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Contributed Paper

Morphology and Phylogeny Reveal Nine New Records of Polypores from Dry Zone of Sri Lanka

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ABSTRACT

Polyporoid fungi, which belong to Basidiomycota are of leathery and woody texture and have poroid hymenophores. Even though several earlier studies have been carried out in Sri Lanka, the fungal names of theses collections have not been revised according to modern taxonomic criteria. During a survey carried out in the dry zone, 44 polyporoid fungi were collected and identified using macromorphological and microscopic characteristics. The identification was confirmed by the phylogenetic analysis of Internal Transcribed Spacer (ITS1 and ITS2) and the 5.8 S rRNA genes. The encountered polypores are reported here with special emphasis on nine species (*Fuscoporia senex*, *Rhodofomitopsis feei*, *Phanerodontia chrysosporium*, *Earliella scabrosa*, *Panus conchatus*, *Panus similis*, *Trametes cubensis*, *Trametes elegans* and *Trichaptum byssogenum*) which are reported from Sri Lanka for the first time. The phylogenetic tree constructed using maximum parsimony and Bayesian phylogenetic analysis of Internal Transcriber Spacers exhibited the diversity of the collection, with species extending over all the four polyporoid clades; core polyporoid clade, *Antroidia* clade, phlebioid clade and residual polyporoid clade as well as hymenochaetoid clade.

Keywords: Polypores, ITS, *Panus, Trametes*, *Trichaptum*, Sri Lanka

1. INTRODUCTION

The global fungal diversity is estimated to be 2.2-3.8 million different species as the second most diverse group of living organisms on earth, after insects [1,2]. Fungal species producing fruit bodies of sufficient size and structure are considered as macrofungi and fall into Classes of Ascomycota and Basidiomycota. Polyporoid fungi, which belong to the division Basidiomycota, are macrofungi with caps or brackets and poroid hymenophores [3]. Usually these are of leathery and woody texture, mostly growing on decaying logs and sticks. The polypores can survive for a longer period due to their unique adaptation of producing new layers of spore producing surfaces which is ensured by continuous supply of food material by elevation above ground. Another factor is that their fruiting

bodies being large and tough, are not readily eaten by insects and animals [4]. Classification of these was initially based on morphology and with current advanced phylogenetic studies it has been subjected to critical changes [5].

These polypores are generally distributed among hymenochaetoid clade and polyporoid clade. The hymenochaetoid clade is dominated by wood-decaying species, majority causing white rot. The fruit body types greatly vary with no clear-cut morphological synapomorphies known for this clade. However, most species have dolipore septa with continuous parenthesomes. Most species also have cystidia in the tissue of fruit body among basidia in the hymenium [5,6]. The members of polyporoid clade represent a diverse group of Agaricomycetes comprising with nearly 1800 described species. Similar to hymenochaetales various basidiocarp types exist and as such there is no morphological synapomorphy. Phylogenetic analysis in Polyporales, using ribosomal RNA genes and protein coding genes supported four major clades; *Antroidia*, core polyporoid, phlebioid & residual, as well as several lineages outside those major clades [7].

Available information on Sri Lankan macrofungi is lacking, specifically on polypores and other aphyllophorales. The earliest record of Sri Lankan fungi is from Hantana in 1783 by J.G. Koenig. Later, J. Berkeley and T. Petch described and recorded over 500 species. In 1905, T. Petch published many accounts in the Annals of the Royal Botanical Gardens, Peradeniya, under the titles of "Revisions of Ceylon fungi" and "Additions to Ceylon Fungi" [8]. The most recent account of Sri Lankan macrofungi, 'A handbook to the macrofungi of Sri Lanka' was published by Coomaraswamy and Kumarasingham in 1988. However, the fungal names of these collections have not been revised according to the modern taxonomic criteria. During a macrofungal survey in the dry zone of Sri Lanka 45 polyporoid fungi were collected and identified using macromorphological and microscopic characteristics. The identification was confirmed by the phylogenetic analysis of Internal Transcribed Spacer (ITS1 and ITS2) and the 5.8 S rRNA gene. Due to the fact that polypores have poorly been studied, much detailed descriptions of many polypores encountered in this study are the first of its kind, which do not have any previous records from Sri Lanka and nine such polyporoid fungi are described in this paper. Furthermore, full descriptions, color photographs of morphological characteristics and a phylogenetic tree to show the placement of all the polypore records are provided.

2. MATERIALS AND METHODS

The studied 44 specimens were collected from Institute of Fundamental Studies (IFS) Sam Popham Arboretum and adjacent woodlands in Dambulla, Sigiriya wilderness and National Parks of Minneriya, Kaudulla & Wasgamuwa. Opportunistic and random sampling were carried out, and while walking through the study site and conspicuous fruit bodies were collected [9]. In forests and National parks, area of 50-100 m radius into the forests from the walking path was included as the study site. During 2012 to 2014, each site was visited twice within a period of one year.

The specimens (air/oven dried and placed inside zip-lock plastic bags incorporated with silica gel) were deposited at the herbarium of the Department of Plant Sciences, University of Colombo, Sri Lanka. Thin Sections from basidiocarp were mounted in Lactophenol Cotton Blue stain and Mezler's reagent and studied at magnifications up to ×1000 using an Olympus CX21FS1 microscope and phase contrast illumination. Size dimensions were determined for 30 basidiospores (Mean spore length [L], mean spore width [W] and variation in the L/W ratios within the specimen studied [Q] are shown in the description of each species), 10 basidia and 10 cystidia from each basidiomata. Photographs of microscopic observations were taken from Canon, Power Shot A2600 camera. Special colour terms

follow reference [10]. Macromorphological features (cap size, shape, color, surface/stem texture, gills color, presence of any special characters including rings, concentric rings, grooves) and microscopic features (hyphal characteristics, presence and nature of cystidia, characteristics of basidia and basidiospores) were noted.

Fungal DNA was extracted from the dried basidiocarps according to the CTAB based method [11]. PCR products were obtained using the mixture containing 0.125 units Go Taq® DNA Polymerase (Promega), 1 x buffer with dye, 200 μM each dNTP, 5 mM $MgCl_2$, 0.2 μM forward primer, 0.2 μM reverse primer and 5-50 ng of template DNA on a Veriti 96 well Thermal Cycler (Applied biosystems). ITS region and 5.8 S rRNA gene were amplified using primers ITS1F (CTT GGT CAT TTA GAG GAA GTA A) and ITS4B (CAG GAG ACT TGT ACA CGG TCC AG) [12]. The PCR procedure was done as follows: initial denaturation at 95 ºC for 12 minutes, followed by 13 amplification cycles of denaturation (95 ºC for 35 s), annealing (55 ºC for 55 s) and extension (72 ºC for 45 s). The extension step was lengthened to up to 120 s from 14^{th} to 26^{th} cycle and 180 s from 27^{th} to 35^{th} cycle. Then the final extension was performed for 10 minutes at 72 ºC [12]. PCR products were sequenced by Macrogen, Inc. (Korea).

