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New records of Ascomycetous fungi from Andaman Islands, India and their molecular sequence data

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Abstract

Information on fungal diversity in Andaman and Nicobar Islands, India is meagre. We are investigating the ascomycetous fungal diversity colonizing decaying plant litter in Andaman Islands. Recent collections have yielded four new records of ascomycetes: *Diaporthae phaseolorum*, *Eutypa flavovirens*, *Rhytidhysteron rufulum* and *Trichoderma peltatum*. These species are reported in this paper supported with morphological and molecular sequencing analyses.

Key words – 4-new records – *Diaporthae phaseolorum* – *Eutypa flavovirens* – *Rhytidhysteron rufulum* – *Trichoderma peltatum* – taxonomy

Introduction

Fungi are poorly investigated from the Andaman and Nicobar Islands (A & N Islands), India with only 446 fungi recorded although 2400 plant species are known from the Islands (Niranjan & Sarma 2018). Of the known fungi, most reports are of lichenized fungi, leaf inhabiting ascomycetes belonging to the order Meliolales, and marine fungi (Chinnaraj 1993, Hosagoudar 2013, Jagadeesh Ram & Sinha 2016, Niranjan & Sarma 2018). We have initiated studies on the diversity of ascomycetous fungi colonizing woody litter from the forests of A & N Islands, since wood degrading ascomycetous fungi have remained virtually unexplored. Examination of dead and decaying twigs on the forest floor has resulted in the discovery of four ascomycetes that are new records to this region. These are reported in this paper supported with photomicrographs and molecular sequence analyses.

Material and Methods

Dead and decaying twig samples on the forest floor in the reserved forests of South, Middle and North Andaman Islands, were collected into zip lock plastic bags, air dried overnight, and packed into new plastic bags for shipment to the laboratory for further processing. Before undertaking microscopic examination, the twigs were placed individually into plastic boxes lined with sterile tissue paper, rehydrated by sprinkling sterile water and incubated for 3 days to 2 months. The samples were then examined using a Stereo Zoom microscope (Optika SZM-LED, Italy) to locate fungal fruiting structures. Hand sections were taken wherever necessary. Fruit

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bodies were cut with a razor and the spore constituents were transferred to a microslide mounted in stains such as lactophenol, lactophenol cotton blue, Lougal's reagent and India ink. These slides were then examined using a Nikon ECLIPSE TiU upright microscope with DIC objectives fitted with Nikon DS-Fi2 digital camera to take photomicrographs. Measurements were taken with Nikon NIS-Elements-Imaging Software version 4.4 program, and photoplates made with Microsoft Power Point, and Adobe Photoshop version 7.0. Morphological identification was carried out by referring to various monographs and individual publications including Pandey (2008), Hyde et al. (2013) and Maharachchikumbura et al. (2016). Herbarium specimens have been deposited at Ajrekar Mycological Herbarium (AMH) Agharkar Research Institute (ARI), Pune, India. The cultures are maintained in our fungal biotechnology laboratory, Department of Biotechnology, Pondicherry University. GeneBank accession numbers are available at https://submit.ncbi.nlm.nih.gov/subs/. The individual ITS sequences obtained were subjected to Blast search tool of NCBI to reveal closely related matches in GeneBank. Multiple sequence alignments were performed in an online software (http://mafft.cbrc.jp/alignment/server/index.html; Katoh & Standley 2013). All the phylogenetic data sets used in this study are mentioned in Table 1.

DNA extraction and PCR

Single spore isolation was performed as outlined by Choi et al. (1999). Four pure axenic cultures were grown on malt extract agar (MEA) for one week at 28 °C, followed by a simple and rapid DNA extraction protocol (Aamir et al. 2015) using FasPrep 24 tissue homogenizer (MP Biomedicals GmbH, Eschwege, Germany). The DNA was resuspended in 50 µL TE buffer and analyzed quantitatively as well as qualitatively by 1% agarose gel electrophoresis. The internal transcribed spacer (ITS) gene was chosen for phylogenetic analysis. This region was amplified by PCR using primer pair ITS4 & ITS5 (White et al. 1990) in a reaction volume of 50 µL. The contents of the reaction mixture were 32 µL PCR grade water (Sigma, St. Louis, MO, USA), 5µL PCR buffer (10×), 4µL of 10 mMdNTPs mix (Sigma-Aldrich), 1 µL of each primer (20 pmol/µL), 1 µL (5 U/µL) of Taq polymerase (Sigma-Aldrich) along with 20–50 ng of template DNA. Amplification was done using an Applied Biosystems ProFlex PCR System (Applied Biosystems, Waltham, MA, USA) following standard cycling conditions: initial denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 95°C for 90 seconds, primer annealing at 52°C, primer extension at 72 °C for 1 min, and a final extension step at 72 °C for 10 min. The amplified products were analyzed on 1.2% agarose gel containing ethidium bromide. The PCR products were purified using an Axygen PCR cleanup kit (Axygen Scientific, CA, USA). Sequencing reactions were performed with a BigDye terminator cycle sequencing kit, ver. 3.1/1.1 (Applied Biosystems). All the sequencing reactions were purified and analyzed on an ABI Avant 3100 automated DNA sequencer (Applied Biosystems).

Phylogenetic analysis

Phylogeny was constructed using the individual and aligned data performed using maximum likelihood, maximum parsimony and Bayesian criteria. Maximum likelihood was performed by using the Randomized Accelerated Maximum Likelihood (RAxML). RAxML-HPC2 on XSEDE (8.2.8) (Stamatakis et al. 2008, Stamatakis 2014) in the CIPRES Science Gateway platform (Miller et al. 2010) using GTR+I+G model of evolution. Phylograms were visualized with FigTree v1.4.0 program (Rambaut 2012) and reorganized in Microsoft Power Point (2007) and Adobe Photoshop (Version 7.0, Adobe®, San Jose, CA).

Maximum parsimony (MP) was performed with PAUP v. 4.0b10 (Swofford 2002), with the following parameters such as characters unordered with equal weight, random taxon addition, branch swapping with tree bisection-reconnection (TBR) algorithm, branches collapsing if the maximum branch length was zero. Alignment gaps were treated as missing characters in the analysis of the combined data set, where they occurred in relatively conserved regions. Trees were inferred using the heuristic search option with 1000 random sequence additions, with max trees 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.

Bayesian analysis was conducted 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 5,000,000 generations and trees were sampled every 1000th generation. 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 1.5 (Rambaut & Drummond 2007). First 20% of generated trees were discarded and remaining 80% of trees were used to calculate posterior probabilities of the majority rule consensus tree. BYPP greater than 0.95 are given above each node. We consider the bootstrap support >75 as strong support, between 50–75 as moderate support and below 50 as minimum support.

Table 1 Culture collection and GenBank accession numbers used in the phylogenetic analysis of *Diaporthe phaseolorum*, *Eutypa flavovirens*, *Rhytidhysteron rufulum* and *Trichoderma peltatum*. Newly recorded isolates are shown in bold.

Species No.	Name	Culture collection No.	ITS
1	*Diaporthe aseana*	MFLUCC12_0299	KT459414
	Diaporthe asparagi	F103	KJ512161
	Diaporthe asparagi	HB5	JQ614001
	Diaporthe caulivora	Dpc1	HM347712
	Diaporthe miriciae	BRIP54736	KJ197282
	Diaporthe miriciae	BRIP56918a	KJ197284
	Diaporthe multigutullata	ZJUD98	KJ490633
	Diaporthe novem	52733	HM347710
	Diaporthe phaseolorum	P48	KX381176
	Diaporthe phaseolorum	**PUFNI 1635**	**MH048874**
	Diaporthe pseudolongicolla	PL42	JQ697843
	Diaporthe pseudolongicolla	PL68	JQ697842
	Diaporthe rostrata	CFCC50062	KP208847
	Diaporthe subclavata	ZJUD95	KJ490630
	Diaporthe ueckerae	SLHX11	KY565425
	Diaporthe ueckerae	SLHX3	KY565424
	Xylaria hypoxylon	CBS122620	KY204024
2	*Anthostoma decipiens*	IPVFW349	AM399021
	Cryptosphaeria ligniota	CBS27387	NR_154799
	Cryptovalsa ampelina	DRO101	GQ293902
	Diatrype enteroxantha	HUEFS155114	KM396624
	Diatrype enteroxantha	HUEFS192141	KM396622
	Diatrypella banksiae	*CPC29118*	KY173402
	Diatrypella verruciformis	UCROK1467	JX144793
	Eutypa flavovirens	CHUNI6	KR092798
	Eutypa flavovirens	**PUFNI 310**	**MH048876**
	Eutypa flavovirens	MFLUCC150899	KU144933
	Eutypa flavovirens	MFLUCC150852	KU144932
	Eutypa lata	ANT12065	KM822754
	Eutypa lata	MI711	AY462557

Table 1 Continued.

