenochaetoid added three fungi to Basidiomycetes from Indian Mangroves.

MATERIALS AND METHODS

Collection and Morph-taxonomical analyses of Hymenochaetoid fungi

Muthupet mangrove is located at the southern end of the Cauvery Delta on the east coast of India. The swamp is occupied by the presence of Acanthus ilicifolius, Aegiceruas corniculatum, Avicennia marina, Excoecaria agallocha, Rhizophora mucronata and Lumnitzera racemosa. Monospecific stands of Prosopis juliflora are found all along the landward margin. Morpho and microscopical analyses were carried out for three isolates.

The basidiomes associated with the host were photographed. The basidiomes were individually tagged with the respective details such as locality, habitat, host, collection number, date of collection, and type of rot. Macro-morphological characters such as shape, size of basidiome, perennial or annual, colour, texture, margin (acute or obtuse), context (homogenous, duplex with or without blackline), tube layer (colour, length, stratification), and pores (size and shape) were examined in the fresh sample and recorded. Colour descriptions are based on the Methuen handbook (Kornerup and Wanscher, 1978). To study the microscopical characters, free-hand sections of dry specimens were mounted in water, 5% KOH (v/w), cotton blue (CB) and Melzer's reagent (IK). Sections were studied, and photos were taken at magnification up to ×1000 using a LABOMED OPTIC-CX BINO LED microscope. Microscopic measurements and illustrations were made in a 5% KOH solution. Specimens in this study were deposited in the Madras University Botany Laboratory (MUBL), Centre for Advanced Studies in Botany, University of Madras, Chennai-600 025, Tamil Nadu, India.

RESULTS AND DISCUSSION

The stem/butt rot of the trees at the mangrove was visually observed. Most trees exhibited severe decay with hollow trunks and branches (**Figure 1**). Most specimens were collected from tree trunks. Detailed microscopical studies were carried out for three species belonging to two genera (*Fulvifomes* and *Inonotus*).



Figure 1: a-c, Hymenochaetoid fungi associated with decaying trees; d-f, Wood decay of Mangrove trees with hollow trunks; g-i, Hymenochaetoid fungi associated with decaying trees: g, *Fulvifomes fastuosus*; h, *Fulvifomes mangroviensis*; i, *Inonotus rickii*.

Taxonomic descriptions

Fulvifomes fastuosus (Lév.) Bondartseva and S. Herrera, Mikologiya Fitopatologiya, **26(1)**:13 (1992)

 $\equiv Phellinus fastuosus (Lév.) Ryvarden, Norwegian Journal of Botany,$ **19**:234 (1972). (Figure 2)

Basidiome perennial, pileate, sessile, soft, to light corky when dry. Pilei applanate, projecting up to 3.4 cm long, 9 cm broad and 1.5 cm thick at the base. Pileal surface brownish yellow (5C8) to tan brown (6E5), velutinate to smooth, azonate, lacking cracks and crust. Margin brownish orange (5C5) to golden yellow (5B7), obtuse, 1 mm in thickness. Pore surface pale orange (5A3) to brownish orange (5C6). Pores round to angular, smooth, 6–9 per mm. Context homogenous, corky, yellowish-brown (5D8), up to 1 cm in thickness. Tube layer yellow (5C6) to light brown (6D8), stratified tubes up to 0.5 cm thickness.

Hyphae system dimitic, tightly interwoven, tissues darkening in KOH without hyphal swelling. Context: hyphae loosely interwoven; Generative hyphae thin to thick-walled, frequently septate, rarely branched, rarely encrusted with simple crystals, golden yellow, 2 - 4.5 µm; Skeletal hyphae thick-walled with narrow to wide lumen, aseptate and unbranched, golden yellow, 2.6-5 µm. Trama hyphae: tightly interwoven; Generative hyphae thin to thick-walled, rarely branched, septate, rarely encrusted with simple crystals, golden yellow, 2-5 µm; Skeletal hyphae dominant, narrow lumen, rarely septate and unbranched, golden yellow, 2.6-4.6 µm. Cystidioles hyaline, sabulate, rare, 5.7–11 \times 2–6.3 µm. Seate and Chlamydospores absent. Basidioles broadly clavate, 10-22 × 3.5-11 µm. Basidia broadly clavate, with four sterigmata, $10-21 \times 4-7 \mu m$. Basidiospores thick-walled, subglobose, golden yellow to rusty brown, acyanophilic, inamyloid, non-dextrinoid, (4.6-) 4.9 - 5.7 (-5.9) × (3-) 3.9 -4.9 (-5) μ m, Q=1.16 (n = 50/2), Q =1.05-1.3.

