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Deniquelata vittalii sp. nov., a novel Indian saprobic marine fungus on *Suaeda monoica* and two new records of marine fungi from Muthupet mangroves, East coast of India

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Abstract

Deniquelata vittalii, a novel species of marine fungi in the genus *Deniquelata*, found saprobic on a decaying woody stem of the halophyte *Suaeda monoica*, collected from Muthupet mangroves, southeast coast of India is described and illustrated in this paper. Morphologically *D. vittalii* resembles *D. barringtoniae*, but it is distinct in having larger ascomata, asci and golden yellow to dark brown ascospores with 3–6 transverse septa. Phylogenetic analyses inferred from combined LSU, SSU and ITS datasets indicate that *D. vittalii* shares a sister relationship with *D. barringtoniae* with high statistical support and forms a strongly supported monophyletic clade. Both morphological differences and DNA based sequence data strongly support the establishment of the new taxon. New records of *Farasanispora avicenniae* and *Hysterium rhizophorae* are also reported in this paper supplemented with molecular sequence data.

Key words – Dothideomycetes – Didymosphaeriaceae – Pleosporales – Mangrove Fungi – Molecular phylogeny

Introduction

Didymosphaeriaceae was introduced by Munk (1953), which is typified by *Didymosphaeria* Fuckel with *D. epidermidis* as the type species. This family is characterized by brown, thick-walled, 1-septate ascospores and trabeculate pseudoparaphyses, which anastomose above the asci in a gelatinous matrix and includes 25 accepted genera (Aptroot 1995, Hyde et al. 2013, Ariyawansa et al. 2014a, b, Wijayawardene et al. 2017). However, very few genera were reported in this family from marine based habitats (Jones et al. 2015).

The monotypic genus *Deniquelata* Ariyawansa & K.D. Hyde was established by Ariyawansa et al. (2013) to accommodate *Deniquelata barringtoniae* Ariyawansa & K.D. Hyde, found as a pathogen on living leaves of *Barringtonia asiatica* (Lecythidaceae). Both morphology and phylogenetic analyses of a concatenated dataset of the SSU and LSU rDNA supported *Deniquelata*

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as a new genus within Montagnulaceae, which was later synonymized and transferred to Didymosphaeriaceae based on its sister group relationship to *Bimuria* D. Hawksw., Chea & Sheridan and *Trematеia* Kohlm., Volkm-Kohlm. & OE. Erikss. (Ariyawansa et al. 2013, 2014b). This genus is characterized by ascomata that are immersed, globose to sub-globose, dark brown to black, asci bitunicate, clavate to broadly-clavate with a short furcate pedicel. Ascospores oblong to narrowly oblong, reddish brown to dark yellowish brown, muriform with three transverse septa and 1–2 vertical septa, verruculose and lacking a sheath (Ariyawansa et al. 2013).

Farasanispora Abdel-Wahab, Bahkali & E.B.G. Jones is a monotypic genus established by Li et al. (2016) to accommodate *Farasanispora avicenniae* Abdel-Wahab, Bahkali & E.B.G. Jones recorded on decaying wood of *Avicennia marina* from Farasan Island. Phylogenetic analysis of a concatenated dataset of the SSU and LSU rDNA supported *Farasanispora* as a new genus within Pleosporales and related to well established families such as Trematosphaeriaceae, Ascocylindricaceae and Morosphaeriaceae that are known to harbour marine fungi. However, any close affinities to other genera or an accurate position within a particular family remained unresolved (Li et al. 2016). Currently the genus is monotypic with *Farasanispora avicenniae*.

The genus *Hysterium* was circumscribed by morphological characteristics such as hysterothecial, pigmented, carbonaceous ascomata and ascospores that are 3 or more transversely-septate (Bisby 1923). The recent molecular studies showed that the species of *Hysterium* are polyphyletic (Schoch et al. 2009). Hyde et al. (2017) reported *Hysterium rhizophorae*, a new species, from *Rhizophora apiculata* based on the differences in the asci and ascospore dimensions in contrast with *H. angustatum*, which also formed a distinct lineage in the phylogenetic analyses.

We have reported some new species from our ongoing studies on biodiversity of marine fungi from Muthupet mangroves, Tamil Nadu, south east coast of India (Devadatha et al. 2017, 2018, Devadatha & Sarma 2018). In the present study, we introduce a new species *Deniquelata vittalii* in *Deniquelata* based on morphological characters and phylogenetic analyses. Further, two new records of marine fungi to India, viz., *Farasanispora avicenniae* and *Hysterium rhizophorae* are also reported in this paper supplemented with molecular sequence data.

Materials & Methods

Sample collection and morphological studies

Decaying mangrove woody stem pieces of the halophyte *Suaeda monoica* Forssk. ex J.F. Gmel and *Aegiceras corniculatum* (L.) Blanco were collected from Muthupet mangroves (10.4 °N, 79.5 °E), Kaveri River Delta, Tamil Nadu, southeast coast of India as detailed in Devadatha et al. (2017). Specimens were incubated in moist chambers and examined under an Optika stereo zoom SZM-LED1 microscope. Hand sections of the ascomata were taken, where necessary, and the spore mass contents were scooped out with the help of forceps or a needle and mounted in sterile sea water and/or Lactophenol to observe the microscopic characters. Images were captured using Nikon ECLIPSE TiU upright microscope with DIC objectives connected to Nikon DS-Fi2 digital camera.

Single spore isolation was performed as described in Chomnunti et al (2014) with the modifications outlined in Devadatha et al. (2017). The herbarium specimens and the type cultures were deposited in the Ajrekar Mycological Herbarium (AMH) and National Fungal culture collection of India (NFCCI), Agharkar Research Institute (ARI), Pune, India. Facesofffungi and MycoBank numbers are provided (Jayasiri et al. 2015, MycoBank 2017).

DNA extraction, PCR amplification and sequencing

Genomic DNA was extracted from the axenic cultures grown on Malt Extract Agar (MEA) medium by using the DNeasy plant DNA extraction kit (QIAGEN, Germany) following the manufacturer's protocol. In this study, several loci were amplified using known universal primer pairs: ITS1 and ITS4 to amplify ITS region and nuclear small subunit rDNA region (SSU) was amplified using NS1 and NS4 (White et al. 1990). Nuclear large subunit rDNA (LSU) was amplified using LR0R and LR5 (Vilgalys & Hester 1990). The translation elongation factor 1-alpha

gene (TEF-1α) was amplified using primers EF1–983F and EF1–2218R (Rehner & Buckley 2005). The RNA polymerase II second largest subunit (RPB2) gene was amplified using the fRPB2-5F and fRPB2-7cR primer (Liu et al. 1999).

The amplifications were performed employing 50µL of Polymerase chain reaction (PCR) mixtures containing 5 µL of 10X Ex Taq buffer, 4 µL of deoxy nucleotide triphosphate mixture (2.5 mM of each dNTP), 1 µL of each primer (10 µM), 2µL of DNA template, 0.25 µL of Takara EX Taq polymerase and 36.75 µL of Nuclease free water. The PCR amplification conditions and amplified PCR amplicons were purified as reported in Devadatha et al. (2018). The purified PCR products were sequenced at Macrogen Inc. (Seoul, Korea).

Phylogenetic analyses

Taxa used in the phylogenetic analyses were obtained based on the BLAST search similarity resulted from LSU and ITS regions and through published literature (Ariyawansa et al. 2013, 2014a, b) for the taxonomic placement of *Deniquelata vittalii*. Multi-gene phylogenetic analyses of combined LSU, SSU, ITS, TEF-1α, RPB2 sequence data were performed for *Farasanispora avicenniae* (Li et al. 2016) and *Hysterium rhizophorae* (Hyde et al. 2017), based on the published data. Multiple sequence alignments for individual regions were generated online at MAFFT server (http://mafft.cbrc.jp/alignment/server/) (Katoh & Standley 2013) and alignments were improved manually using BioEdit, where necessary. The individual sequence datasets (LSU, SSU, ITS, TEF-1α, RPB2) were combined using BioEdit v.7.0.5.2 (Hall 1999). Three different datasets were prepared for the multigene phylogenetic analyses.

Maximum-parsimony analysis was performed by using PAUP v.4.0b10 (Swofford 2002) software to generate the most parsimonious trees. Trees were inferred using the heuristic search option with 1000 random sequence additions, with maxtrees set at 1000. Descriptive tree statistics for parsimony; Tree Length (TL), Consistency Index (CI), Retention Index (RI), Relative Consistency Index (RC) and Homoplasy Index (HI) were calculated for trees generated under different optimality criteria. The Kishino-Hasegawa tests (Kishino & Hasegawa 1989) were performed in order to determine whether trees were significantly different. Maximum parsimony bootstrap values (MP) equal or greater than 70 % are given above each node for Figure 1 and equal or greater than 75 % for Figs 2, 3.