Species No.	Name	Culture collection No.	ITS
	Halodiatrype avicenniae	MFLUCC150953	KX573916
	Halodiatrype salinicola	MFLUCCl51277	KX573915
	Monosporascus cannonballus	ATCC26931	NR_111370
	Monosporascus cannonballus	CMM3646	JX971617
	Peroneutypa alsophila	EL58C	AJ302467
	Peroneutypa kochiana	EL53M	AJ302462
	Xylaria hypoxylon	CBS121680	AM993138
3	*Escovopsioides nivea*	JSP3004	KR093934
	Escovopsis aspergilloides	CBS42393	NR_137160
	Escovopsis trichodermoides	VEM001	KJ485699
	Hypocreopsis lichenoides	286342	JN006756
	Hypocreopsis rhododendri	1064036	JN006754
	Hypomyces peltigericola	CBS141848	KY088202
	Hypomyces samuelsii	CBS127157	NR_121430
	Mycogone perniciosa	CBS64882	FJ904634
	Sepedonium ampullosporum	CBS39252	NR_111031
	Sepedonium laevigatum	CBS101645	NR_119422
	Trichoderma asperellum	D19	GQ131397
	Trichoderma aureoviride	CCTCC_AV487	KT588281
	Trichoderma brevicompactum	CTCCSJ_ASD50416	KU89634
	Trichoderma crassum	CTCCSJ_AXM50487	KU896365
	Trichoderma lixii	DAOM231617	AY605754
	Trichoderma lixii	CCTCCAF340	KT588249
	Trichoderma peltatum	GJS091550	HM535607
	Trichoderma peltatum	KRCF1077	AB742527
	Trichoderma peltatum	**PUFNI1745**	**MH048875**
	Trichoderma peltatum		EF392732
	Trichoderma peltatum	GJS091512	HM466660
	Trichoderma peltatum	GJS10105	HM466663
	Trichoderma spirale	CTCCSJ_AYM50457	KU896358
	Trichoderma stromaticum	CTCCSJ_ASC50265	KU896315
	Trichoderma virens	CTCCSJ_ASC50261	KU896313
	Trichoderma voglmayrii	8196	KJ783308
	Protocrea illinoensis	TFC9698	EU703930
4	*Gloniopsis praelonga*	CBS119332	EU552133
	Glonium pusillum	CBS119348	EU552134
	Hysterium angustatum	MFLU161179	KX611363
	Hysterium pulicare	CBS119331	EU552137
	Hysterobrevium constrictum	JCM2753	LC228641
	Hysterobrevium mori	MFLUCC140520	KY496739
	Hysterographium minus	JCM2758	LC228642
	Hysterographium pulchrum	_____	DQ402184
	Ostreichnion centramurum	chuni70	KM272258
	Psiloglonium colihuae	MFLUCC110178	KP744466
	Psiloglonium sasicola	MFLUCC100565	KP744467
	Rhytidhysteron neorufulum	MFLUCC130216	KU377561
	Rhytidhysteron neorufulum	MFLUCC13_0221	KU37756

Table 1 Continued.

Species No.	Name	Culture collection No.	ITS
	Rhytidhysteron neorufulum	MFLUCC17_2236	MH062956
	Rhytidhysteron rufulum	MFLUCC120013	KJ418112
	Rhytidhysteron rufulum	MFLUCC14077	KU377560
	Rhytidhysteron rufulum	**PUFNI 1634**	**MH077555**
	Rhytidhysteron rufulum	534A	EU020046
	Rhytidhysteron rufulum	539A	EU020056
	Rhytidhysteron rufulum	544A	EU020045
	Rhytidhysteron thailandicum	MFLUCC14_0503	KU377559
	Rhytidhysteron thailandicum	S4S208B	MH037556

Results

Molecular phylogeny

In the *Diaporthe phaseolorum* phylogeny (Fig. 1), 16 different taxa belonging to *Diaporthe* were represented along with our taxon and *Xylaria hypoxylon* as an out group (Table 1). RAxML analysis yielded a minimum scoring tree with a final ML optimization likelihood value of -1914.568767. The matrix had 155 distinct alignment patterns with 23.51% of undetermined characters or gaps. Estimated base frequencies were as follows; A = 0. 245016, C = 0. 268387, G = 252215, T = 0. 234382; substitution rates AC = 1.003617, AG = 1.585937, AT = 1.632817, CG = 0.701228, CT = 4.132200, GT = 1.000000. Proportion of invariable sites I = 0.345847; gamma distribution shape parameter α = 0.624702. The maximum parsimonious dataset consists of 558 characters of which 379 were constant, 70 parsimony-informative and 109 parsimony-uninformative. The parsimony analysis of the data matrix resulted in one thousand equally parsimonious trees with a length of 250 steps (CI = 0.856, RI = 0.812, RC = 0.695, HI = 0.144) in the first tree. The phylogenetic analysis showed that our *Diaporthae phaseolorum* PUFNI1635 nested with *Diaporthae phaseolorum* P48 with strong bootstrap support 90% MP, 0.91 BYPP, and minimum support in ML.

Partial ITS nucleotides sequence dataset comprises 20 taxa (Table 1), including our strain from Diatrypaceae, that were used to determine the placement of *Eutypa flavovirens* (Fig. 2). ML, MP and BYPP analyses yielded best scoring trees with a final ML optimization likelihood value of –3355.156032. The matrix had 257 distinct alignment patterns with 4.98% of undetermined characters or gaps. Estimated base frequencies were as follows; A = 0. 232670, C = 0. 251266, G = 238956, T = 0. 277108; substitution rates AC = 1.134630, AG = 3.473540, AT = 2.319370, CG = 0.923198, CT = 4.267756, GT = 1.000000. Proportion of invariable sites I = 0.309764; gamma distribution shape parameter α = 0.698082. The maximum parsimonious dataset consists of 576 characters of which 326 were constant, 166 parsimony-informative and 84 parsimony-uninformative. The parsimony analysis of the data matrix resulted in one thousand equally parsimonious trees with a length of 588 steps (CI = 0.631, RI = 0.630, RC = 0.397, HI = 0.369). The phylogenetic analysis showed that our *Eutypa flavovirens* PUFNI310 nested with *Eutypa flavovirens* CHUNI6 with strong bootstrap support (100% ML, 100% MP, 1.00 BYPP).

In the phylogenetic tree for *Rhytidhysteron rufulum* (Fig. 3), 22 taxa from Hysteriaceae with *Glonium pusillum* as an out group were used (Table 1). ML, MP and BYPP analysis yielded best scoring trees with a final ML optimization likelihood value of –4840.530790. The matrix had 379 distinct alignment patterns with 9.22% of undetermined characters or gaps. Estimated base frequencies were as follows; A = 0. 222386, C = 0. 282278, G = 272787, T = 0. 222550; substitution rates AC = 1.095411, AG = 1.801505, AT = 0.907615, CG = 0.798350, CT = 3.163606, GT = 1.000000. Proportion of invariable sites I = 0.217189; gamma distribution shape parameter α = 0.658626. The maximum parsimonious dataset consists of 612 characters of which

248 were constant, 256 parsimony-informative and 108 parsimony-uninformative. The parsimony analysis of the data matrix resulted in one thousand equally parsimonious trees with a length of 1017 steps CI = 0.628, RI = 0.660, RC = 0.415, HI = 0.372. The phylogenetic analysis showed that our *Rhytidhysteron rufulum* PUFNI 1634 nested with *Rhytidhysteron rufulum* MFLUCC140577 with strong bootstrap support (97% ML, 87% MP and 0.97% BYPP).

In the case of *Trichoderma peltatum* (Fig. 4), 27 taxa from Hypocreaceae were included with *Protocera illinoensis* as an outgroup (Table 1). ML, MP and BYPP analysis yielded a best scoring trees with a final ML optimization likelihood value of –892.537055. The matrix had 25 distinct alignment patterns with 5.87% of undetermined characters or gaps. Estimated base frequencies were as follows; A = 0. 250351, C = 0. 255610, G = 255610, T = 0. 238429; substitution rates AC = 0.954014, AG = 1.910453, AT = 2.065143, CG = 0.000100, CT = 1.002539, GT = 1.000000. Proportion of invariable sites I = 0.000100; gamma distribution shape parameter α = 29.506646. The maximum parsimonious dataset consists of 644 characters of which 357 were constant, 190 parsimony-informative and 97 parsimony-uninformative. The parsimony analysis of the data matrix resulted in one thousand equally parsimonious trees with a length of 716 steps CI = 0.641, RI = 0.744, RC = 0.477, HI = 0.359. The phylogenetic analysis showed that our *Trichoderma peltatum* PUFNI1745 clustered with *Trichoderma peltatum* GJS091550 in BS values.