Specimen examined: India, Tamil Nadu, Thiruvarur district, Muthupet, 10°42'N 79°50'E, basal trunk of *Aegiceras corniculatum*, 18 March 2018, Sugantha Gunaseelan (MSK-MP18).

Notes: Morphologically, *Fulvifomes fastuosus* from our study is similar to *F. fastuosus* from China, India, and Sri Lanka by sharing sessile, pileate, velutinate young basidiome turning smooth, with age, homogenous context, smaller pores (7–9/10 per mm), distinct tube layers, dimitic hyphal system and coloured basidiospores (Dai, 2010; Ediriweera *et al.*, 2014; Sundari *et al.*, 2018; Wu *et al.*, 2022). However, previously reported *F. fastuosus* lacks cystidioles, but cystidioles were abundant and variously shaped in all our examined specimens. The size of the basidiospores varies slightly from the earlier reports, which can be caused by the effect of temperature on drying, modes of nutrition or other environmental factors (Kaserud *et al.*, 2008; Hu *et al.*, 2022).

Fulvifomes mangroviensis S. Gunaseelan, K. Raja, K. Kezo and M. Kaliyaperumal, Persoonia, **49**:283 (2022) (Figure 2)

Basidiome annual, pileate, applanate, sessile, soft, to light corky when dry. Pilei dimidiate, convex, projecting up to 4.2 cm long, 5.7 cm broad and 1.5 cm thick at the base. Pileal surface brownish yellow (5C8) to tan brown (6E5), tomentose to smooth, azonate, lacking cracks and crust. Margin greyish orange (5B5), acute to obtuse, 1 mm in thickness. Pore surface pale orange (5A3) to dark brown (6F8). Pores round to angular, smooth, 3–5 per mm. Context homogenous, corky, yellowishbrown (5D8), up to 1 cm in thickness. Tube layer hard corky, yellow (5C6) to light brown (6D8), tubes up to 0.6 cm thickness.



Figure 2: *Fulvifomes fastuosus.* a, Basidiocarp; b, Pore surface; c, Contextual hyphae; d, Cystidioles; e. Basidioles; f, Basidia; g, Basidiospores. Scale bar d-i = 5μ m; *Fulvifomes mangroviensis.* h, Basidiocarp; i, Pore surface; j, Contextual hyphae; k, Tramal hyphae; l, Cystidioles; m, Basidioles; n, Basidia; o, Basidiospores. Scale bar d-i = 5μ m.

Hyphal system dimitic in trama, subdimitic to dimitic in context, tissue darkening in KOH without hyphal swelling. Context hyphae loosely interwoven, generative hyphae thin to thick-walled, frequently septate, rarely branched, infrequently encrusted, hyaline to brown, $2-7\mu$ m. Skeletal hyphae or Skeletoid hyphae thick walled with narrow to wide lumen, dominant, occasionally with secondary septa, branched, golden yellow, 2.5–8.2 µm. Trama hyphal system tightly interwoven. Generative hyphae thin to thick-walled, branched, septate, hyaline to brown, rarely encrusted with simple crystals, 2–4.5 μ m. Skeletal hyphae dominant, narrow lumen, aseptate, unbranched, yellow to golden yellow, 2.6–6.2 μ m. Setae and chlamydospores absent. Cystidioles yellow, subulate, rare, 5.7–14 × 2–4.7 μ m. Basidioles broadly clavate 6–12 × 3.9–5.3 μ m size. Basidia broadly clavate, yellow, four sterigmata, 6.2–14 × 3.9–6.2 μ m. Basidiospores thick-walled, broadly ellipsoid to subglobose, golden yellow to brown, acyanophilic, inamyloid, non-dextrinoid, (4.6–) 4.9–5.7 (–5.9) × (3–) 3.9–4.9 (–5) μ m, Q=1.14, (n = 50/2), Q =1.05–1.3.