The evolutionary models for phylogenetic analyses were selected for each gene using MrModeltest v. 2.3 (Nylander 2004) under the Akaike Information Criterion (AIC) as implemented in both PAUP v. 4.0b10 and Mr. Bayes v.3.1.2. GTR+I+G model was selected as the most appropriate in each locus for Bayesian analysis and maximum-likelihood by AIC in MrModeltest as the best-fit model.

Bayesian analysis was performed with MrBayes v. 3.1.2 (Huelsenbeck & Ronquist 2001) to evaluate Bayesian posterior probabilities (BYPP) (Rannala &Yang 1996, Zhaxybayeva & Gogarten 2002) by Markov Chain Monte Carlo sampling (BMCMC). GTR+I+G was used in the command. Six simultaneous Markov chains were run for 1,000,000 generations and trees were sampled every 100th generation (resulting in 10001trees). The distribution of log-likelihood scores was examined to determine stationary phase for each search and to decide if extra runs were required to achieve convergence, using the program Tracer 1.4 (Rambaut & Drummond 2007). First 20% of generated trees were discarded and remaining 80% trees were used to calculate posterior probabilities in the majority rule consensus tree. BYPP greater than 0.95 are given above each node (Figs. 1, 2, 3).

RAxML-HPC2 on XSEDE (8.2.8) (Stamatakis et al. 2008, Stamatakis 2014) in the CIPRES Science Gateway platform (Miller et al. 2010) was used to construct a maximum likelihood (ML) tree. Maximum Likelihood bootstrap values greater than 70% were given above each node for (Fig. 1) and equal or greater than 75 % for (Figs. 2, 3).

The Phylogenetic trees were viewed in FigTree v1.4.0 program (Rambaut 2012) and reorganized in Microsoft power point (2016) and Adobe Illustrator® CS5 (Version 15.0.0, Adobe®, San Jose, CA). Novel sequences generated in the current study were deposited in GenBank (Tables

1, 2, 3) and the finalized alignment and tree of *Deniquelata vittalii* were deposited in TreeBASE, submission ID: 22390 (http://www.treebase.org/).

Results

Phylogenetic analyses

The first phylogenetic analyses were carried out with 24 sequences with our new taxon and strains from Didymosphaeriaceae, with *Stemphylium vesicarium* and *Stemphylium botryosum* as the outgroup taxa using combined LSU, SSU and ITS gene datasets (Table 1). Three different alignments corresponding to each individual gene and a combined alignment of the three genes were analyzed. The maximum parsimony dataset consists of 2332 characters with 1848 characters as constant, 311 characters were counted as parsimony-informative and 173 characters as parsimony-uninformative. The parsimony analyses resulted in two equal parsimonious trees with a tree length of 974 steps, CI = 0.633, RI = 0.661, RC = 0.419, HI = 0.367 values. RAxML analysis yielded a best scoring tree (Fig. 1) with a final ML optimization likelihood value of -8221.245191. The matrix had 586 distinct alignment patterns, with 16.69% of undetermined characters or gaps. Estimated base frequencies were as follows; A = 0.247437, C = 0.227513, G = 0.277892, T = 0.247158; substitution rates AC =1.689545, AG = 2.522196, AT = 1.252478, CG = 0.689774, CT = 6.758270, GT = 1.000; proportion of invariable sites I = 0.576761; gamma distribution shape parameter α = 0.582903. The Bayesian analyses resulted in 10001 trees after 1,000,000 generations. The first 2000 trees, representing the burn-in phase of the analyses, were discarded, while the remaining 8001 trees were used for calculating posterior probabilities in the majority consensus tree for all the phylogenetic analyses (Figs 1, 2, 3). Phylogenetic trees obtained from ML, MP and Bayesian analyses yielded trees with similar topology and congruent with previous work-based on MP, ML and Bayesian analyses (Ariyawansa et al. 2013, 2014b). MP tree is shown with the bootstrap support (BS) values of MP and ML (≥70 %) and Bayesian posterior probabilities (BYPP) greater than 0.95 are shown above the internal branches respectively (Fig. 1).

Table 1 GenBank and culture collection accession numbers of species included in the phylogenetic study of *Deniquelata vittalii*. Sequences generated in this study are in blue.

Taxon	Voucher/ Culture	GenBank Accessions		
		ITS	LSU	SSU
Alloconiothyrium aptrootii	CBS 980.95	JX496121	JX496234	-
Bimuria novae-zelandiae	CBS 107.79	-	AY016356	AY016338
Deniquelata barringtoniae	MFLUCC 11-0257	KM213997	KM214000	KM214003
Deniquelata barringtoniae	MFLUCC 11-0422	JX254654	JX254655	JX254656
Deniquealta vittalii	**NFCCI4249**	**MF406218**	**MF182395**	**MF622059**
Didymocrea sadasivanii	CBS 438.65	-	DQ384103	DQ384066
Didymosphaeria rubi-ulmifolii	MFLUCC 14-0023	-	KJ436586	KJ436588
Kalmusia spartii	MFLUCC 14-0560	KP744441	KP744487	KP753953
Karstenula rhodostoma	CBS 691.94	-	AB807531	AB797241
Laburnicola muriformis	MFLUCC 16-0290	KU743197	KU743198	KU743199
Letendraea cordylinicola	MFLUCC 11-0150	KM213996	KM213999	KM214002
Leptosphaerulina australis	CBS 317.83	GU237829	FJ795500	GU296160
Montagnula cirsii	MFLUCC 13-0680	KX274242	KX274249	KX274255
Neokalmusia brevispora	KT 2313	LC014574	AB524601	AB524460
Paraphaeosphaeria michotii	MFLUCC 13-0349	KJ939279	KJ939282	KJ939285
Paraconiothyrium hawaiiense	CBS 120025	JX496027	JX496140	EU295655
Phaeodothis winteri	CBS 182.58	-	DQ678073	DQ678021
Pseudopithomyces chartarum	UTHSC 04-678	HG518060	HG518065	-
Stemphylium vesicarium	CBS 191.86	KC584239	GU238160	GU238232
Stemphylium botryosum	CBS 714.68	KC584238	KC584345	KC584603
Pseudocamarosporium corni	MFLUCC 13-0541	KJ747048	KJ813279	-
Spegazzinia tessarthra	Yone 211	JQ673429	AB807582	AB797294
Tremateia arundicola	MFLUCC 16-1275	KX274241	KX274248	KX274254
Xenocamarosporium acaciae	CPC 247.55	KR476724	KR476759	-

Table 2 GenBank and culture collection accession numbers of species included in the phylogenetic study of *Farasanispora avicenniae*. Sequences generated in this study are in blue.

Taxon	Voucher/ Culture	GenBank Accessions				
		LSU	SSU	ITS	TEF1α	RPB2
Aegeanispora elanii	MAW-2017	KY026052	KY026051	-	-	-
Aquilomyces patris	CBS135661	KP184041	KP184077	NR137961	-	-
Ascocylindrica marina	MD6011	-	KT252907		-	-
Ascocylindrica marina	MD6012	KT252906	-		-	-
Camarographium koreanum	CBS 117159	JQ044451	-	JQ044432	-	-
Clypeoloculus akatensis	KT788	AB807543	AB797253	AB809631	AB808519	-
Halomassarina thalassiae	BCC 17054	GQ925849	GQ925842	-	-	-
Halomassarina thalassiae	BCC 17055	GQ925850	GQ925843	-	-	-
Helicascus elaterascus	KT2673	AB807533	AB797243	AB809626	AB808508	-
Helicascus nypae	BCC36751	GU479788	GU479754		GU479854	GU479826
Falciformispora lignalitilis	BCC21118	GU371827	GU371835	KF432943	GU371820	
Falciformispora senegalensis	CBS19679	KF015631	KF015636	KF015673	KF015687	KF015717
Falciformispora tompkinsii	CBS20079	KF015625	KF015639	NR132041	KF015685	KF015719
Farasanispora avicenniae	MF1207	KT950962	KT950961	-	-	-
Farasanispora avicenniae	**NFCCI-4220**	**MG844277**	**MG844281**	**MG844285**	**MG948548**	**MG973031**
Macrodiplodiopsis desmazieri	CBS123812	KR873269	-	KR873234	-	-
Massarina eburnea	H3953	AB521735	AF164367	-	AB808517	-
Massarina ignaria	-	DQ810223	DQ813511	-	-	-
Medicopsis romeroi	CBS 122784	EU754208	EU754109	KF366447	KF015679	KF015707
Morosphaeria ramunculicola	BCC18404	GQ925853	GU479760	-	-	-
Morosphaeria velatispora	BCC17058	GQ925851	GQ925840	-	-	-
Lentithecium fulviatile	CBS 123090	FJ795450	FJ795493	-	-	FJ795467
Lentithecium arundinaceum	CBS123131	GU456320	GU456298	-	GU456281	-
Pseudochaetosphaeronema larense	CBS639.94	KF015610	KF015651	KF015655.	KF015683	-
Trematosphaeria hydrela	CBS88070	KF314116	-	-	KF314136	-
Trematosphaeria pertusa	CBS122368	FJ201990	-	NR132040	-	-
Ulospora bilgrami	AFTOLD1598	DQ678076	DQ678025	-	DQ677921	FJ795476
Verriculina enalia	BCC18402	GU479803	GU479771	-	GU479864	DQ677974

Table 3 GenBank and culture collection accession numbers of species included in the phylogenetic study of *Hysterium rhizophorae*. Sequences generated in this study are in blue.