The electropherograms were analyzed and edited by the BioEdit software and contiguous sequences were obtained. The resultant sequences were subjected to DNA homology search using GenBank database at National Center for Biotechnology Information (NCBI) using Basic local alignment search tool (BLAST). The newly generated sequences were submitted to the GenBank.

The sequences were aligned by MUSCLE multiple alignment software [13], using default parameters. Gaps in the alignments were treated as missing data. A Maximum Parsimony (MP) analysis was conducted using software PAUP* 4.0a (Sinauer Associates; Inc.; Sunderland; MA; USA). The tree was generated through a heuristic search using 1000 bootstrapping replications with 10 random sequence additions using tree bisection reconnection (TBR) branch swapping. Max trees were set to 1000, branches of zero length collapsed and all parsimonious trees were saved. Descriptive tree statistics, tree length (TL), consistency index (CI), retention index (RI), rescaled consistency index (RC) and homoplasy index (HI) were calculated for each tree generated. *Agaricus* sp. (ITS-KT186161) was selected as the outgroup. In addition, another agaricus, *Pleurotus tuber-regium* (ITS-KP734199) and a species from Russulales family, *Stereum hirsutum* (ITS-KP715550) were included in the analysis. Bayesian analysis used the MrBayes version 3.2.7 software package [14] and Bayesian posterior probabilities for each clade were presented. Bayesian analysis, implementing the Markov Chain Monto Carlo (MCMC) technique was performed. The parameters in MrBayes were set as follows: lset nst = 6, and rates = gamma and resulted in an average standard deviation of split frequencies of 0.0125.

3. Results and discussion

Out of a total number of 44 polypores collected and identified during the study (Table 1), nine polypore species were reported from Sri Lanka for the first time and detailed descriptions are given below. In some of the descriptions certain characters such as spores, basidia and other microscopic details are missing since they could not be observed as the fruitbodies collected were of poor quality.

3.1 Taxonomy

1. *Fuscoporia senex* (Nees & Mont.) Ghob.-Nejh., in Ghobad-Nejhad & Dai, Mycotaxon 101: 208 (2007) Figure 1.

Basidiocarp solitary, semi-circular, applanate, broadly attached, 1.1-7.8 cm long from base to margin and 1.9-11.0 cm wide. Upper surface umber, bay to dark brick, narrow concentric zones, velvety. Lower surface dark brick to rusty tawny, margin

Table 1. List of polypores collected and identified in the study reported herein.

Classification				Scientific Name	
Phylum	**Order**	**Family**	**Genus**		
Basidiomycota	Hymenochaetales	Hymenochaetaceae	*Fuscoporia*	*1.*	*Fuscoporia senex*
			Hydnoporia	*2.*	*Hydnoporia tabacina*
			Fomitiporia	*3.*	*Fomitiporia repanda*
			Inocutis	*4.*	*Inocutis porrecta*
			Phellinus	*5.*	*Phellinus fastuosus*
			Phellinus	*6.*	*Phellinus gilvus*
		Incertae sedis	*Trichaptum*	*7.*	*Trichaptum byssogenum*
	Polyporales	Incertae sedis	*Australohydnum*	*8.*	*Australohydnum dregeanum*
			Fuscopostia	*9.*	*Fuscopostia fragilis*
			Phanerodontia	*10.*	*Phanerodontia chrysosporium*
		Fomitopsidaceae	*Fomitopsis*	*11.*	*Fomitopsis* sp.
			Rhodofomitopsis	*12.*	*Rhodofomitopsis feei*
		Irpicaceae	*Flavodon*	*13.*	*Flavodon flavus*
		Panaceae	*Panus*	*14.*	*Panus conchatus*
			Panus	*15.*	*Panus similis*
		Podoscyphaceae	*Podoscypha*	*16.*	*Podoscypha petalodes*
		Phanerochaetaceae	*Porostereum*	*17.*	*Porostereum spadiceum*
		Polyporaceae	*Cellulariella*	*18.*	*Cellulariella warnieri*
			Coriolopsis	*19.*	*Coriolopsis byrsina*
			Cerrena	*20.*	*Cerrena caperata*
			Dichomitus	*21.*	*Dichomitus* sp.
			Earliella	*22.*	*Earliella scabrosa*
			Favolus	*23.*	*Favolus philippinensis*
			Favolus	*24.*	*Favolus tenuiculus*
			Funalia	*25.*	*Funalia aspera*
			Funalia	*26.*	*Funalia* sp.
			Ganoderma	*27.*	*Ganoderma applanatum*
			Ganoderma	*28.*	*Ganoderma carnosum*
			Ganoderma	*29.*	*Ganoderma tsugae*
			Ganoderma	*30.*	*Ganoderma* sp.
			Lentinus	*31.*	*Lentinus arcularius*
			Lentinus	*32.*	*Lentinus squarrosulus*
			Lentinus	*33.*	*Lentinus swartzii*
			Microporellus	*34.*	*Microporellus* sp.
			Perenniporia	*35.*	*Perenniporia* sp.
			Pilatotrama	*36.*	*Pilatotrama ljubarskyi*
			Polyporus	*37.*	*Polyporus dictyopus*
			Pseudofavolus	*38.*	*Pseudofavolus tenuis*
			Trametes	*39.*	*Trametes apiaria*
			Trametes	*40.*	*Trametes coccinea*
			Trametes	*41.*	*Trametes cubensis*
			Trametes	*42.*	*Trametes elegans*
			Trametes	*43.*	*Trametes maxima*
			Trametes	*44.*	*Trametes vernicipes*