Taxonomy

Diaporthe phaseolorum (Cooke & Ellis) Sacc. Fig. 5
Sylloge Fungorum 1: 692 (1882)

Saprobic on decaying twigs. Teleomorph – *Ascomata* 230–300 × 250–380 µm, perithecial, immersed, single to grouped, globose, coriaceous, black, ostiolate, papillate. *Neck* 220–300 × 49–90 µm, periphysate. *Peridium* 12.5 µm wide, two layered, outer brown layer of *textura epidermoidea* cells and inner layer of thin *textura angularis* cells. *Hamathecium* paraphyses filamentous, hypha-like, no clear septa, branched. *Asci* 27.5–37.5 × 5–7.5 µm ($\bar{X}$= 32.5 × 7.0) (n=25), unitunicate, 8-spored, cylindrical to clavate, sessile, apically flat and blunt, with an ocular chamber, J–ve apical ring, smooth-walled, deliquescent. *Ascospores* 10–12.5 × 2.5–3.75 µm ($\bar{X}$=11×2.8) (n= 23), hyaline, overlapping uniseriate, fusoid, 1-septate with a constriction, 2-pseudoseptate, acute ends, rarely 1 true septum, smooth-walled, rarely guttulate. Anamorph – Hyaline, intermediary chlamydospore-like structures observed in the culture (Fig. 6).

Culture characteristics – Colony morphology – white colonies on MEA in 1 week old cultures, radial, undulated margin with a ring pattern, surface slightly raised (Fig. 6).

Known distribution – Many countries in Asia, Africa, Europe, North America and South America. New record to India and Andaman & Nicobar Islands.

Material examined – INDIA, Andaman & Nicobar Islands, South Andaman, Kalatan (1˚47’52”N 92˚42’50”E), Isolated from an unidentified twig, (AMH-9955), living culture PUFNI 1635, 10 August 2016, M. Niranjan.

Notes – *Diaporthe phaseolorum* has been reported as endophytic, saprobic and plant pathogenic (Gomes et al. 2013) from Brazil, New Zealand and USA. It causes stem canker disease (Costamilan et al. 2008, Li et al. 2017) in soybean plants (*Glycine max*) and *Euphorbia neriifolia* var. *cristata*. In the present study this fungus has been found as saprobic, colonizing dead and decaying twigs. The present collection forms a new record of this fungus for the A & N Islands, thus extending its geographic range. Our strain is different from *D. phaseolorum* var. *caulivora* strain CH 40/06 (Costamilan et al. 2008) in having smaller ascomata and asci and larger ascospores.

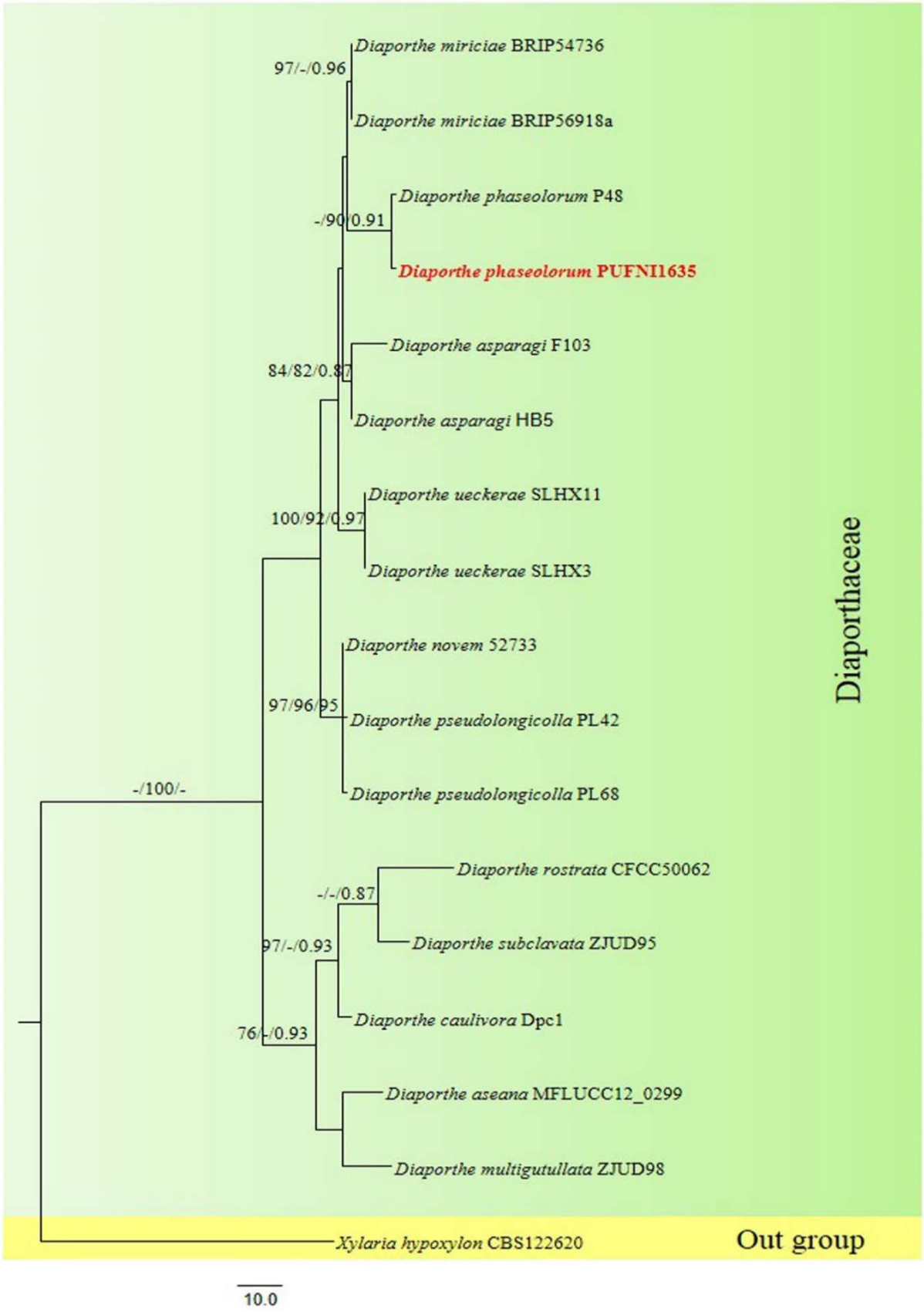

Fig. 1 – Phylogram generated from the best scoring of the MP tree based on ITS sequence data. Bootstrap support values for ML and MP higher than 75% and BYPP values greater than 0.86 are given above each branch, respectively. The tree is rooted with *Xylaria hypoxylon* CBS122620. The existing names *Phomopsis* = *Diaporthe* are added due to their similar sequence names. New isolate is indicated in red.