Taxon	Voucher/ Culture	GenBank Accessions			
		LSU	SSU	ITS	RPB2
Hysterium angustatum	CBS 236.34	FJ161180	GU397359.	-	FJ161117
Hysterium angustatum	CBS123334	FJ161207	FJ161180	-	GU566751
Hysterium angustatum	GKM5211	GQ221906	-	-	-
Hysterium angustatum	SMH5216	GQ221908	-	-	-
Hysterium angustatum	GKM243a	GQ221899	-	-	-
Hysterium angustatum	CMW:20409	FJ161194	FJ161153.	-	-
Hysterium hyalinum	CBS:237.34	FJ161181	FJ161141	-	-
Hysterium barrianum	ANM1495	GQ221885	-	-	-
Hysterium barrianum	ANM1442	GQ221884	-	-	-
Hysterium pulicare	ANM85	GQ221898	-	-	-
Hysterium pulicare	EB 0238	FJ161201	FJ161161	-	FJ161127
Hysterium pulicare	CBS:119331	-	-	EU552137	-
Hysterium pulicare	AFTOL-ID 1254	DQ678055	DQ678002	-	FJ238433
Hysterium pulicare	ANM1455	GQ221904	-	-	-
Hysterium vermiforme	GKM1234	GQ221897	-	-	-
Hysterium rhizophorae	MFLU 16-1179	KX611364	KX611365	KX611363	-
Hysterium rhizophorae	**NFCCI-4250**	**MG844276**	**MG844280**	**MG844284**	**MG968956**
Psiloglonium clavisporum	CBS:123338	FJ161197	-	-	-
Psiloglonium clavisporum	CBS:123340	FJ161205	-	-	-

The phylogenetic analyses show that our new taxon *Deniquelata vittalii* clustered together with the strains of *D. barringtoniae* with a strong statistical support in a monophyletic clade (100%

ML, 100%MP, 1.00 BYPP) in the family Didymosphaeriaceae. *Deniquelata vittalii* shares a sister relationship with *D. barringtoniae* with significant statistical support (88% ML, 89% MP, 1.00 BYPP, Fig. 1).

The second multigene phylogenetic analyses include 27 in-group taxa from different genera from Pleosporales, including our taxon, while *Verruculina enalia* served as the outgroup taxon (based on LSU, SSU, TEF-1α, ITS and RPB2 sequence data, Table 2). RAxML analysis yielded a best scoring tree (Fig. 2) with a final ML optimization likelihood value of -22392.941365. The matrix had 1629 distinct alignment patterns, with 42.78% of undetermined characters or gaps. Estimated base frequencies were as follows; A =0.249321, C = 0.235804, G = 0.273367, T = 0.241508; substitution rates AC = 1.425242, AG =3.166282, AT = 1.605461, CG = 1.085865, CT = 7.895025, GT = 1.000000; proportion of invariable sites I = 0.436939; gamma distribution shape parameter α =0. 442521. The maximum parsimony dataset consisted of 4613 characters with 3128 characters as constant, 1009 characters were counted as parsimony-informative and 476 characters as parsimony-uninformative. The parsimony analyses resulted in one equal parsimonious tree with a tree length of 3657 steps, CI = 0.596, RI = 0.484, RC = 0.288, HI = 0.404 values. Phylogenetic analyses indicate that both strains of *Farasanispora avicenniae* cluster together with high bootstrap support (100% ML, 100% MP, 1.00 BYPP, Fig. 2). Our phylogenies generated herein, under different criteria, yielded similar results as previously reported in connection to the uncertain familial placement of *Farasanispora avicenniae* (Li et al. 2016).

The phylogenetic analyses inferred from the third dataset include 19 taxa from *Hysterium* including our strain, while two strains of *Psiloglonium clavisporum* served as outgroup taxa (Table 3). RAxML analysis yielded a best scoring tree (Fig. 3) with a final ML optimization likelihood value of -9131.172652. The matrix had 297distinct alignment patterns, with 50.91% of undetermined characters or gaps. Estimated base frequencies were as follows; A = 0.253481, C = 0.222684, G = 0.285514, T = 0.238321; substitution rates AC = 0.906683, AG =1.276344, AT = 0.828696, CG = 0.793370, CT = 2.357133, GT = 1.000000; proportion of invariable sites I = 0.492153; gamma distribution shape parameter α = 586.774160. The maximum parsimonious dataset consists of 19 taxa with 3632 characters, of which 2500 were constant, 312 were parsimony-informative and 820 parsimony-uninformative. The parsimony analysis of the data matrix resulted in one hundred and ninety-eight equally parsimonious trees with a length of 1523 steps (CI = 0.922, RI = 0.711, RC = 0.656, HI = 0.078). The molecular phylogeny indicates that both the strains of *H. rhizophorae* (MFLUCC161179 and NFCCI4250) nested together with significant bootstrap support of 77% in MP, 0.96% in BYPP and moderate support of 64% in ML (Fig. 3). Phylogenetic trees resulted from ML, MP, BYPP analyses were in congruent to the earlier study (Hyde et al. 2017).

Taxonomy

Deniquelata vittalii Devadatha, V.V Sarma, E.B.G Jones, sp. nov. Fig. 4

MycoBank number: MB820842; Facesoffungi number: FoF04375

Etymology – In honour of Professor B.P.R. Vittal, formerly Professor in the University of Madras, India for his contributions to Indian mycology

Holotype – AMH-9888

Saprobic on decaying woody stem of the halophyte *Suaeda monoica*. Sexual morph: *Ascomata* 95–360 μm high, 75–350 μm in diameter ($\bar{x}$ = 225 × 212 μm, n = 10), immersed to semi-immersed, erumpent, globose to sub-globose, dark brown to black, aggregated to solitary, obpyriform, coriaceous, fused with the host tissues, with a papillate to depressed ostiole. *Peridium* 10–40 μm ($\bar{x}$ = 23 μm, n = 10) wide at the base, 10–55 μm ($\bar{x}$ = 30 μm, n = 10) at the sides, comprising 3–5 layers of thin-walled hyaline to pale brown cells inwardly and thick-walled pale brown to dark brown cells of *textura angularis* outwardly fused with the host tissue. *Hamathecium* composed of 1.5–3 μm wide ($\bar{x}$ = 2 μm, n = 10), cellular, profusely branched, hyaline, septate