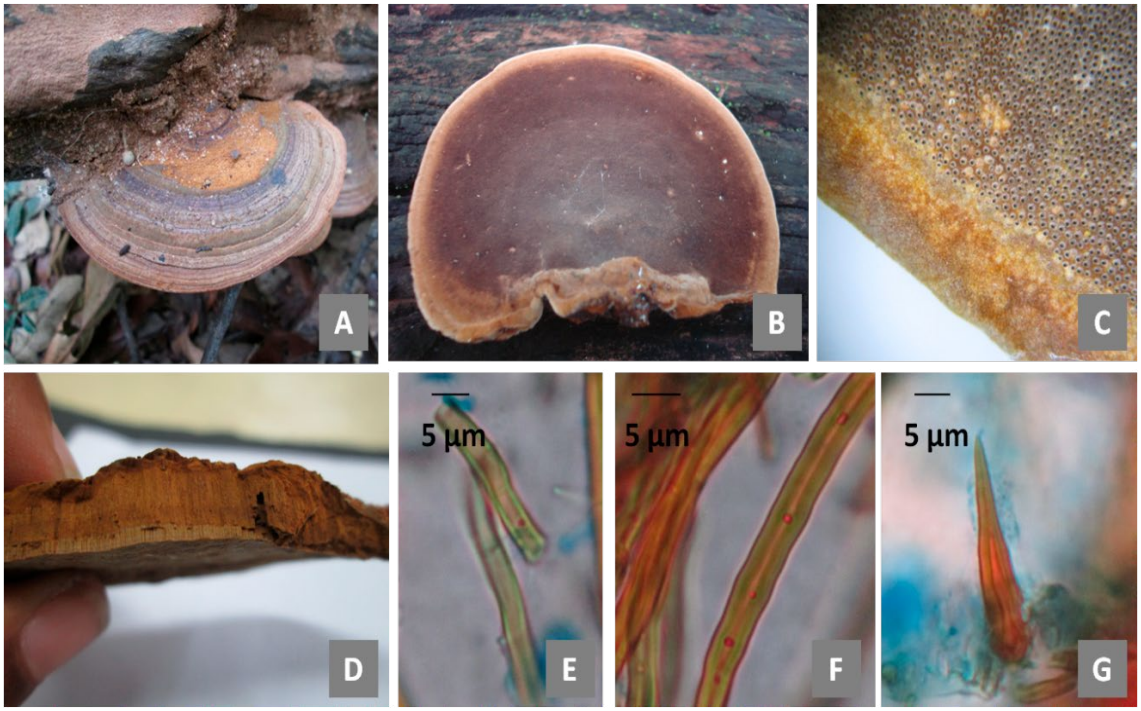

Figure 1. *Fuscoporia senex*; Fruit body of *Fuscoporia senex* (A), Lower surface (B), Lower surface × 50 (C), Cross section of the fruit body (D), Generative hyphae (E), Skeletal hyphae (F), Setae (G).

fulvous, sterile, round pores, invisible to naked eye, 8-11 pores/mm. Context fulvous, >1-2 mm thick, tube layer umber, 0.3-3 mm thick. The hyphal system dimitic, generative hyphae simple septate, yellow, 2.3-3.2 µm wide, skeletal hyphae brown, thick walled, 1.8-3.7 µm thick. Setae 5.5-6.4 × 31.2-44.0 µm in size.

Remarks: This fungus has been recorded from Iran, China, Taiwan, Japan, Vietnam and Korea. *Fuscoporia* Murrill is a heterogeneous group in terms of morphology and anatomy. However, the phylogenetic analysis of *Phellinus* and *Inonotus* have shown that *Fuscoporia* forms a monophyletic group [15]. This species is distinguished by the pileal surface with indistinct zones, brownish margin and pore surface [16]. There is a distinct margin in the pore surface as well.

Specimens examined: Sri Lanka, Sigiriya wilderness, Minneriya, Kaudulla National Parks, on fallen angiosperm wood, collectors: S. S. Ediriweera, R.L.C. Wijesundera, C.M. Nanayakkara, N.W. Gunasekara & M.D.M. Fernando; February & July 2013, Herbarium Specimen Nos.: UOC:SIGWI:S56, UOC:MINNP:M11, UOC:KAUNP:MK41; GenBank Accession Nos.: KR818821, KP794600.

2. *Trichaptum byssogenum* (Jungh.) Ryvarden, *Norw. Jl Bot.* 19 (3-4): 237 (1972) Figure 2.

Basidiocarp solitary or fused, sessile, semi-circular, 1.0-5.0 cm from base to margin and 1.5-6.5 cm wide. Upper surface purple, buff to olivaceous buff, rough hair present. Lower surface livid vinaceous in colour, angular pores and maze like, pore size 1-3 mm. Hyphal system dimitic,

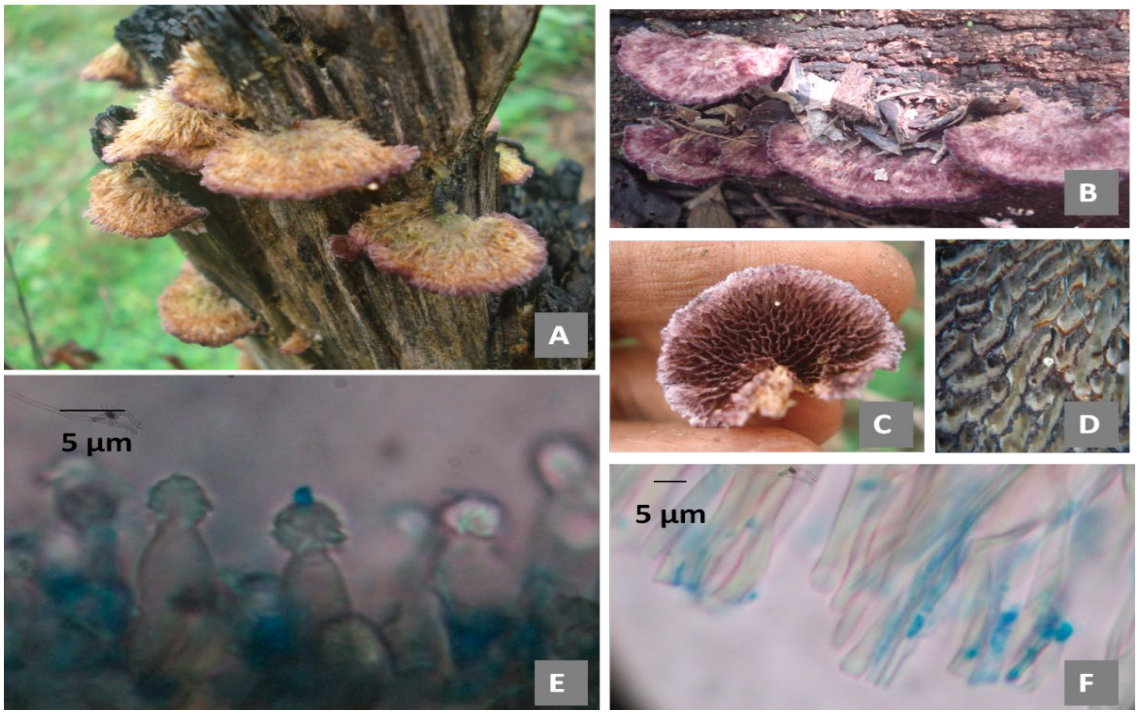

Figure 2. *Trichaptum byssogenum*; Fruit body (A,B), Lower surface (C), Lower surface × 05 (D), Cystidia (E), Generative hyphae & skeletal hyphae (F).

generative hyphae rarely clamped, hyaline, 2.0-4.0 µm thick; skeletal hyphae pale yellow, thick walled to solid, 3.5-4.5 µm thick. Hyphal pegs present. Cystidia lecythiform with a rounded crystal-like apex, hyaline, 3.5-5.5 × 12.0-15.0 µm in size.