Specimen examined: INDIA, Tamil Nadu, Thiruvarur district, Muthupet, 10°46'N 79°51'E, basal trunk of *Aegiceras corniculatum*, 18 March 2018, K. Malarvizhi (MSK-MP41).

Notes: F. mangroviensis shares similar character with other reported Asian mangrove inhabiting fungi, F. halophilus, F. mangrovicus, F. merrillii, F. siamensis and F. xylocarpicola, in having applanate, sessile, broadly attached pilei and soft corky basidiomes but the former differs by annual, brownish yellow to tan brown pilei, azonate, lacking cracks, smooth to tomentose, larger pores (3-5 per mm), Subdimitic to dimitic hyphal system in context and dimitic in trama, presence of cystidioles and variations in basidiospores size (4.6–5.9 × 3–5 µm) (Hattori et al., 2014). F. mangroviensis differs from F. robiniae in pileus character, pore per mm, hyphal system, shape and size of basidiospore (Salvador-Montoya et al., 2018).

Inonotus rickii (Pat.) Reid, Kew Bulletin, 12:141 (1957)

 $\equiv Xanthohrous rickii$ Pat., Bulletin de la Société Mycologique de France, 24 (1908) (Figure 3)

Basidiome semi-spherical or cushion-shaped, soft and fleshy when fresh, velvety to the touch, orange yellow (4B8), golden yellow (5B7), brownish yellow (5C8) to golden brown (5D7), soft, velutinate, exudates present over the surface of the Ptychogastroid basidiocarp. Pore surface yellowish brown (5E8). Pores round to angular, 2–4 per mm. Context brown (6D6) to golden brown (5D7), spongy, fibrous, concentrically zonate when fresh, turning rust brown (6F8), brittle when dry, crumbling into a mass of chlamydospores. Tube layer yellowish brown (5E8), not stratified, tubes up to 0.6 cm thickness.

Hyphal system monomitic; tissue darkening of the tissues in KOH, without hyphal swelling. Context: Generative hyphae golden yellow to yellowish brown, interwoven, thin to thick-walled, $2.5-6 \mu m$ wide, septate, occasionally branched. Trama: Generative hyphae hyaline to golden yellow, thin-

walled to thick-walled, septate, branched, 2.6-5.7 um.



Figure 3: *Inonotus rickii.* a,b, Basidiocarp; c,d, Setal hyphae; e,f, Chlamydospores; g, Basidia; h, Basidiospores (BS) and Chlamydospore (CS). Scale bar $c-e = 5 \mu m$; f-h = $5 \mu m$.

Setal hyphae brown, abundant, present in the context and dissepments, running parallel to the tube, thick-walled with narrow and wide lumen, acuminate to subulate, aseptaete, tapering towards the tip, $48.4-429 \times 5.7-11.27$ µm. Hymenial seate golden brown to brown, infrequent, thick-walled, with narrow and wide lumen, subulate to ventricose in water and reddish brown to rust brown in KOH, 19–45 \times 5.7–8.36 $\mu m.$ Basidioles clavate to subclavate, 5–12 \times 3–5 μ m. Basidia broadly clavate, with four sterigmata, $17.1-26.6 \times 5.7-7.12$ μm, sterigmata up to 2.8 μm long. Basidiospores hyaline to pale yellow, broadly ellipsoid, thickwalled, smooth, golden yellow to reddish brown in KOH, cyanophilic, inamyloid, non-dextrinoid, (4.3-) 5.2 - 8.1 $(-9.3) \times (4.7-)$ 5.3 - 6.9 $(-7.3) \mu m$ (Q=1.2). Chlamydospores abundant, varies in shape, thick-walled, smooth, yellow to brown, cyanophilic, inamyloid, non-dextrinoid, 7.2-35.6 × 5.7–25.2 μm.

Specimen examined: INDIA, Tamil Nadu, Thiruvarur district, Muthupet, 10°32'N 78°12'E, trunk of *Pongamia pinnata*, 18 March 2018, Kezhocuyi Kezo (KMS-MP48).