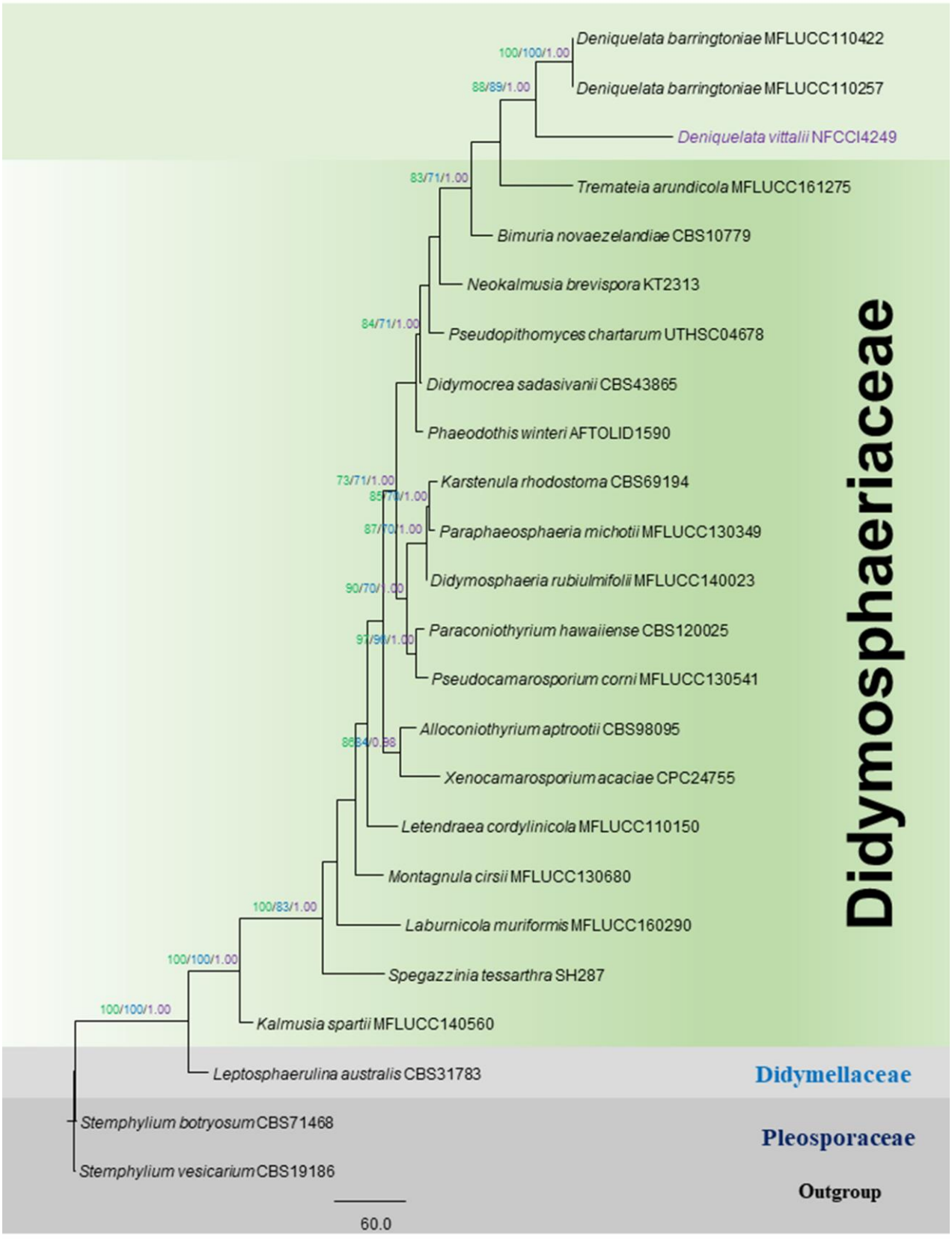

Figure 1 – Phylogram generated from maximum parsimony analysis based on a combined dataset of LSU, SSU and ITS. Bootstrap support values for maximum likelihood (ML, green), maximum parsimony (MP, blue) equal to or greater than 70 % and the values of Bayesian posterior probabilities (BYPP, purple) equal to or greater than 0.95 are given above each branch, respectively. The new species is in purple colour. The tree is rooted with *Stemphylium vesicarium* and *Stemphylium botryosum*.

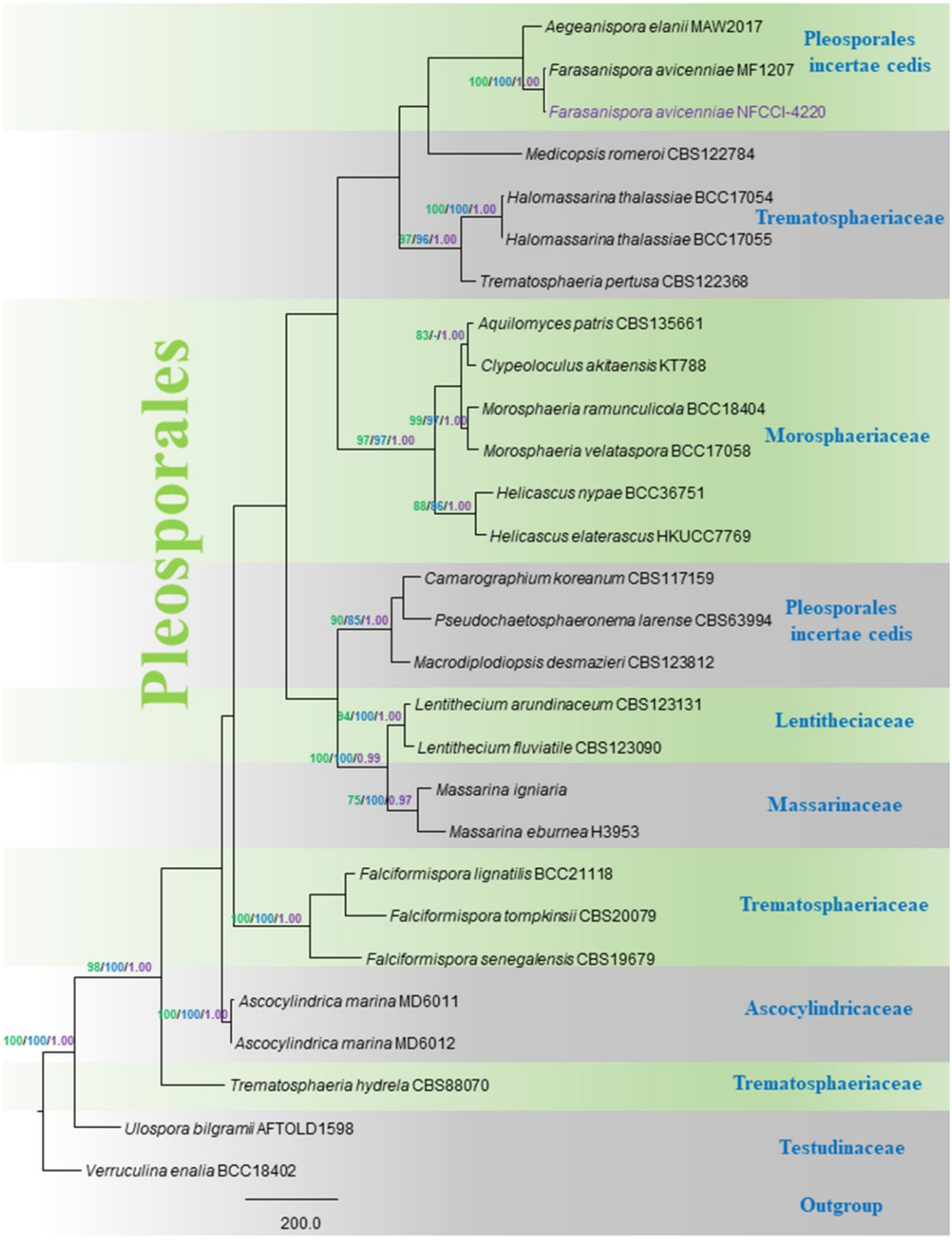

Figure 2 – Phylogram generated from maximum parsimony analysis based on combined LSU, SSU, TEF1, ITS and RPB2 sequence data of *Farsanispora avicenniae* NFCCI-4220 and other related taxa in Pleosporales. *Verruculina enalia* is the outgroup taxon. Bootstrap support values for maximum likelihood (ML, green), maximum parsimony (MP, blue) equal to or greater than 75 % and the values of Bayesian posterior probabilities (BYPP, purple) equal to or greater than 0.95 are given above each branch, respectively. The new record is in purple colour.