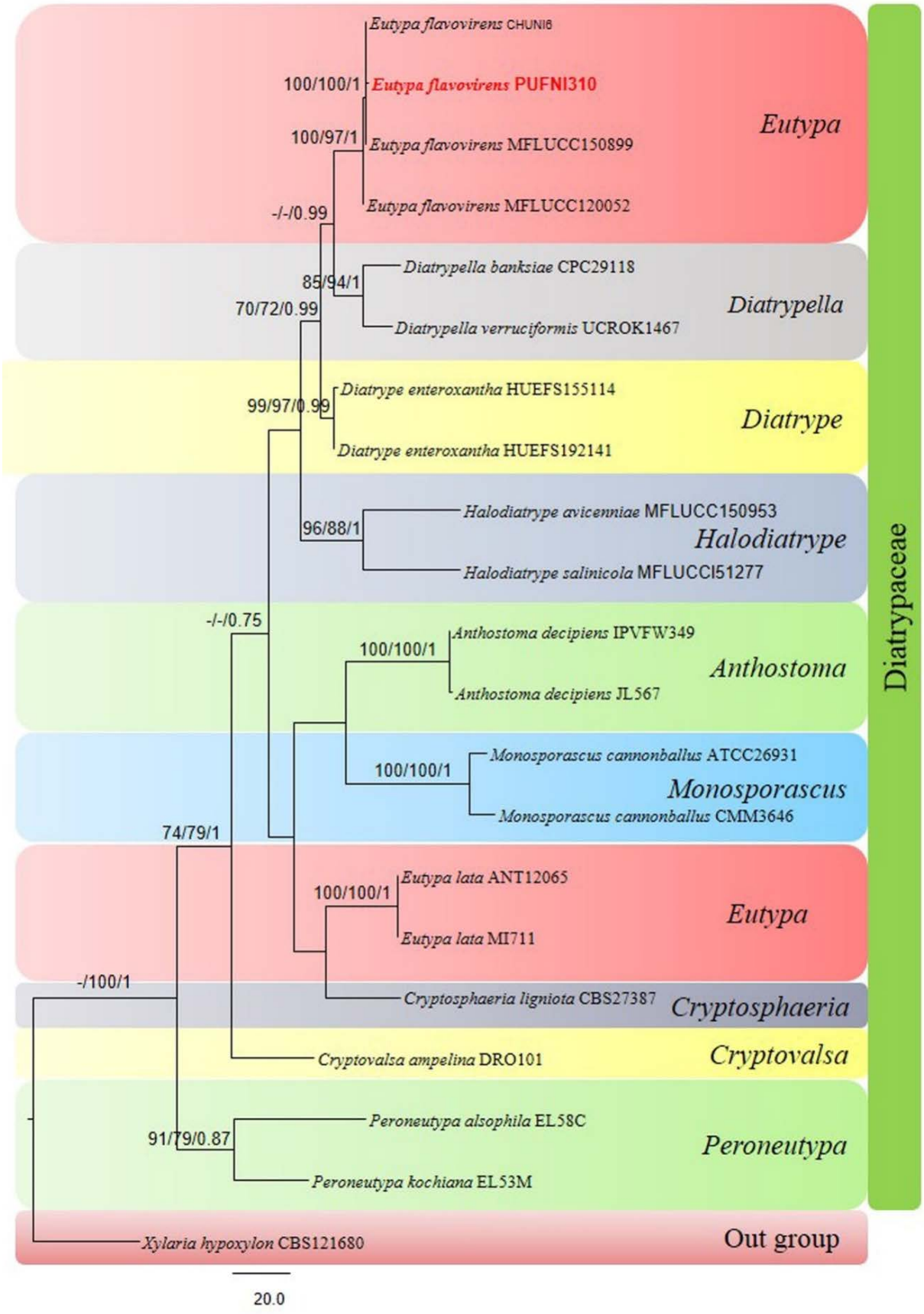

Fig. 2 – Phylogram based on the maximum parsimony analysis of an ITS rDNA sequence dataset. Bootstrap support values for ML and MP higher than 75% and BYPP values greater than 0.75 are given above each branch, respectively. The new isolate is represented in red. The tree is rooted to *Xylaria hypoxylon* CBS 121680.

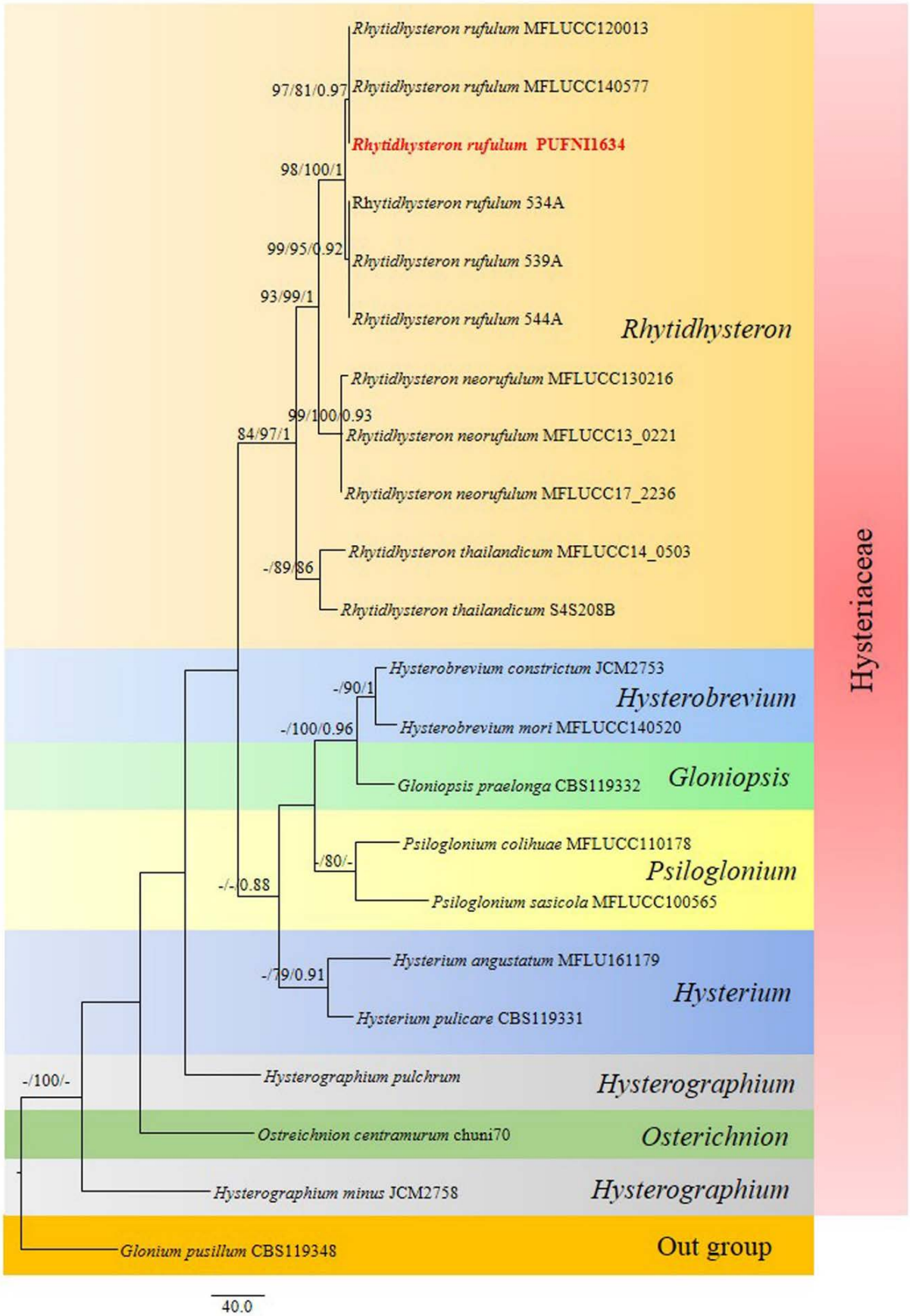

Fig. 3 – Maximum parsimony tree based on a ITS sequence data. Bootstrap support values for maximum likelihood greater than 75% and Bayesian posterior probabilities greater than 0.75 are given below and above the nodes. *Glonium pusillum* CBS 119348 is the out group taxon. Newly generated strain in this study is indicated in red.

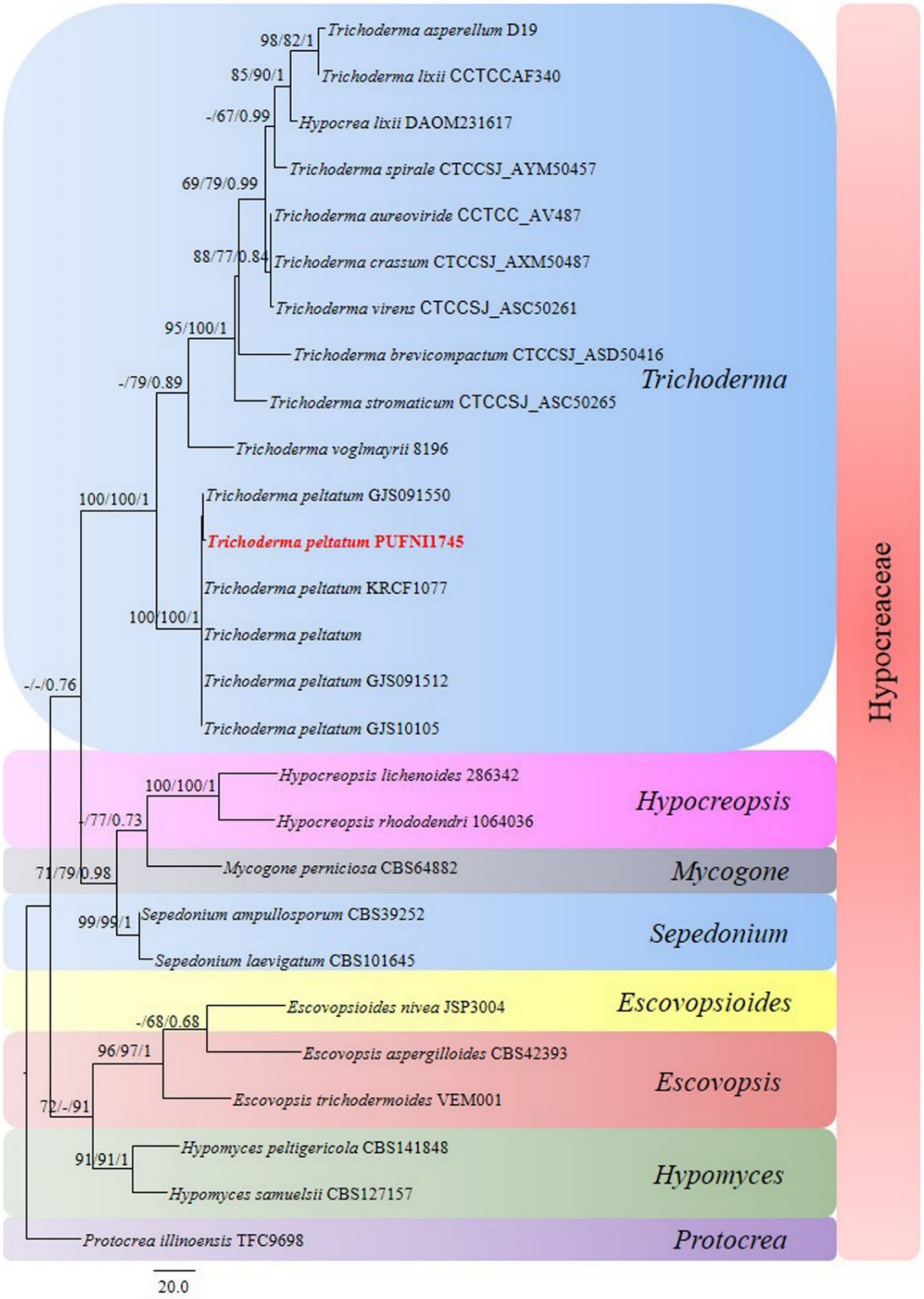

Fig. 4 – Phylogram generated from the best scoring of the MP tree based on ITS sequence data. Bootstrap support values for ML and MP higher than 75%and BYPP values greater than 0.75 are given above each branch, respectively. The tree is rooted with *Protocera illinoensis* TFC9698. New strain is indicated in red.