Remarks: The genus *Trichaptum* is characterized by a purplish to violet pore surface in actively growing fruitbodies, paling to buff or pale brown with age and on drying. *Trichaptum* has an imperforate parenthosome in the doliphore apparatus which is a feature that has been reported in polyporoid fungi of the Hymenochaetaceae so far. The phylogenetic analysis of 18S rDNA sequences have shown that *Trichaptum* species were closer to Hymenochaetaceae than to other genera of the Polyporaceae [17] and it is evident from the phylogenetic analysis of the present study as well (Figure 10). The characteristic features of this species are the tomentose to hispid or strigose pileus, large pores (1-2 per mm) and apically incrusted cystidia [18].

Specimens examined: Sri Lanka, Sigiriya wilderness; Kaudulla National Parks, on fallen angiosperm wood, collectors: S.S. Ediriweera, R.L.C. Wijesundera, C.M. Nanayakkara, N.W. Gunasekara & M.D.M. Fernando; February July 2013 & July 2013, Herbarium Specimen No.: UOC:SIGWI:S10; GenBank Accession No.: KR265130.

3. *Phanerodontia chrysosporium* (Burds.) Hjortstam & Ryvarden, *Syn. Fung.* (Oslo) 27: 28 (2010) Figure 3.

Basidiocarp effused reflexed or resupinate and loosely attached. Upper surface fulvous to brick colour, margin white to yellow, concentrically

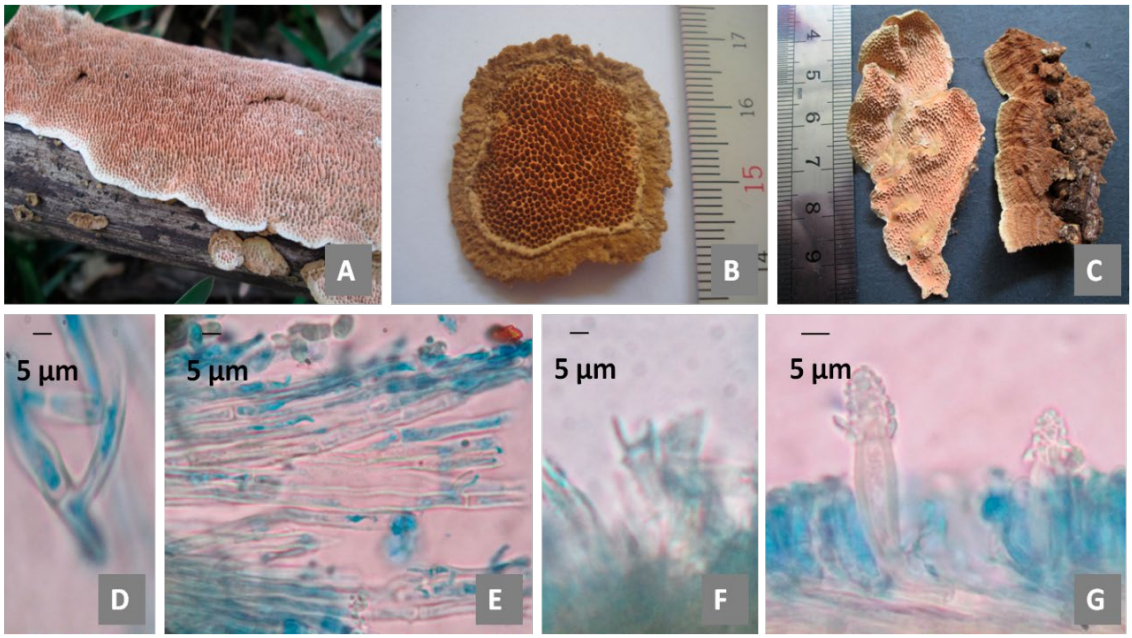

Figure 3. *Phanerodontia chrysosporium*; Fruit body (A-C), Generative hyphae (D,E), Basidia (F), Cystidia (G).

zoned, radially striate. Lower surface straw, buff to salmon colour, angular pores, 1-2 pores/mm, sterile margin. Spore print white. Hyphal system monomitic, hyphae clamped, hyaline, pale yellow in context, branched at right angles, simple septate or rarely clamped, thin walled, 3.7-8.3 µm thick. Basidia cylindrical, 5.5-6.4 × 9.0-11.0 µm in size. Cystidia fusiform to cylindrical, with apical crystals, thick walled, 5.5-7.3 × 23.0-27.0 µm in size.

Remarks: The genus *Phanerodontia* includes 4 species where fruit bodies are formed undersides of dead tree trunks and belongs to family Phanerochaetaceae. This family members are placed among phlebioid clade and primarily composed of corticoid species (Figure 10). This species is distinguished by their long, broad, cylindrical, smooth cystidia and complete lack of clamp connections. Due to the rapid growth rate on Malt Extract Agar and numerous conidia it is readily identified in the culture. This species is reported from southern Arizona, Eastern North America and temperate zones.

Specimens examined: Sri Lanka, Sigiriya wilderness & Kaudulla National Park, on fallen angiosperm wood, collectors: S.S. Ediriweera, R.L.C. Wijesundera, C.M. Nanayakkara, N.W. Gunasekara & M.D.M. Fernando; February 2013 & July 2013, Herbarium Specimen No.: UOC:SIGWI:S44, UOC:KAUNP:MK67, UOC:KAUNP:MK76; GenBank Accession No.: KR265131, KP771707.

4. *Rhodofomitopsis feei* (Fr.) B.K. Cui, M.L. Han & Y.C. Dai, in Han, Chen, Shen, Song, Vlasák, Dai & Cui, *Fungal Diversity* 80: 366 (2016) Figure 4.