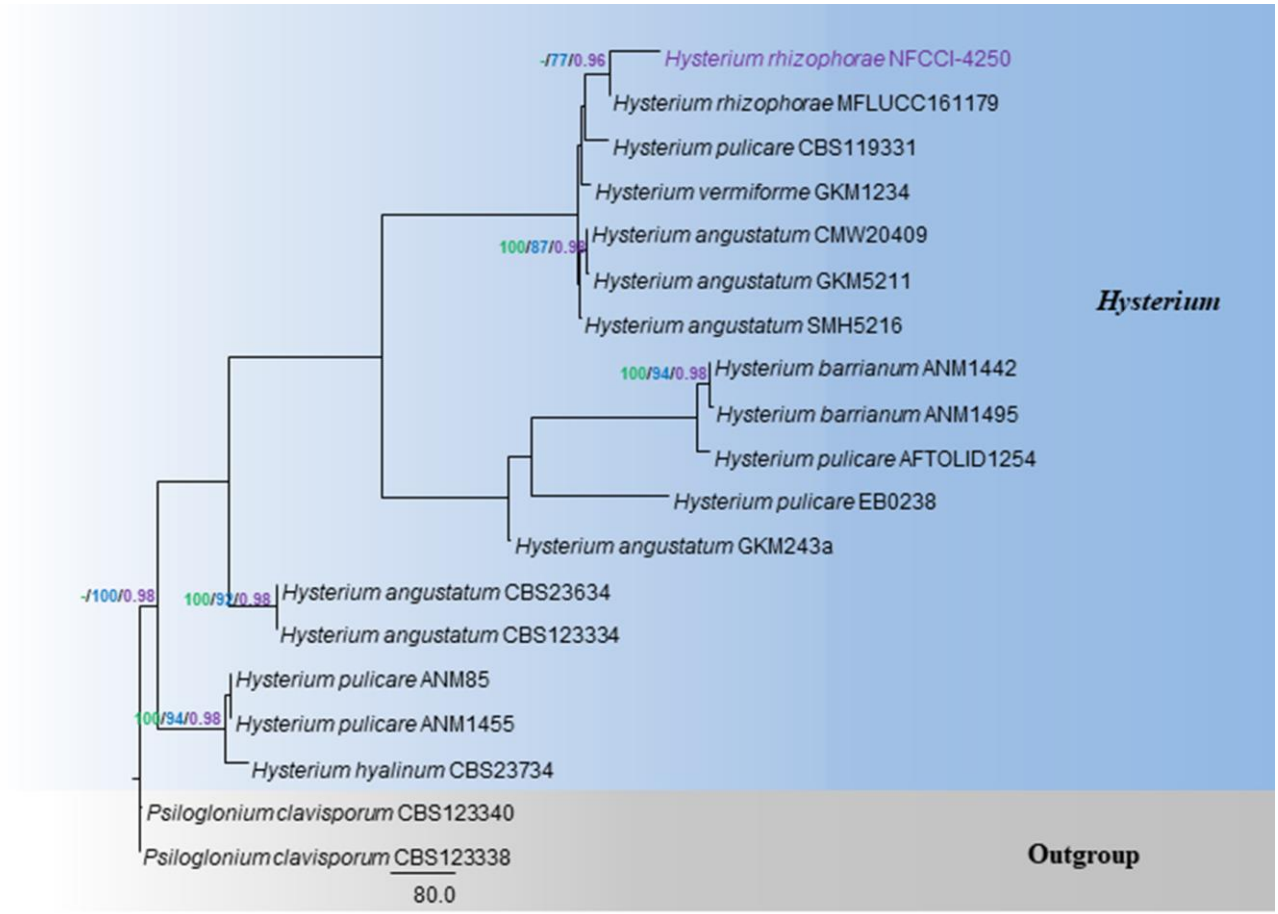

Figure 3 – Phylogram generated from maximum parsimony analysis based on combined LSU, SSU and RPB2 sequence data of selected taxa. Bootstrap support values for maximum likelihood (ML, green), maximum parsimony (MP, blue) equal to or greater than 75 % and the values of Bayesian posterior probabilities (BYPP, purple) equal to or greater than 0.95 are given above each branch, respectively. The new record is in purple. The tree is rooted with *Psiloglonium clavisporum*.

pseudoparaphyses, anastomosing above the asci, enclosed in a gelatinous matrix. *Asci* 75–135 × 10–19 µm ($\bar{x}$ = 98 × 15 µm, n = 30), 8-spored, bitunicate, fissitunicate, cylindric-clavate to clavate, apically rounded with an ocular chamber, with a 7–15 µm long and broad furcate pedicel. *Ascospores* 18–26 × 7.5–13.5 µm ($\bar{x}$ = 22.5 × 10.7 µm, n = 50), 1–2 seriate, muriform, hyaline when young, developing into golden yellow to dark brown at maturity, rarely guttulate, ellipsoidal to broadly oblong, planate, flat, verrucose, deeply constricted in the middle, slightly curved to straight, apically conical to elliptical, with 1–2 longitudinal septa in each cell, 3–6 transverse septate, lacking mucilaginous sheath. Asexual morph: Undetermined.

Culture characteristics – Ascospores germinating on 50% sea water agar producing germ tubes at both ends of the ascospores within 24 hours. Colonies on MEA showed moderate growth attaining 20–24 mm in diameter within a week and reached 35–50 mm after 20 days of incubation at 25°C, front pale ochraceous salmon, reverse capucine buff, with pale yellow diffusible pigments, margin filamentous, pulvinate, circular, cottony and fluffy.

Material examined – INDIA, Tamil Nadu, Tiruvarur, Muthupet mangroves (10.4°N 79.5°E), on decaying woody stem of the halophyte *Suaeda monoica* (Amaranthaceae) 15 August 2015, B. Devadatha (AMH-9888, holotype), ex-type living culture NFCCI-4249.

Notes – Multigene analyses of LSU, SSU and ITS sequence data show that our new taxon belongs to the family Didymosphaeriaceae and is closely related to *Deniquelata barringtoniae* with a strongly-supported monophyletic clade (Fig. 1). Morphologically *Deniquelata vittalii* resembles the generic type *Deniquelata barringtoniae* in having sub-globose to globose ascomata, shorter

papilla with a depressed ostiole; asci with short furcate pedicel and ascospores that are oblong, straight or slightly curved, muriform with 1–2 vertical septa, verruculose (Ariyawansa et al. 2013, 2014b). However, *Deniquelata vittalii* can be easily distinguished from *D. barringtoniae* in having larger ascomata, asci and ascospore dimensions (Fig. 4, Table 4). *Deniquelata barringtoniae* is distinct in having smaller, reddish-brown ascospores (13–16 × 5–7), with 3-transverse septa whereas *D. vittalii* has larger, brown ascospores (17.5–25 × 7.5–13.5) with 3–6 transverse septa (Fig. 4, o–s). Further, *D. barringtoniae* is pathogenic on living leaves of *Barringtonia asiatica* from terrestrial environments (Ariyawansa et al. 2013), while *D. vittalii* is saprobic on decaying woody stem of the halophyte *Suaeda monoica* from marine environments. Distinct nucleotide differences between *Deniquelata vittalii* and *D. barringtoniae* were noted across various gene regions analyzed [35 within ITS; 34 within LSU; 12 within SSU; 46] which comply with recommendations outlined by Jeewon & Hyde (2016) to establish new species based on DNA sequence data. Hence the new species *D. vittalii* has been proposed to be accommodated in the genus *Deniquelata*.

Farasanispora avicenniae Abdel-Wahab, Bahkali & E.B.G. Jones, Fungal Diversity. 78: 63 (2016) Fig. 4

Facesoffungi number: FoF01635

Saprobic on decaying woody stem of the halophyte *Suaeda monoica*. Sexual morph: *Ascomata* 170–330 µm high, 165–345 µm diam. ($\bar{x}$ = 239 × 240 µm, n = 10), immersed to erumpent, sub globose, solitary to gregarious, coriaceous, dark brown to black, ostiolate. *Peridium* unequal in thickness, 18–40 µm ($\bar{x}$ = 28 µm, n = 10) wide at the sides comprising two different cell layers, outer layer of brown to hyaline polygonal cells fused with host tissue and thick inner layer composed of several hyaline flattened cells of *textura angularis*. *Peridium* less developed at the base 15–30 µm ($\bar{x}$ = 22.5 µm, n = 10) wide, hyaline to light brown cells fused with the host tissue. *Hamathecium* composed of 1.5–2.5 µm ($\bar{x}$ = 2.1 µm, n= 20) wide, numerous, septate, branched, filamentous pseudoparaphyses resembling hyphae embedded in a gelatinous matrix, anastomosing above the asci. *Asci* 70–135 × 20–32 µm ($\bar{x}$ = 105 × 26 µm, n = 40), 8-spored, bitunicate, fissitunicate, cylindrical to clavate, short pedicellate, apically rounded and thickened with an ocular chamber. *Ascospores* 30–37 × 7–15 µm ($\bar{x}$ = 34 × 11 µm, n = 50), biseriately arranged, constricted at the septa, rugose, hyaline, fusiform,1–3 septate, the septum is sub-median, upper cell longer and wider, slightly curved, guttulate, lacking a mucilaginous sheath. Senescent ascospores are larger 35–42 × 10–15 µm ($\bar{x}$ = 37 × 12 µm, n = 10), light brown, flattened, distinctly constricted at the middle septum, striate, verruculose, 2–3 septate.

Asexual morph: After 25 days of fermentation in Czapek-Dox broth, oval to ellipsoidal chlamydospores were found, 8–25 × 10–20 µm ($\bar{x}$ = 15 × 12.5 µm, n = 10), hyaline to purple colour filamentous hyphae, pinkish diffusible pigments produced as extracellular metabolites (Fig. 6).