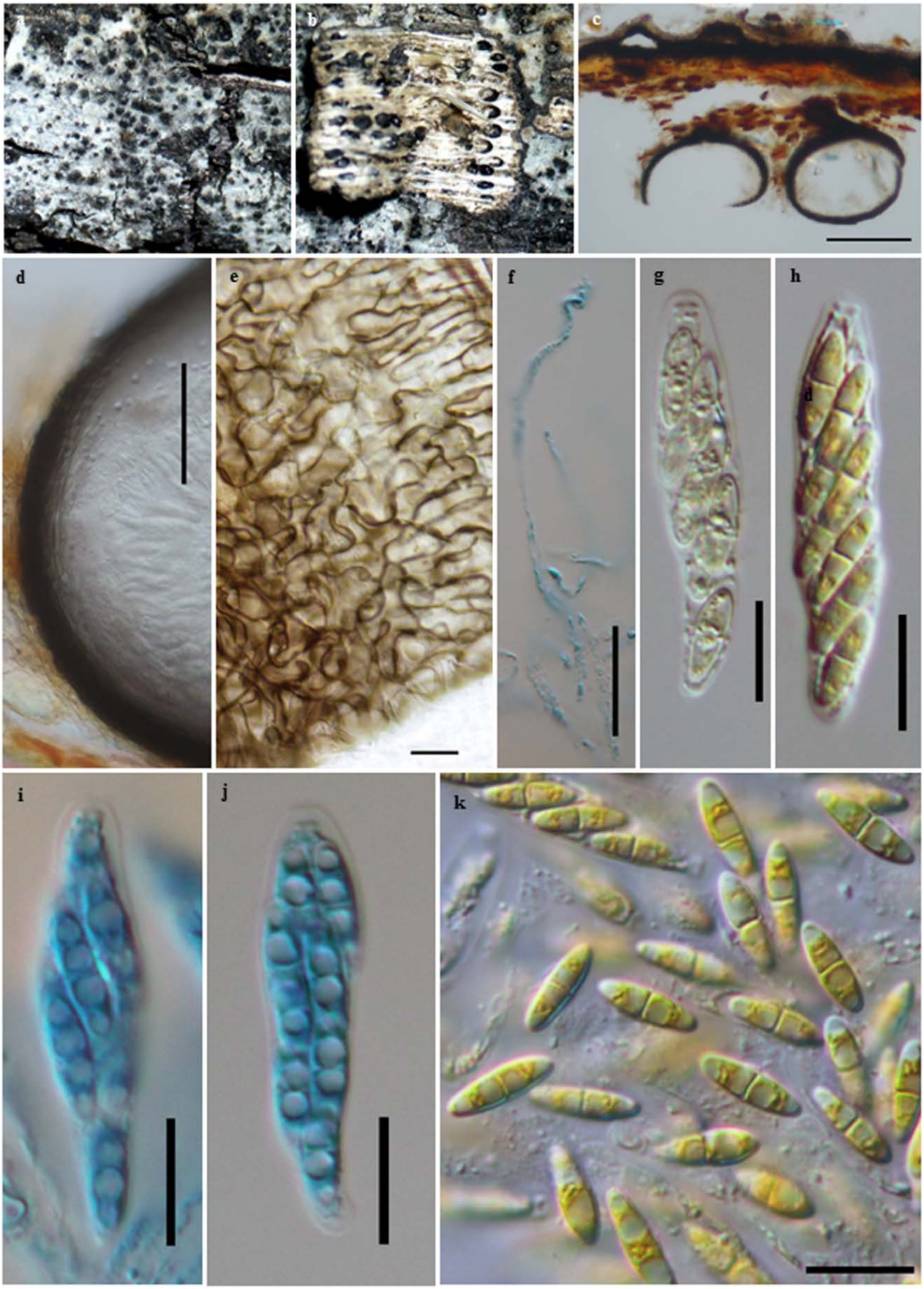

Fig. 5 – *Diaporthe phaseolorum* (PUFNI1635). a, b Ascomata on an unknown twig. c section of ascomata. d Peridium. e Peridium of *textura epidermoidea*. f Paraphyses. g–j Asci. k Ascospores in Lougal's solution. Scale bars – c = 100 *µm*, d = 50 *µm*, e = 10 µm, f = 20 *µm*, g–k = 10 *µm*.

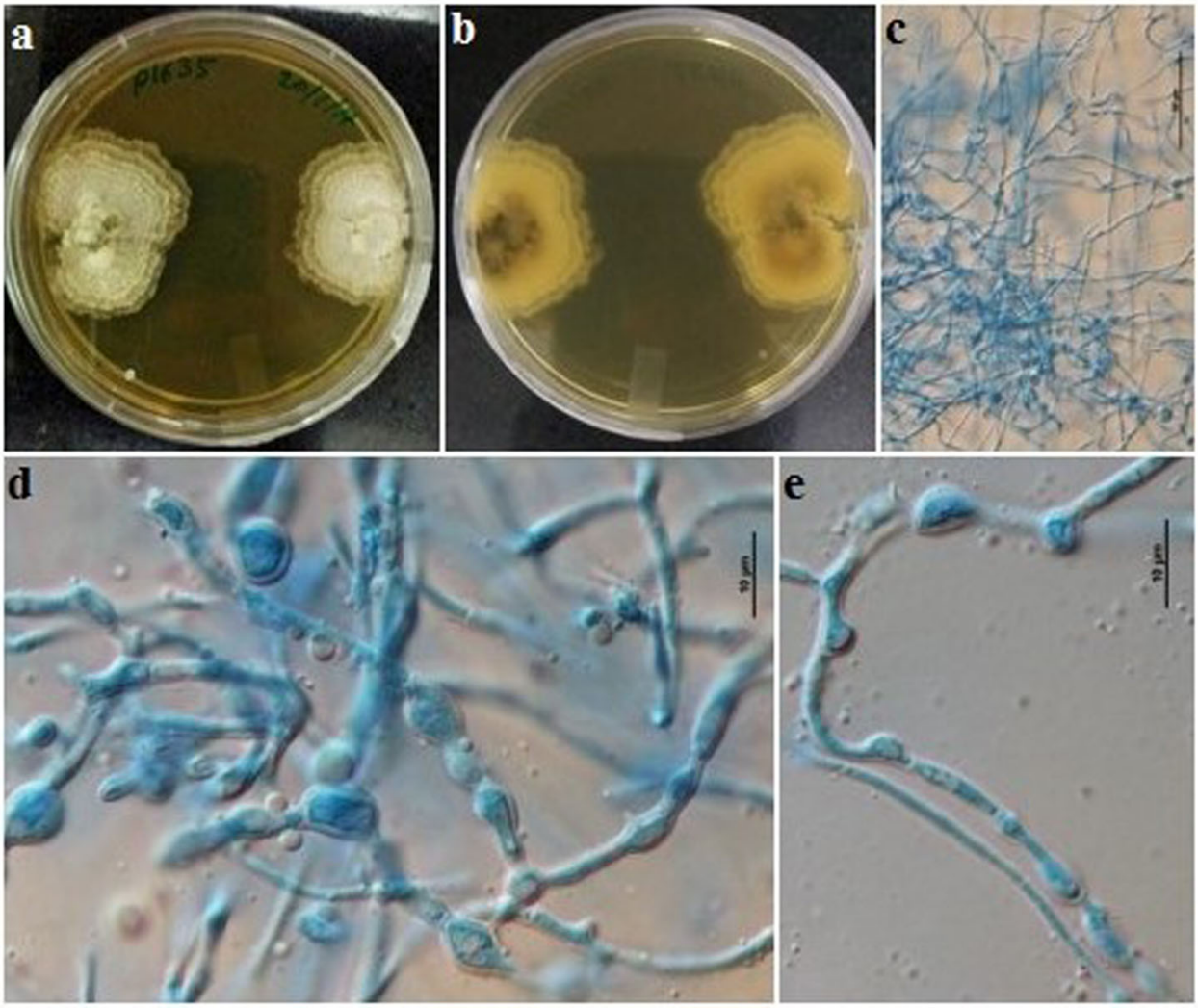

Fig. 6 – Anamorph of *Diaporthe phaseolorum.* a, b Culture plate. c-e Hyphae. Scale bar– c = 50 µm, d,e = 10 µm.