Notes: Indian *Inonotus rickii* (MLCASB003 and MLCASB025) showed similar morphology with the earlier reported isolates from Argentina (Gottileb *et al.*, 2002), China (Dai, 2010), India (Sharma, 2012), and Uruguay (Melo *et al.*, 2012). However, the Chinese isolates lack hymenial setae, whereas our Indian isolate has hymenial setae of

19–45 \times 5.7–8.36 μm length, which is longer than the earlier Indian report (15–25 μm \times 5–8 μm) (Sharma, 2012).

Hymenochaetoid fungi associated with decay or disease in mangrove trees include Fulvifomes (F. fastuosus (Brazil and Puerto Rico), F. halophilus (Thailand), F. magrovicus (Brazil), F. merrellii (Micronesia), F. rimosus (Brazil), F. siamensis (Thailand), F. swieteniae (Panama) and F. xylocarpicoloa), Fuscoporia (F. Callimorpha (Panama), F. gilva (USA and Brazil), and F. senex (Brazil)), Fomtiporella (*F*. caryophylli (Micronesia) and F. punctata (Brazil)), Inonotus (I. hispidus (Bangladesh), dryadeus, Ι. Ι. luteoumbrinus (Micronesia), I. pachyphloeus (India, Kenya and Philippines) and I. porrectus (Puerto Rico)) and Phellinus (P. adherens Р. lamensis (Bangladesh)) (Panama) and (Chalermpongse, 1991; Das and Aminuzzaman, 2017; Gilbert et al., 2008; Baltazar et al., 2009a,b; Sakayaroj et al., 2012). The present study documented the first report of F. fastuosus and I. rickii from Indian Mangrove and a new species, F. mangroviensis (Tan et al., 2022). Most of the Basidiomycetes associated with rots in Muthupet belong to the family Hymenochaetaceae. However, further sampling is required to analyse the hymenochaetoid species diversity in Indian mangroves.

ACKNOWLEDGEMENTS

Malarvizhi Kaliyaperumal and Sugantha Gunaseelan thank Extramural Research-SERB, DST (EMR/2016/003078), Government of India, for the financial assistance. They are also grateful to 'The PCCF' of the Tamil Nadu Forest Department for providing permission (E2/20458/2017), assistance and support during field visits in the Eastern Ghats of Tamil Nadu. The authors thank Prof. N. Mathivanan, Director, Centre for Advanced Studies in Botany, University of Madras, for providing laboratory facilities.

REFERENCES

- Baltazar, J.M., Trierveiler-Pereira, L., Loguercio-Leite, C. 2009b. A checklist of xylophilous basidiomycetes (Basidiomycota) in mangroves. *Mycotaxon*, **107**:221-224; doi: 10.5248/107.221.
- Baltazar, J.M., Trierveiler-Pereira, L., Loguercio-Leite, C., et al., 2009a. Santa Catarina Island mangroves 3: a new species of Fuscoporia. Mycologia, 101:859-863; doi: 10.3852/08-082.
- Chalermpongse, A. 1991. Fungal diseases in mangrove ecosystem. In: Proceeding, the 5th Silviculture Seminar in Thailand, Division of Silviculture, Royal Forest Department, Bangkok, Thailand, pp. 307-338.