Culture characteristics – Ascospores germinating on seawater agar within 24 hours, germ tubes arising from terminal ends of the ascospores. Colonies on malt extract agar moderately growing, reaching 45–60 mm diameter after 25 days of incubation at room temperature, initially white to grey becoming dark grey to brown in older cultures, flexuous on surface, zonate, undulate, effuse, flattened, medium dense, reverse pink, producing diffusible pigments into media.

Material examined – INDIA, Tamil Nadu, Tiruvarur, Muthupet mangroves, decaying woody stem of the halophyte *Suaeda monoica* Lam. (Amaranthaceae), (10.4°N 79.5°E), 29 October 2016, B. Devadatha, (AMH-9911**)** living culture (NFCCI-4220).

Notes – Multigene phylogenetic analyses show that *Farasanispora avicenniae* (NFCCI-4220) belongs to the order Pleosporales sharing sister relation to Trematosphaeriaceae and Morosphaeriaceae and distantly related to *Massarinaceae* (Fig. 2). However, it did not group with any known family and formed a distinct monophyletic clade with the existing *Farasanispora avicenniae* (MF1207) with a high bootstrap support from (100% ML, 100%MP, 1.00 BYPP, Fig. 2) and shares a sister group relation to *Aegeanispora elanii* with a lower bootstrap support. Morphological characteristics of *Farasanispora avicenniae* occurring on the decaying woody stems

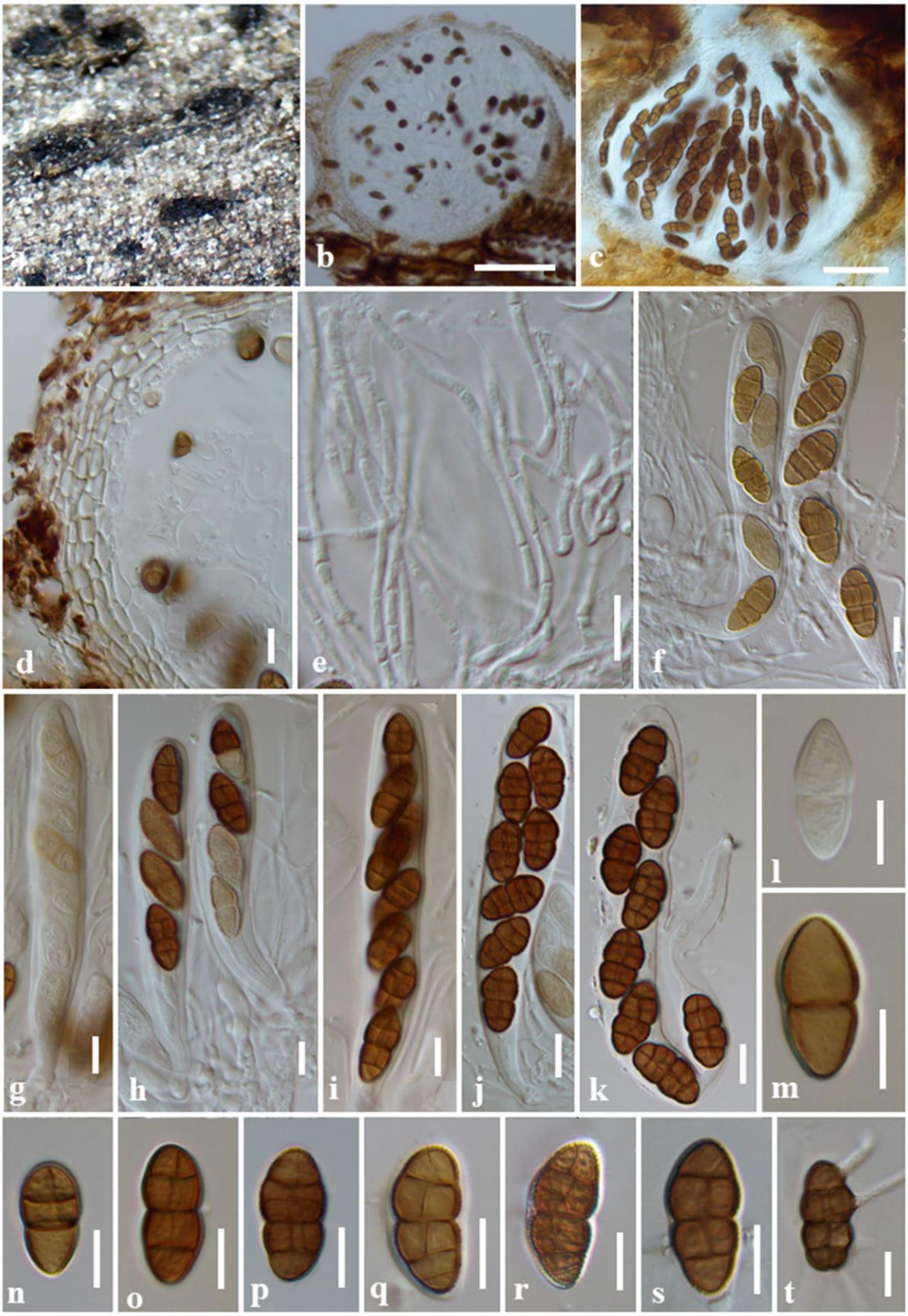

Figure 4 – *Deniquelata vittalii* (AMH-9888, holotype). a Ascomata semi-immersed in the decaying woody stem of the halophyte *Suaeda monoica*. b–c Longitudinal sections of ascomata d Section of peridium. e Cellular and hyaline pseudoparaphyses. f-k Immature and mature asci. l-s Ascospores. t Germinating ascospores. Scale bars: b = 100 µm, c = 50 µm, d-t = 10 µm.

Table 4 Synopsis of morphological differences between *Deniquelata vittalii* and *D. barringtoniae*

Species	*Deniquelata barringtoniae*	*Deniquelata vittalii*
Host	*Barringtonia asiatica*	*Suaeda monoica*
Life mode	Parasitic on leaves	Saprobic on decaying woody stems
Habitat	Terrestrial	Marine
Ascomata (µm)	150–180 × 164–189	100–350 × 75–350
Peridium thickness (µm)	9–17	10–40
Asci (µm)	67–78 × 10–15	70–140 × 10–20
Ascospores (µm)	13–16 × 5–7, oblong, reddish brown to dark yellowish brown, 3-transverse septa and 1–2 longitudinal septa	17.5–25 × 7.5–13.5, golden yellow to dark brown, deeply constricted in the middle, 3–5 transverse septate, 1–2 longitudinal septa in each cell
References	Ariyawansa et al. (2013)	This study

of halophyte *Suaeda monoica*, reported in the present study, are similar to *Farasanispora avicenniae* reported from *Avicennia marina* (Li et al. 2016). However, its occurrence on *Suaeda monoica*, in India, constitute new host and geographic records. *Farasanispora avicenniae* shares similar morphological characters with *Halomassarina thalassiae* (Kolhm. & Volkm -Kohlm.) Suetrong et al. (Suetrong et al. 2009). However, it differs from *Halomassarina thalassiae* (Suetrong et al. 2009, Li et al. 2016) in having smaller ascomata, without a clypeus or papillae and in the absence of periphyses in the ostiolar canal, lacking senescent ascospores and a prominent gelatinous sheath (Fig. 5) (Kohlmeyer & Kohlmeyer 1987). Prior to the present study, only LSU and SSU gene sequence data of *F. avicenniae* were available (Li et al. 2016). This study provided ITS, TEF-1α and RPB2 in addition to LSU and SSU (Table 2).

Hysterium rhizophorae Dayarathne & K. D. Hyde, in Fungal Diversity 87:42 (2017) Fig. 7

Facesoffungi number: FoF02911

Saprobic on decaying wood of *Aegiceras corniculatum*. Sexual morph *Ascomata* hysterothecial, 650–2100 µm long ($\bar{x}$ = 1060 µm, n = 5), 100–400 µm high, 170–200 µm wide ($\bar{x}$ = 243 × 199 µm, n = 5), erumpent to superficial with base immersed, solitary to gregarious, straight to flexuous, ellipsoid or elongate, with pointed ends, opening by a depressed longitudinal slit, in vertical section sub-globose to globose, carbonaceous, black. *Peridium* 25–75 ($\bar{x}$ = 50 µm, n = 5) µm wide, carbonaceous, comprising an outer layer of dark brown cells of *textura globosa* and an inner layer of hyaline to pale brown cells of *textura globosa*. *Pseudoparaphyses* 1–2 µm (n = 30) wide, cellular, septate, flexuous, branched. *Asci* 40–65 × 6–12 µm ($\bar{x}$ = 48 × 10 µm, n = 20), 8-spored, bitunicate, cylindric to claviform, short pedicellate. *Ascospores* 10–17 × 3–5 µm ($\bar{x}$ = 14 × 4 µm, n = 30), overlapping biseriate, light brown, ellipsoidal, straight to slightly curved, with 3-transverse septa, often slightly constricted at the median septum, with or without guttules. Asexual morph: Undetermined.