Eutypa flavovirens (Pers.) Tul. & C. Tul. Fig. 7
Selecta Fungorum Carpologia, Tomus Secundus. Xylariei – Valsei – Sphaeriei 2: 57 (1863)

Saprobe on an unidentified twig. Teleomorph – *Stromata* erumpent, superficial, with 3 layers in a vertical section, outer thin carbonaceous layer (14–25 µm high) interspersed with discoid rings indicating the area of protruding neck of the ascoma, middle green cell layer (68–75 µm high) and an inner white layer. *Ascomata* 212.5–396 × 184.6–363 µm ($\bar{X}$=311.2 × 244.6) (n=6, 10), multi-peritheciate, 2–12 in a stroma, globose to broadly ovoid, immersed in stroma, soft, ostiolated. Neck 130–180 × 935–135 µm ($\bar{X}$=156.6 × 117.8) (n=6), with septate periphyses oriented towards apical direction. *Peridium* 14.5–19.3 µm ($\bar{X}$=16.7 µm) (n=7) wide, consists of two strata, an outer carbonaceous stratum and an inner stratum of several dark brown to hyaline layers of *textura angularis* cells. *Hamathecium* paraphyses 5.2–8.9 µm, sparsely present, septate, unbranched. *Asci* 75–110 × 6.1–8.8 µm ($\bar{X}$=88.80 × 7.1) (n=25), unitunicate, persistent, clavate, with J–ve apical ring, ascospores placed sub apically within asci, long pedicellate, smooth-walled. *Ascospores* (7.6–)8.1–10(–10.5) × (1.6–)1.8–2.4(–2.6) µm ($\bar{X}$=9 × 2) (n=25), 8-spored, triseriately arranged in the sub-apical region, allantoid, rounded ends with one or two guttules, smooth-walled. Anamorph – Not seen.

Colony morphology on MEA – colonies white, irregular, initially flat becoming raised and again falling flat, margin entire, surface shows light circular patters with white and pale brown color. One-month old colony produces droplets on mycelial surface.

Known distribution – Throughout the world

Material examined – INDIA, A & N Islands, Middle Andaman, Nimbudera. Isolated from an unidentified twig, (AMH-9954), 3 February 2016, M. Niranjan & V.V. Sarma, living culture PUFNI 310.

Notes – *Diatrypaceae* family is characteristic of producing allantoid ascospores. The genera *Diatrype* and *Eutypa* are closely related and are very difficult to delineate (Vasilyeva & Stephenson 2004). The key differences are that *Diatrype* has a compact and effuse stroma without penetrating into the host, while in *Eutypa* the ascomata are erumpent through the host tissues with individual ostioles protruding out. *Eutypa flavovirens* is one of the most common fungi occurring throughout the world. It has pale yellow to green stromatic tissues, many species have been identified with little differences in size of the asci and ascospores. This is the first record of this fungus from A & N Islands, thus extending its geographic range.

Rhytidhysteron rufulum (Spreng.) Speg. Fig. 8

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Saprobic on decaying twigs. Teleomorph –*Ascomata* 600–900 × 1600–1850 µm, superficial, hysteriothecial, single to aggregated, a central slit straight in the middle and bent at ends, stromatic crust with longitudinal cracks, peridium two layered, outer layer thick, dark brown and the inner layer thick, pale brown of *textura angularis* cells. *Epithecium* paraphyses hyaline excepting at apex, apically rounded. *Hamathecium* pesudoparaphyses numerous, septate, unbranched, end cells dark and globose, longer than asci. *Asci* 172.5–225 × 10–15 ($\bar{X}$=187.5 × 13) (n=21), 8-spored, bitunicate, cylindrical, with an ocular chamber, smooth-walled, short-pedicellate, persistent. *Ascospores* 27–32.5 × 10–11.2 ($\bar{X}$= 30 × 10.4) (n=25), uniseriate to overlapping uniseriate, hyaline to sub-hyaline when young, dark reddish brown at maturity, immature spores 1-septate, mature spores 3-septate, middle cells darker than apical cells, smooth-surface to slightly verruculose, straight or slightly curved. Anamorph – Hyaline, intermediary chlamydospore-like structures observed (Fig. 9).

Known distribution – Throughout the world

Material examined – INDIA, South Andaman, NIOT, Coco plantation (11˚38’34.6” N92˚42’17.7”E), Isolated from an unidentified decaying twig (AMH-9956), 9 August 2016 M. Niranjan & V.V. Sarma, living culture PUFNI 1634.

Notes – In a recent study 20 species have been accepted in the genus *Rhytidhysteron* (Soto-Medina & Lücking 2017) including saprobic and pathogenic taxa. The genus is characterized by ascomata that are conspicuous with lateral cracks, mostly brown ascospores with transverse septa and sub-muriform septa. In *R. rufulum* also the ascomata are brown and ascospores 3-septate and reddish brown. It is closely related to *R. discolor* but is different in lacking orange-brown ascomata with discs and brown ascospores. This is the first record of this fungus from A & N Islands, thus increasing its geographic range.

Trichoderma peltatum (Berk.) Samuels, Jaklitsch & Voglmayr Fig. 10

Mycotaxon 126: 151 (2014)

Saprobe on *Pterocarpus dalbergioides* twig. Teleomorph – *Stromata* 1–1.9 cm wide, pale brown, multi-peritheciate, contains *textura epidermoidea* cells, *Ascomata* 320–370 × 210–260 µm, globose to sub-globose, immersed in stromata, ostiolate, 75–80 × 62.5–75 µm ($\bar{X}$=78.5 × 70.5) (n=5), ostiole conical, thickened, periphysate. *Peridium* comprises merged layers of *textura angularis* cells, 17.5 µm wide. *Hamathecium* paraphysoids aseptate, unbranched, attached at both top and bottom of ascoma, narrower towards apex, 1–4 µm wide. *Asci* 50–80 × 3–5 µm ($\bar{X}$=65.6 × 3.8) (n=25), unitunicate, cylindrical, persistent, J–ve in Lougal’s solution, rounded apically, smooth-walled, short-pedicellate. *Ascospores* 8-spored, breaking into 16-part spores, part spores 4.3–5.1 × 2.6–3.6 µm in dia. ($\bar{X}$=4.6 × 3.2) (n=25), uniseriate, hyaline, globose, verruculose. Anamorph–Undetermined.

Colony morphology – Circular, flat elevation, undulated margin, grey colored (Fig. 11).