Basidiocarp annual, solitary or fused, applanate, flabelliform, 0.6-2.0 cm long from base to margin and 0.7-4.0 cm wide. Upper surface clay pink, brick to rusty tawny, concentrically zoned, smooth and velvet like, white margin. Lower surface white to buff, round pores, 5-6 pores/mm, sterile margin. Hyphal system trimitic, generative hyphae clamped, hyaline, 0.9-1.4 µm thick; skeletal hyphae pale yellow,

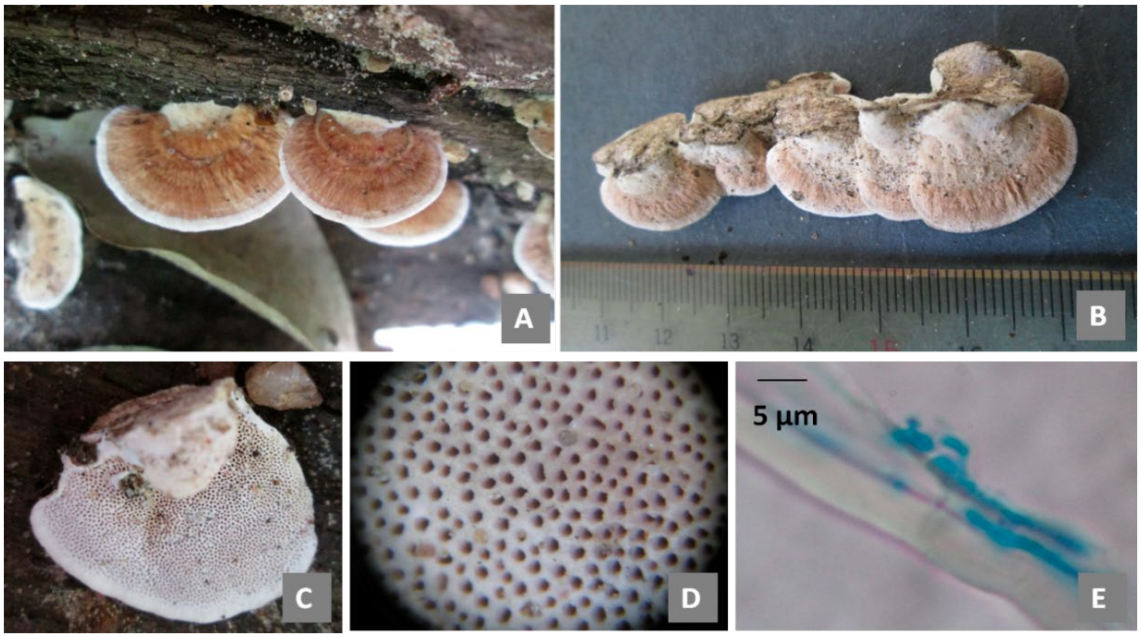

Figure 4. *Rhodofomitopsis feei*; Fruit body (A, B), Lower surface (C), Lower surface × 50 (D), Generative and skeletal hyphae (E).

thick walled to solid, 3.6-5.5 µm thick; binding hyphae pale yellow, solid, rare, 3.6-5.5 µm thick.

Remarks: Based on a taxonomic and phylogenetic studies carried out on brown rot fungi, *Fomitopsis s.l.* and its related genera, six new genera including *Rhodofomitopsis* were proposed. *Rhodofomitopsis* formed a single lineage. Morphologically it differs from *Fomitopsis s.l.* due to its rose, violaceous to pinkish-brown pore surface and context, absence of cystidioles and living on angiosperm wood [19]. This species is distributed in North, Central and South America, Caribbean Islands, Asia, Australia and New Zealand. The characteristic features of this species are pinkish brown basidiomata, circular to angular pores and cylindrical oblong spores [20].

Specimens examined: Sri Lanka, Minneriya National Park, on fallen angiosperm wood, collectors: S.S. Ediriweera, R.L.C. Wijesundera, C.M. Nanayakkara, N.W. Gunasekara & M.D. M. Fernando; July 2013, Herbarium Specimen No.: UOC:MINNP:MK18; GenBank Accession No.: KP780437.

5. *Panus conchatus* (Bull.) Fr., *Epicr. Syst. Mycol.* (Upsaliae): 396 (1838) Figure 5.

Basidiocarp solitary, when immature umblicate with central depression, mature fruit body funnel shaped. Upper surface hairy, covered with thick rough hairs when immature, smooth when mature. Brown colour, diameter 1.0-5.5 cm, margin curved towards gill surface. Gill surface decurrent and purplish brown to brown. Stem 1.2-3.0 cm long, 0.3 cm in diameter with fine hair present. Hyphal system dimitic, generative hyphae clamped, hyaline, 2.8-5.5 µm thick; skeletal hyphae hyaline, thick walled to solid, 1.8-4.6 µm thick. Basidia spherical, 6.3-7.3 × 9-11 µm in size. Caulocystidia clavate, 3.5-6.0 × 12.0-15.0 µm in size. Pleurocystidia clavate, 5.5-6.4 × 13.0-20.0 µm in size.