Culture characteristics – Ascospores germinated on sea water agar within 24 hours, germ tubes arisen from terminal ends of the ascospore. Colonies on malt extract agar reaching 30–75 mm diameter after 25 days of incubation at room temperature, initially hyaline becoming ash grey in older cultures and reverse light brown, irregular, undulate, floccose.

Material examined – INDIA, Tamil Nadu, Tiruvarur, Muthupet mangroves, on intertidal wood of *Aegiceras corniculatum* (Primulaceae), (10.4°N 79.5°E), 29 October 2016, B. Devadatha (AMH-9947), living culture (NFCCI-4250).

Notes – Combined phylogenetic analyses of LSU, SSU, ITS and RPB2 sequence data placed our taxon (*Hysterium rhizophorae* NFCCI4250) in the family Hysteriaceae and clustered together with *Hysterium rhizophorae* (MFLUCC161179) in a monophyletic clade with a significant bootstrap support from MP 77%, BYPP 0.96% and moderate support in ML 64% (Fig. 3). This *Hysterium* species was found on the decaying wood of *Aegiceras corniculatum* having overlapping

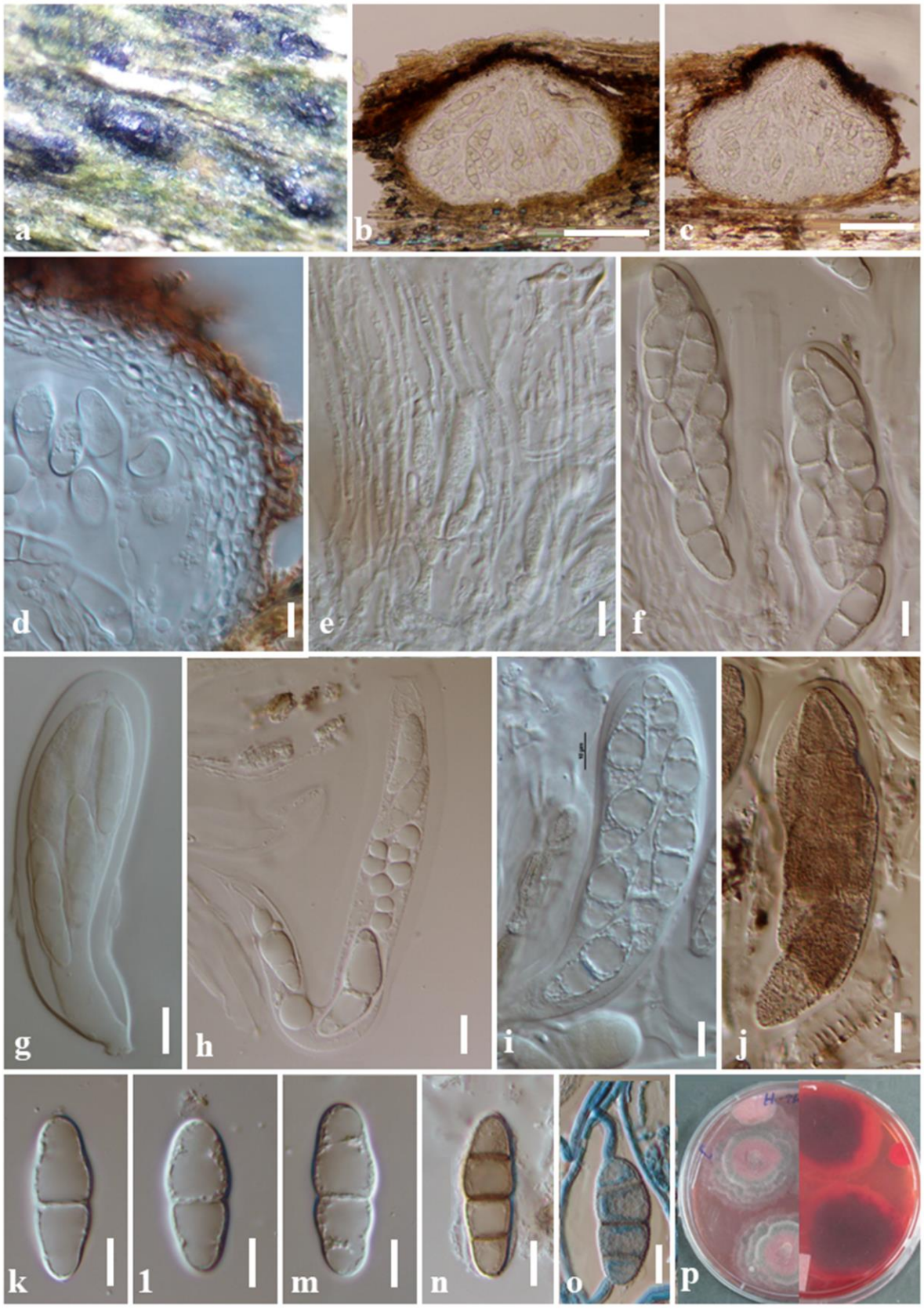

Figure 5 – *Farasanispora avicenniae* (AMH-9911). a Ascomata erumpent on the decaying wood of *Suaeda monoica*. b-c Longitudinal sections of ascomata d Section of peridium e filamentous pseudoparaphyses. f-j Immature and mature asci. k-m Hyaline ascospores. n Mature senescent ascospore. o Germ tubes developed from terminal ends of ascospore p Culture. Scale bars: b–c = 100 μm, d–o = 10 μm.

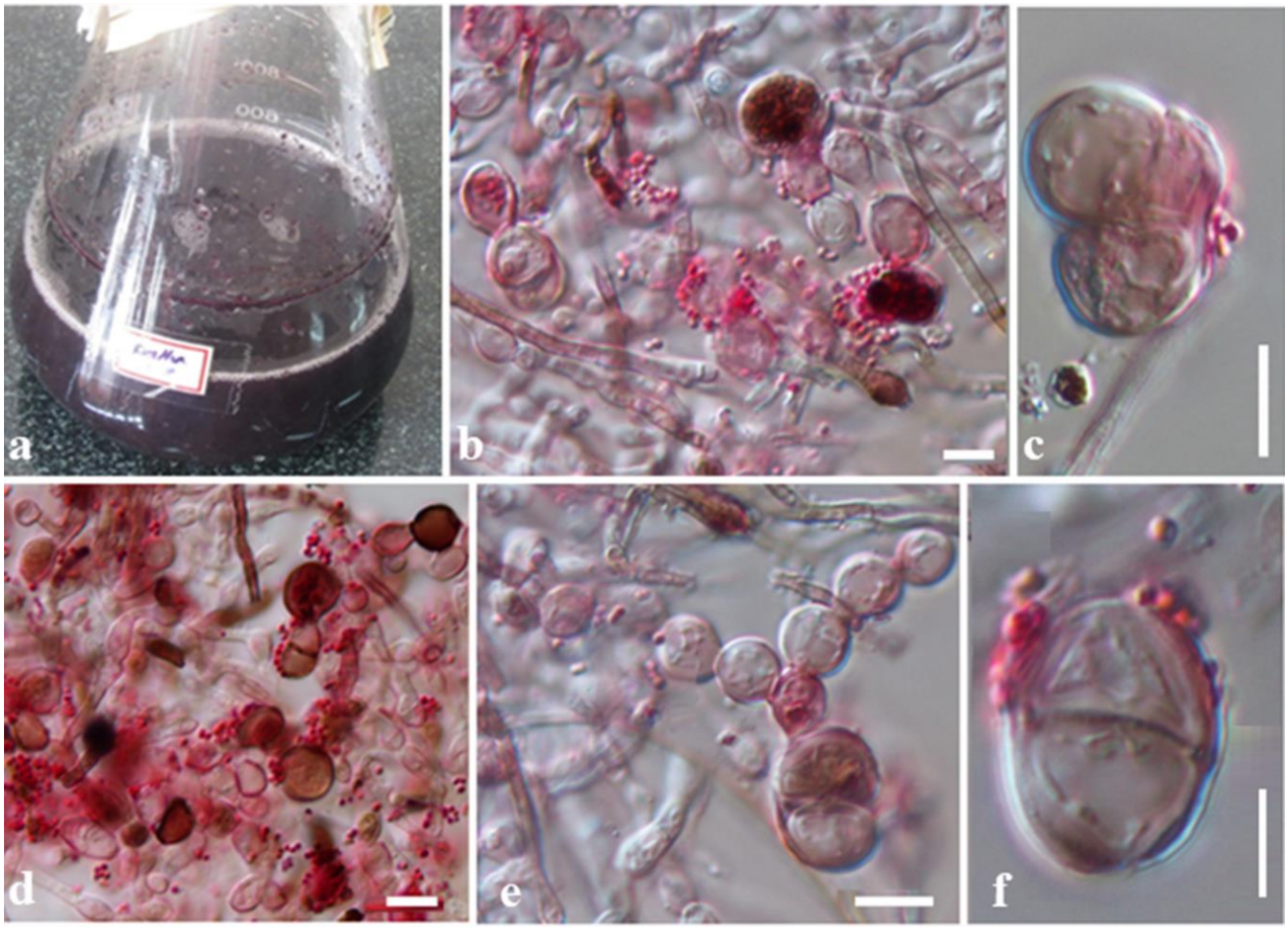

Figure 6 – *Farasanispora avicenniae* (NFCCI-4220). a Fermented culture in Czapek-Dox broth. b, d hyphae showing development of chlamydospores and pink pigments. c, e-f chlamydospores. Scale bars: b–c = 100 μm, d–o = 10 μm.