- Dai, Y.C. 2010. Hymenochaetaceae (Basidiomycota) in China. *Fungal Diversity*, **45**:131-343.
- Das, K., and Aminuzzaman, F.M. 2017. Morphological and Ecological Characterization of Xylotrophic Fungi in Mangrove Forest Regions of Bangladesh. Journal of Advances in Biology and Biotechnology, **11(4):**1-15; doi: 10.9734/ JABB/2017/30971.
- Ediriweera, S.S., Wijesundera, R.L.C., Nanayakkara, C.M. et al., 2014. A new record of Fulvifomes fastuosus from Sri Lanka. Journal of the National Science Foundation of Sri Lanka, **42**:375-377.
- Fiasson, J.L., and Niemelä, T. 1984. The Hymenochaetales a revision of the European poroid taxa. *Karstenia*, **24**:14-28.
- Fox, R.T.V. 2001. Fungal foes in your garden. 51. Butt Rot Mycologist, **15**:184-185.
- Gilbert, G.S., Gorospe, J., Ryvarden, L. 2008. Host and habitat preferences of polypore fungi in Micronesian tropical flooded forests. *Mycological Research*, **112**:674-680; doi: 10.1016/j.mycres.2007.11.009.
- Gottlieb, A.M., Wright, J.E., Moncalvo, J.M. 2002. *Inonotus* s.l. in Argentina – Morphology, cultural characters and molecular analyses. *Mycological Progress*, **1**:299-313; doi: 10.1007/s11557-006-0028-5.
- Hattori, T., Sakayaroj, J., Jones, E.B.G. *et al.*, 2014. Three species of *Fulvifomes* (Basidiomycota, Hymenochaetales) associated with rots on mangrove tree *Xylocarpus granatum* in Thailand. *Mycoscience*, **55**:344-354; doi: 10.1016 /j.myc.2014.01.001.
- Hu, Y., Karunarathna, S.C., Li, H., Galappaththi, M.C.A., *et al.*, 2022. The Impact of Drying Temperature on Basidiospore Size. *Diversity*, **14**:239; doi: 10.3390/d14040239.
- Indian state of forest report, 2021; https://fsi.nic.in/forest-report-2021-details
- Intini, M. 2002. First report of *Inonotus rickii* causing canker rot on boxelder in Europe. *Plant Disease*, **86**:922; doi: 10.1094 /PDIS.2002.86.8.922C.
- Kauserud, H., Colman, J.E, Ryvarden, L. 2008. Relationship between basidiospore size, shape and life history characteristics: a comparison of polypores, *Fungal Ecology*, **1**(1):19-23; doi: 10.1016/j.funeco.2007.12.001.

- Kornerup, A., and Wanscher, J.H. 1978. Methuen handbook of colour, 3rd ed. Eyre Methuen, London.
- Melo, I., Ramos, P., Caetano, M.F.F. 2002. First record of *Inonotus rickii* (Basidiomycetes, Hymenochaetaceae) in Portugal. *Portugaliae Acta Biologica*, **20**:265-269.
- Sakayaroj, J., Preedanon, S., Suetrong, S., et al., 2012 Molecular characterization of basidiomycetes associated with the decayed mangrove tree Xylocarpus granatum in Thailand. Fungal Diversity, 56:145-156; doi: 10.1007/s13225-012-0195-4.
- Salvador-Montoya, C.A, Popoff, O.F., Reck, M. et al., 2018 Taxonomic delimitation of Fulvifomes robiniae (Hymenochaetales, Basidiomycota) and related species in America: F. squamosus sp. nov. Plant Systematics and Evolution, 304:445-459; doi: 10.1007/s00606-017-1487-7.
- Sarma, V.V., and Devadatha, B. 2020. Fungal diversity in mangroves of India and a note on their medicinal potential. In:

Biotechnological utilization of mangrove resources (Eds.: Patra, J.K., Mishra, R.R., Thatoi, H). Elsevier, pp.153-224.

- Sharma, J.R. 2012. Aphyllophorales of Himalayas. Botanical Survey of India, Calcutta, India.
- Sundari, T.M., Anand, A.A.P., Jenifer, P., et al., 2018. Bioprospection of Basidiomycetes and molecular phylogenetic analysis using internal transcribed spacer (ITS) and 5.8S rRNA gene sequence. Scientific Reports, 8:10720; doi: 10.1038/s41598-018-29046-w.
- Tan, Y.P., Bishop-Hurley, S.L., Shivas, R.G., *et al.*, 2022. Fungal Planet description sheets: 1436-1477. *Persoonia*, **49**:261-350; doi: 10.3767/persoonia.2022.49.08.
- Tomlinson, P.B. 1986. The Botany of Mangroves. Cambridge University Press, pp.1-413.
- Wu, F., Zhou, L.W., Vlasák, J. et al., 2022. Global diversity and systematics of Hymenochaetaceae with poroid hymenophore. Fungal Diversity, 113:1-192; doi: 10.21203/rs.3.rs-719853/v1.