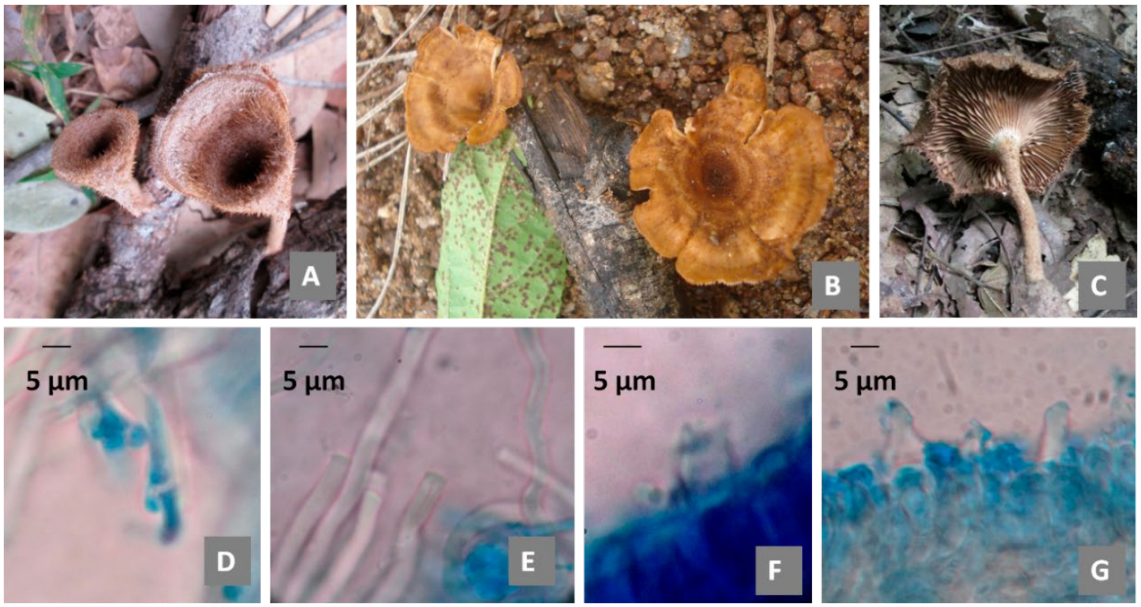

Figure 5. *Panus conchatus*; Fruit body (A), Fruit bodies matured and dried (B), Lower surface (C), Generative hyphae (D), Skeletal hyphae (E), Basidia (F), Pleurocystidia (G).

Remarks: *Panus* sp. are regarded as free gilled mushrooms and form a monopyletic clade with *Lentinus* and *Polyporus*. The genus *Panus* Fr. was introduced by Fries (1838), and *P. conchatus* is considered as the type species. *P. conchatus* is widely distributed in subtropical to tropical regions, temperate, and boreal regions. This species is characterized by its concave, smooth, deeply decurrent gills, with distinctive purple grey to greyish magenta basidiocarps [21].

Specimens examined: Sri Lanka, Sigiriya wilderness & Minneriya National Park, on fallen angiosperm wood, collectors: S.S. Ediriweera, R.L.C. Wijesundera, C.M. Nanayakkara, N.W.Gunasekara & M.D.M. Fernando; February 2013 & July 2013, Herbarium Specimen Nos.: UOC:SIGWI:S24, UOC:MINNP:M13, UOC:MINNP:MK66; GenBank Accession No.: KR818817, KP776992.

6. *Panus similis* (Berk. & Broome) T.W. May & A.E. Wood, *Mycotaxon* 54: 148 (1995) Figure 6.

Basidiocarp solitary, umblicate with a central depression, diameter 3.8-4.0 cm. Upper surface umber to fawn, radially striate, fine hair present, thick hair present in the margin. Lower surface buff colour. Stipe vinaceous buff, covered with fine hairs, up to 6 cm long, and up to 4.0 mm in diameter. Spore ellipsoidal to oval, hyaline, 3.7-4.8 × 4.7-7.5 µm in size (L= 5.76, W= 4.12, Q= 1.40).

Remarks: Even though *Panus* sp. are gilled mushrooms they are placed in Polyporaceae. According to reference [7], the genus *Panus* represents an independent origin of the agaricoid habit in the Polyporales. As such *Panus* species are placed in an outer sub group from core polypores (Figure 10). A characteristic feature of *P. similis* is the strongly plicate striate pileus [22]. This species is reported from Malaya, Borneo regions, Africa and India.

Specimens examined: Sri Lanka, Sigiriya wilderness, on fallen angiosperm wood, collectors: S.S. Ediriweera, R.L.C. Wijesundera, C.M. Nanayakkara,

Figure 6. *Panus similis*; Fruit body (A), Lower surface (B), Basidiospores (C).

N.W. Gunasekara & M.D.M. Fernando; February 2013, Herbarium Specimen No.: UOC:SIGWI:S38; GenBank Accession No.: KR818820.

7. *Earliella scabrosa* (Pers.) Gilb. & Ryvarden, *Mycotaxon* 22(2): 364 (1985) Figure 7.

Basidiocarp solitary or clustered, resupinate and effused-reflexed, 2.0-4.0 cm long and 3.0-9.0 cm wide. Upper surface red maroon, white margin, concentrically grooved, black with KOH. Lower surface yellowish white, pores spherical to elongated, 1-4 pores/mm. Spore print white. Hyphal system trimitic, generative hyphae-hyaline, clamped, 3.5-5.5 µm thick; skeletal hyphae hyaline, thick walled, 3.5-6.0 µm; binding hyphae hyaline, solid, 3.5-5.0 µm. Spore cylindrical, hyaline, thin walled, 2.0-3.0 × 7.5-11.3 µm in size (L= 9.31, W= 2.43, Q= 3.83). Basidia cylindrical, 4 sterigmata, 25.0 to 28.0 µm long. Cystidia cylindrical and fusiform, 4.5-10.0 × 22.0-31.0 µm in size.

Remarks: This species is widespread and common in tropical and subtropical areas, especially in open and degraded forests. Species are recorded from China, Japan, Taiwan, Russia Thailand and Vietnam. It can be easily recognized due to its effused-reflexed, tough basidiocarps with reddish cuticle and irregular, elongated pores. According to the phylogenetic analysis *Earliella scabrosa* is placed among the core polyporoid clade with closer relationship *Ganoderma, Coriolopsis* and *Lentinus* species. (Figure 10).

Specimens examined: Sri Lanka, Dambulla IFS Sam Popham Arboretum, Sigiriya wilderness & Minneriya National Park, on fallen angiosperm wood, collectors: S.S. Ediriweera, R.L.C. Wijesundera, C.M. Nanayakkara, N.W. Gunasekara & M.D.M. Fernando; August 2012, February 2013 & July 2013, Herbarium Specimen No.: UOC:MINNP:M19; GenBank Accession Nos.: KR706165, KR706167, KP734204.