morphological characters with *Hysterium rhizophorae* reported from *Rhizophora apiculata* (Hyde et al. 2017). The occurrence of *H. rhizophorae* on a new host i.e. *A. corniculatum* increases its host range and also this is the first report of this fungus from India thus expanding its geographical range. ITS pairwise alignment resulted in a very low (3) base pair difference with ITS sequence data of the two strains of *H. rhizophorae* (Jeewon & Hyde 2016). Previously only ITS, LSU, SSU and TEF-1α gene sequences were carried out (Hyde et al. 2017). The present study provides additional information on RPB2 sequence in the GenBank (RPB2: MG968956) in addition to the ITS, LSU and SSU gene sequence data for the new record.

Discussion

The monotypic genus *Deniquelata* typified by *D. barringtoniae* was established by Ariyawansa et al. (2013) who reported this fungus as a pathogen on living leaves of *Barringtonia asiatica* with brown spots and fruiting bodies scattered in the necrotic tissues (Ariyawansa et al. 2013, 2014b). The pathogenic nature of *Deniquelata barringtoniae* was proved by *in vitro* pathogenicity testing of healthy leaves of *Barringtonia asiatica* (Ariyawansa et al. 2013).

Multi-gene analyses showed that *Deniquelata vittalii* nested together with *D. barringtoniae* with significant bootstrap support (88% ML, 89% MP, 1.00 BYPP, Fig. 1) and separated from *Bimuria novae-zelandiae* and *Tremateia arundicola* sister groups. *Deniquelata* and *Bimuria* share similarities in having scattered, semi-immersed, sub-globose ascomata with muriform ascospores and are saprobic (*D. vittalii* and *B. novae-zelandiae*). *Deniquelata* species distinct from *Bimuria* typified by *B. novae-zelandiae*, in having shorter papilla with a depressed ostiole; 8-spored asci with short furcate pedicel and ascospores that are oblong, straight or slightly curved, muriform with

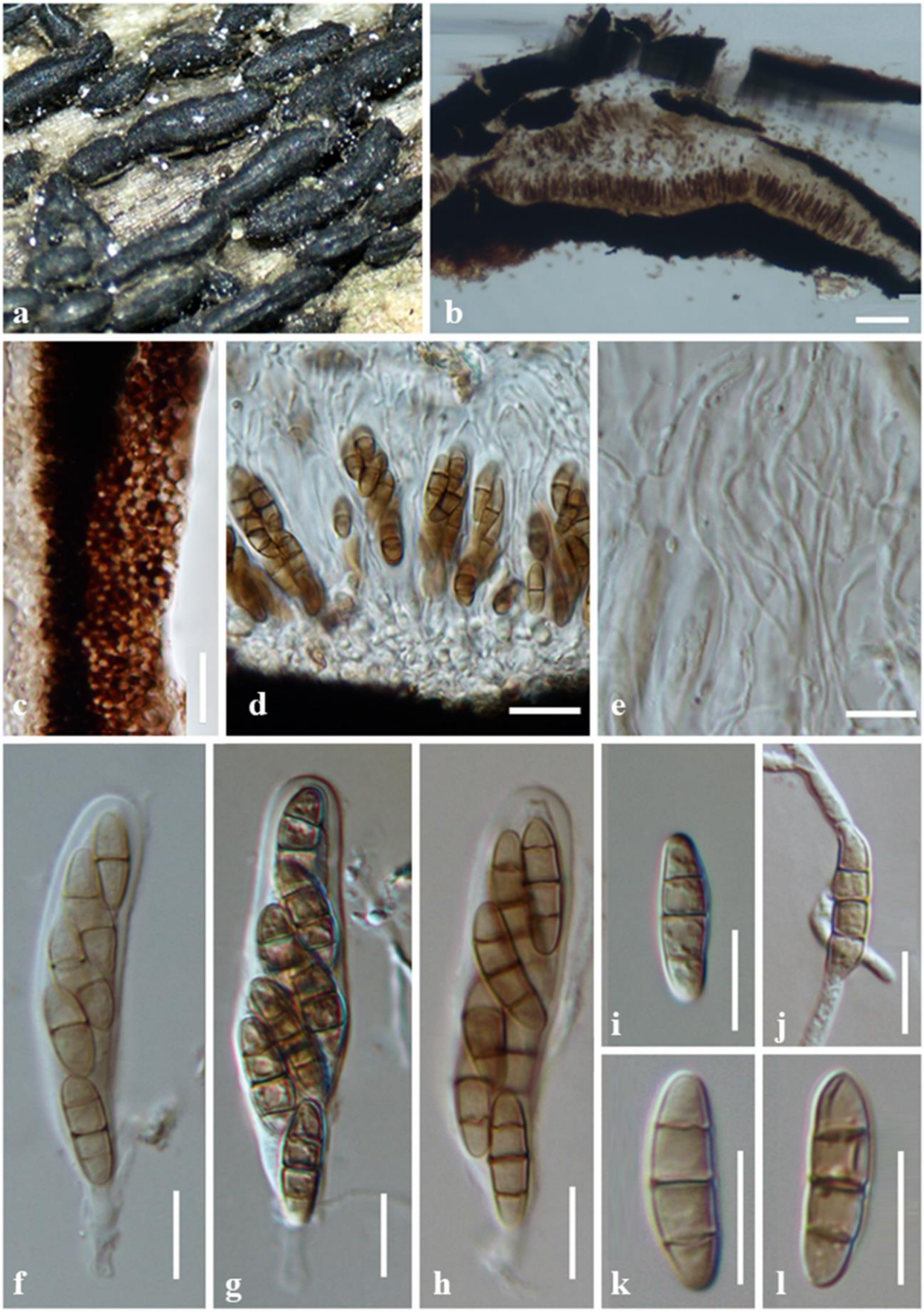

Figure 7 – *Hysterium rhizophorae* (AMH-9947) a Appearance of hysterothecia on host. b Vertical section through hysterothecium. c, d Peridium. e Pseudoparaphyses. f–h Asci. i, k–l Ascospores. j Germinating ascospores. Scale bars: b = 100 μm, c-k = 10 μm.

3–6 transverse septa, 1–2 vertical septa, verruculose. *Bimuria novae-zelandiae* has 2–3-spored asci with short and small knob-like pedicel and comparatively larger ascospores with 5–7 transverse septa, without vertical septa, verrucose and by occurring in terrestrial habitat (Hawksworth 1979, Ariyawansa et al. 2013). The species in *Trematеia* can be clearly distinguished from *Deniquelata* species in having clavate to broadly clavate, short pedicellate, long asci and ellipsoid ascospores with 3–6 transverse septa and 1 vertical septum in each row (Kohlmeyer et al 1995, Hyde et al 2016).

This study provides sequence data of protein coding genes RPB2 and TEF1α (MF168942, MF182398) in GenBank for *Deniquelata vittalii* whereas we lack these sequences data of *D. barringtoniae* for a comparison. This is the first report of a *Deniquelata* species from marine habitats (Jones et al. 2015). Recently (Devadatha & Sarma 2018) reported a new species, *Pontoporeia mangrovei* from decaying woody stem of the halophyte *Suaeda monoica*. By adding the present new taxon, *Suaeda monoica* could be considered as a host that supports several novel marine fungi.

Farasanispora avicenniae and *Hysterium rhizophorae* were recorded for the first time from India and hence constitute new geographic records. Also, their occurrence on new hosts extend their host range. The present study provides new sequences data for these two-known species.

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