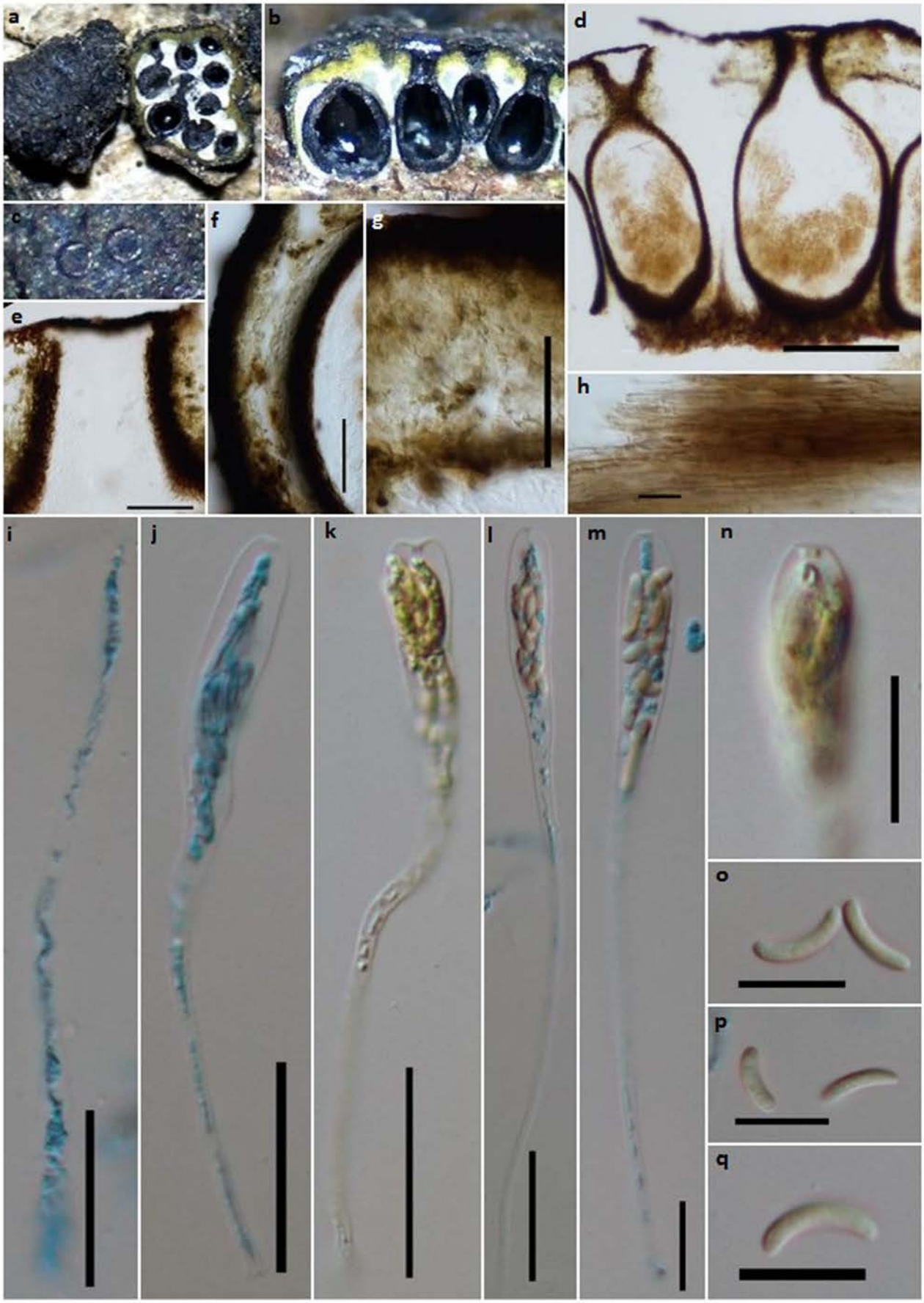

Fig. 7 – *Eutypa flavovirens* (PUFNI 310). a–c Ascomata on natural substrate. d Vertical section of ascoma. e Peridium. f Neck. g Outer and middle layers of stroma. h *Textura porrecta*. i Paraphyses. j–m Asci. n–p Ascospores. Scale bars: d = 200 *µm*, e–g = 50 *µm*, i–l = 20 *µm*, h, m–p = 10 *µm*.

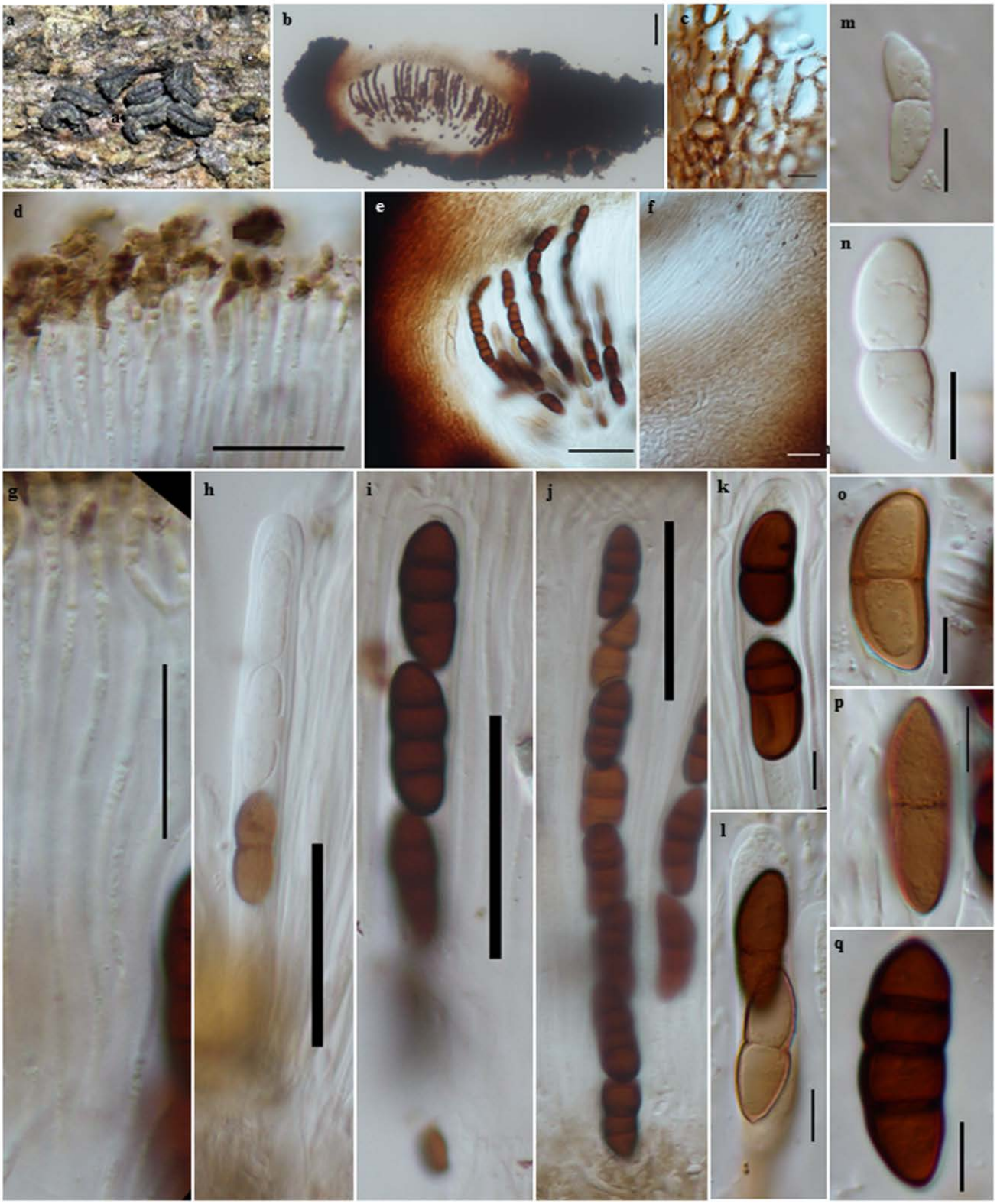

Fig. 8 – *Rhytidhysteron rufulum* (AMH-9956). a Ascomata on decaying wood. b Section of Ascomata. c *Textura angularis*. d Pseudoparaphyses. e Peridium. f Hamathecium. g–j Asci. h, l Apical ring. m–q Ascospores. Scale bars: b = 100 µm c = 10 µm d, g = 20 µm e = 50 µm f = 10 µm h–l = 20 µm m–q = 10 µm.

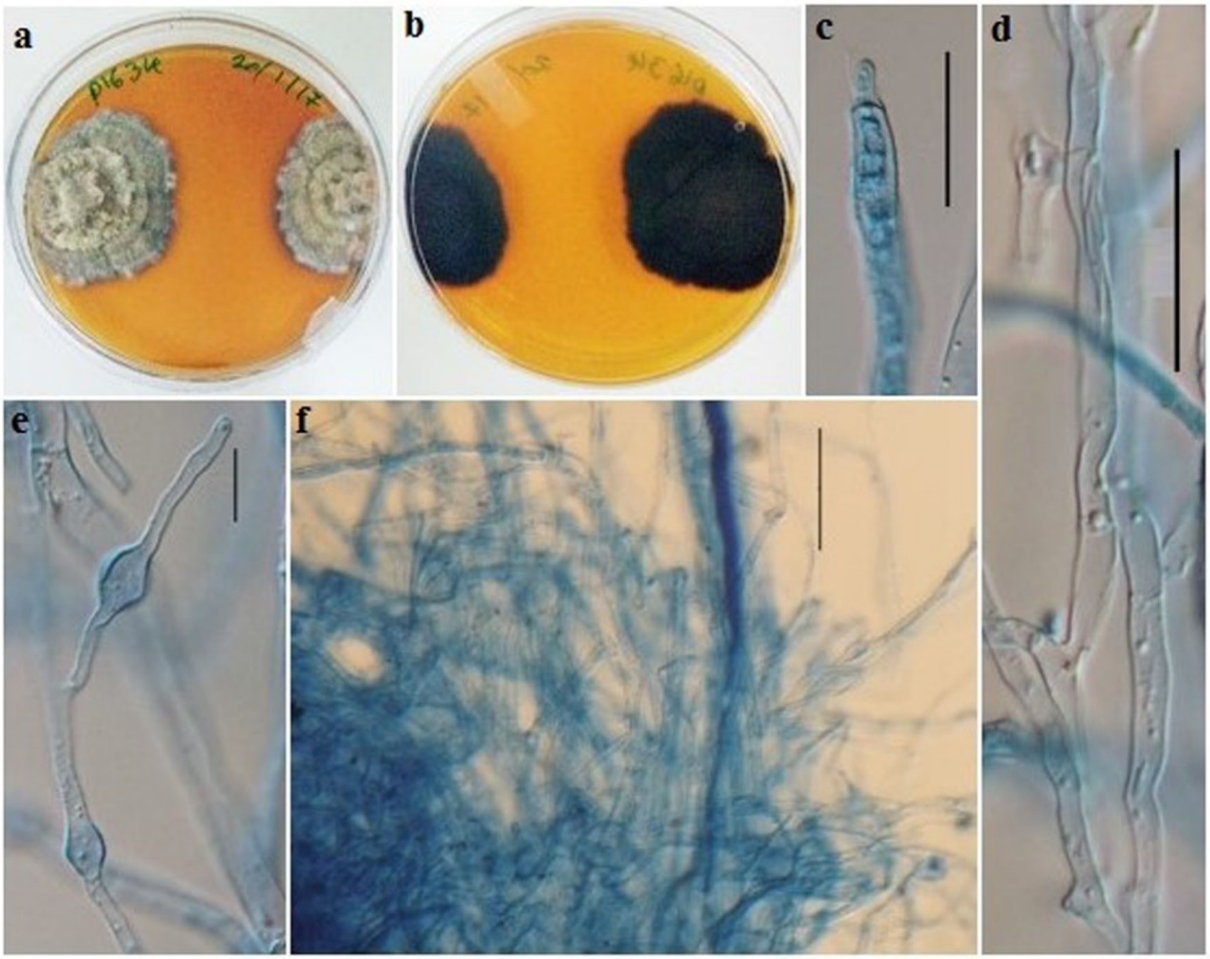

Fig. 9 – Anamorph of *Rhytidhysteron rufulum* (PUFNI 1634). a, b Culture plate. c, d Pseudoparaphyses. e, f Chlamydospores, Scale bars: f = 50 µm, c, d = 20 µm, e = 10µm.