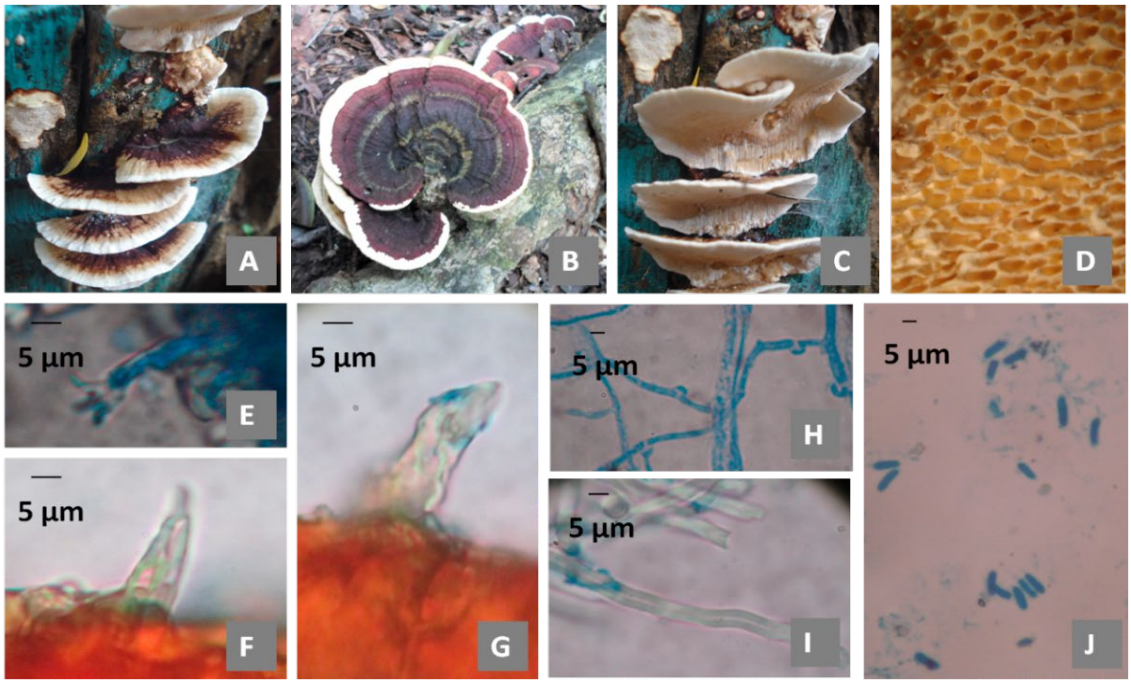

Figure 7. *Earliella scabrosa*; Fruit body (A, B), Lower surface (C), Lower surface × 10 (D), Basidia (E), Cystidia (F, G), Generative hyphae (H), Skeletal hyphae (I), Basidiospores (J).

8. *Trametes cubensis* (Mont.) Sacc., *Syll. Fung.* (Abellini) 9: 198 (1891) Figure 8.

Basidiocarp annual, solitary or fused, flabelliform, 7.0-15.0 cm long from base to margin, 11.0-25.0 cm in diameter with round margin. Upper surface white to smoke grey, faint concentric zones, glabrous. Lower surface white to pale yellow, round pores, 2-3 pores/ mm. Context white, 0.3-1.2 cm thick. Tube layer 0.2-0.7 cm thick. Hyphal system trimitic, generative hyphae clamped, hyaline, 0.5-2.0 µm thick; skeletal hyphae pale yellow, thick walled to filled, 3.5-6.5 µm thick; binding hyphae pale yellow, filled, 3.2-3.5 µm thick.

Remarks: *Trametes* is a genus proposed by Fries and comprises about more than 50 species which are characterized by its sessile to effuse-reflexed, poroid hymenial surface with round, angular to irregular pores, trimitic hyphal system and ellipsoid to allantoid, hyaline smooth basidiospores that do not react to Melzer's reagent. According to the studies on phylogeny of *Trametes* species and other related genera in 2011 [23], carried out using ribosomal markers and protein coding genes, it was found that *Trametes cubensis* is closely related to *Pycnoporus* sp., which is also evident from the current analysis (Figure 10). This species can be recognized in the field by the dimidiate basidiomata with a reddish cuticle from the base [24].

Specimens examined: Sri Lanka, Sigiriya wilderness, on fallen angiosperm wood, collectors: S.S. Ediriweera, R.L.C. Wijesundera, C.M. Nanayakkara, N.W. Gunasekara & M.D.M. Fernando; February 2013, Herbarium Specimen No.: UOC:SIGWI:S26; GenBank Accession No.: KP771708.

9. *Trametes elegans* (Spreng.) Fr. *Epicr. Syst. mycol.* (Upsaliae): 492 (1838) Figure 9.

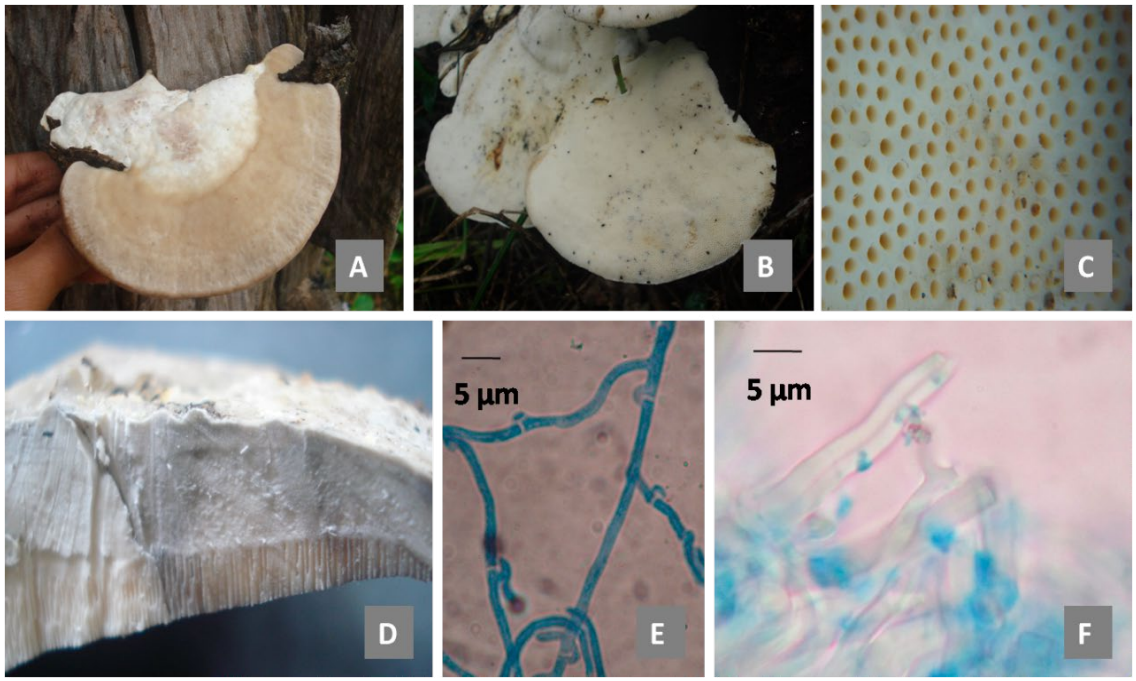

Figure 8. *Trametes cubensis*; Fruit body (A), Lower surface of the fruit body (B), Lower surface × 5 (C), Cross section of the fruit body (D), Generative hyphae (E), Binding & skeletal hyphae (F).