Known distribution – Throughout the world

Material examined – INDIA, A & N Islands, North Andaman, near Mohan Nagar. (N 12˚13’21.8”E 92˚48’15.7”). Isolated from *Pterocarpus dalbergioides* twig, 6 January 2017, M. Niranjan & V.V. Sarma, living culture PUFNI 1745.

Notes – This species has a worldwide distribution but this is the first time that it is reported from A & N Islands, India and hence extends its geographical range.

Discussion

In a recent compilation of fungi recorded from A & N Islands, 446 fungi have been reported (Niranjan & Sarma 2018). Most of these belong to leaf inhabiting Meliolalean fungi, lichenized fungi and marine fungi from a single collection (Niranjan & Sarma 2018). In the above compilation it was found that the wood degrading ascomycetous fungi are almost nil. Though slight differences were found in the dimensions of ascomata, asci and ascospores of the four new records of the fungi, the molecular phylogenetic analyses clearly show their identity being matching with the existing species. The overall topology of the phylogenetic trees resulted from ML, MP and BYPP were similar and in congruent with earlier studies (Murillo et al. 2009, Udayanga et al. 2012, Jaklitsch & Voglmayr 2015, Senwanna et al. 2017). So far there are only two species for which the molecular sequence data are available from A & N Islands and these are basidiomycetes, *Lentinus sajor-caju* (Sharma et al. 2015) and a hyphomycete, *Argopericonia indirae* (Pratibha & Prabhugaonkar 2017). The present study provides information about both morphology and phylogeny of four ascomycetous fungi from A & N Islands thus extending their geographic range.

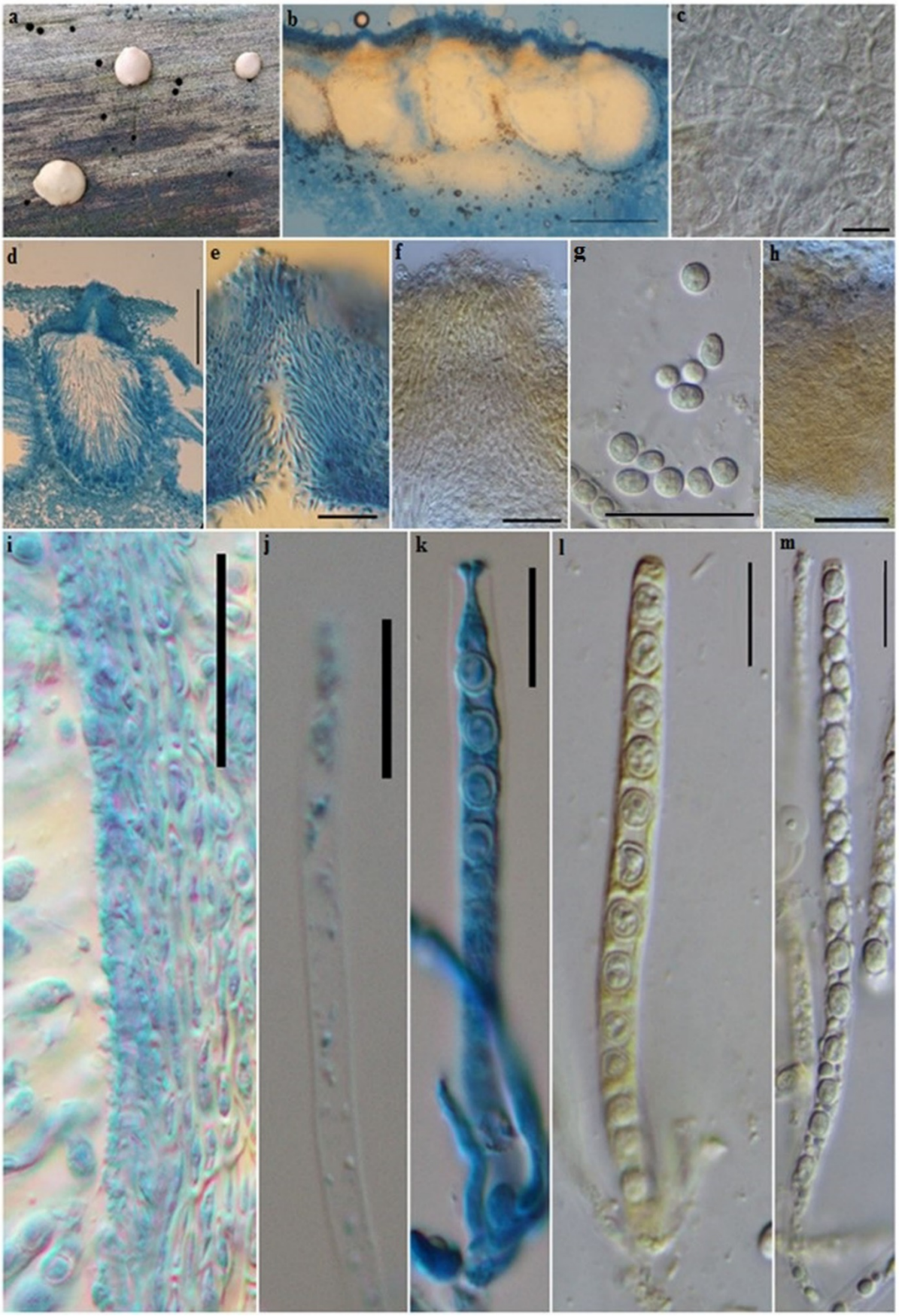

Fig. 10 – *Trichoderma peltatum* (PUFNI1745). a Ascomata on decaying log. b, d Vertical section of ascomata. c *Textura angularis.* e, f Ostiolated neck. h Stroma section. g Ascospores. i Peridium. j Paraphysoids. k–m Asci. Scale bars–b = 200 µm, d = 100 *µm* e,f,h = 50 *µm*, g,i = 20 µm, c,j–m = 10 *µm*.

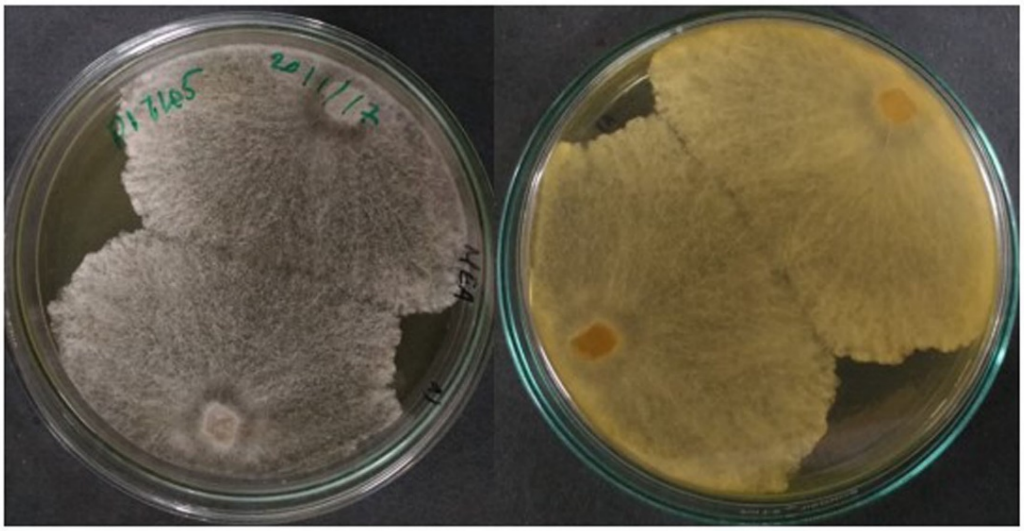

Fig. 11 – Colony morphology of *Trichoderma peltatum.*

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