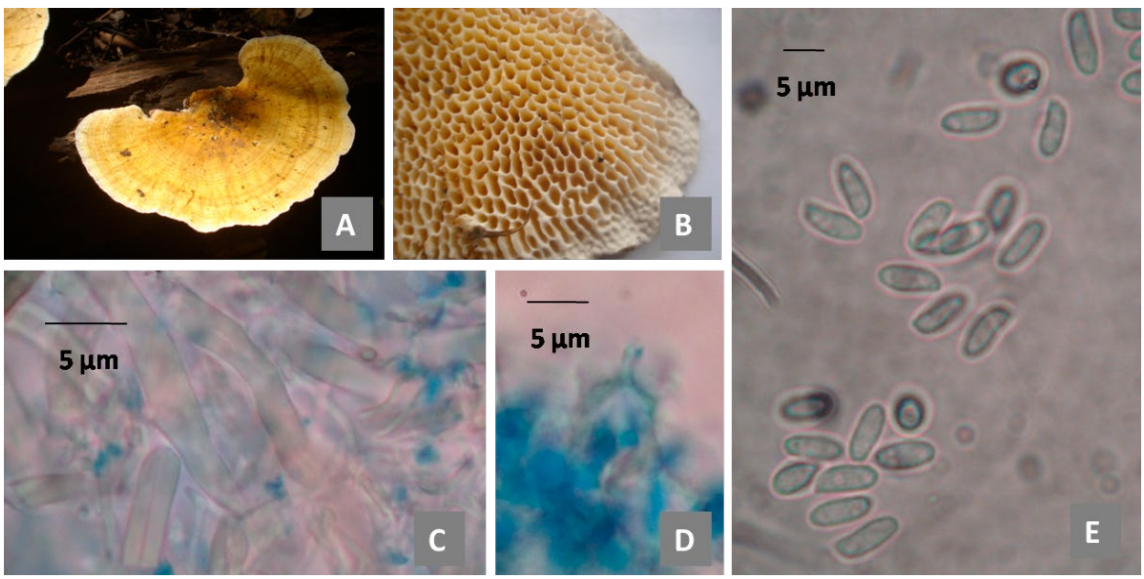

Figure 9. *Trametes elegans*; Fruit body (A), Lower surface of the fruit body (B), Binding & skeletal hyphae (C), Basidia (D), Basidiospores (E).

Basidiocarp solitary and fused, sessile, irregularly bracket shaped, applanate, 4.0-12.0 cm from base to margin, 6.0-20.0 cm in diameter. Upper surface white to buff, turns smoke grey with maturity, concentrically grooved, rough. Lower surface white to straw, pores round to angular, maze like and gill like, 3 pores/mm. Context buff colour, tube layer is 0.2-1.0 cm thick. Spore print white. Hyphal system trimitic, generative hyphae hyaline, 1.0-2.3 μm thick; skeletal hyphae hyaline, thick walled to filled, 3.2-6.0 μm thick; binding hyphae pale yellow, filled, 1.5-3.5 μm. Basidia spherical, with 4 sterigmata, 9.0-8.0 × 11.0-15.0 μm in size. Spore cylindrical, hyaline, thin walled, 3.0-4.8 × 5.5-9.5 μm in size (L= 7.13, W= 3.74, Q= 1.91).

Remarks: *Trametes elegans* is a widespread in tropical and subtropical environments. The hymenophore is highly variable from lamellate to poroid even within the same species [25]. Due to this variability this species was earlier classified as *Lenzites* but was later transferred to *Trametes* [23]. *T. elegans* is easy to recognize because of the narrow lamellae or sinuous pores and the frequently variable hymenophore on the same specimens.

Specimens examined: Sri Lanka, Sigiriya wilderness; Minneriya Kaudulla & Wasgamuwa National Parks, on fallen angiosperm wood, collectors: S.S. Ediriweera, R.L.C. Wijesundera, C.M. Nanayakkara, N.W. Gunasekara & M.D.M. Fernando; February July 2013 & August 2014, Herbarium Specimen Nos.: UOC:SIGWI:S25, UOC:SIGWI:S49, UOC:KAUNP:K06, UOC:MINNP:MK08; GenBank Accession No.: KP780433, KP780434.

3.2 Phylogenetic Analysis

The ITS rDNA dataset of 72 sequences comprised 1107 characters with 459 constants, 146 variables and 502 parsimony-informative characters. The tree length was 3545 steps with consistence index (CI) = 0.3543 and retention index (RI) = 0.5794. The phylogenetic analysis resulted in two major clades; Polyporoid clade and Hymenochaetoid clade (Figure 10). The Hymenochaetoid clade, include members of family hymenochaetaceae with a unique character: presence of setae. In addition, it includes *Trichaptum* sp. Even though setae are not present the septal ultrastructure, suggests that *Trichaptum* is closely related to the Hymenochaetaceae having imperforate parenthosomes [26].

Within the polyporoid clade four informally named subgroups were revealed as an outcome of an analysis in 2004 on order Polyporales using four ribosomal DNA markers in approximately 124 species; core polyporoid clade, *Antroidia* clade, phlebioid clade and residual polyporoid clade [27]. Those four clades could be observed in the phylogenetic analysis performed during this study as well. Most taxa in the core polyporoid clade produce white rot, the hyphal system is dimitic or trimitic and have a tetrapolar mating system [27]. The species in families Polyporaceae and Ganodermataceae are distributed within the core-polyporoid clade. Except for *Ganoderma* all have smooth, inamyloid, cylindrical spores. It is believed that *Ganoderma* type spores (thick walled, dark) are apomorphic within the Polyperaceae having been derived from the smooth cylindrical spores [26]. Almost all the members of *Antroidia* clade causes brown rot. This group includes species of *Antrodia, Auriporia, Daedalea, Fomitopsis, Laetiporus, Oligoporus, Postia, Neolentiporus, Phaeolus, Piptoporus, Sparassis* [28]. In the present analysis this clade is represented by three species, namely *Fuscopostia fagilis, Fomitopsis* sp. and *Rhodofomitopsis feei*. According to one reference [27], the phlebioid clade was introduced with majority of it consisting of corticoid species and resupinate taxa and producing white rot. Similarly, species of family Meruliaceae and Phanerochaetaceae are combined to one clade in the present analysis. There are few species within the polyporoid clade that cannot be placed in any of the three groups above, as their characteristics are contradictory to the original residuals in these groups. Accordingly, *Panus* sp. and *Podoscypha petalodes* forms a separate clade namely the residual polyporoid clade in this study

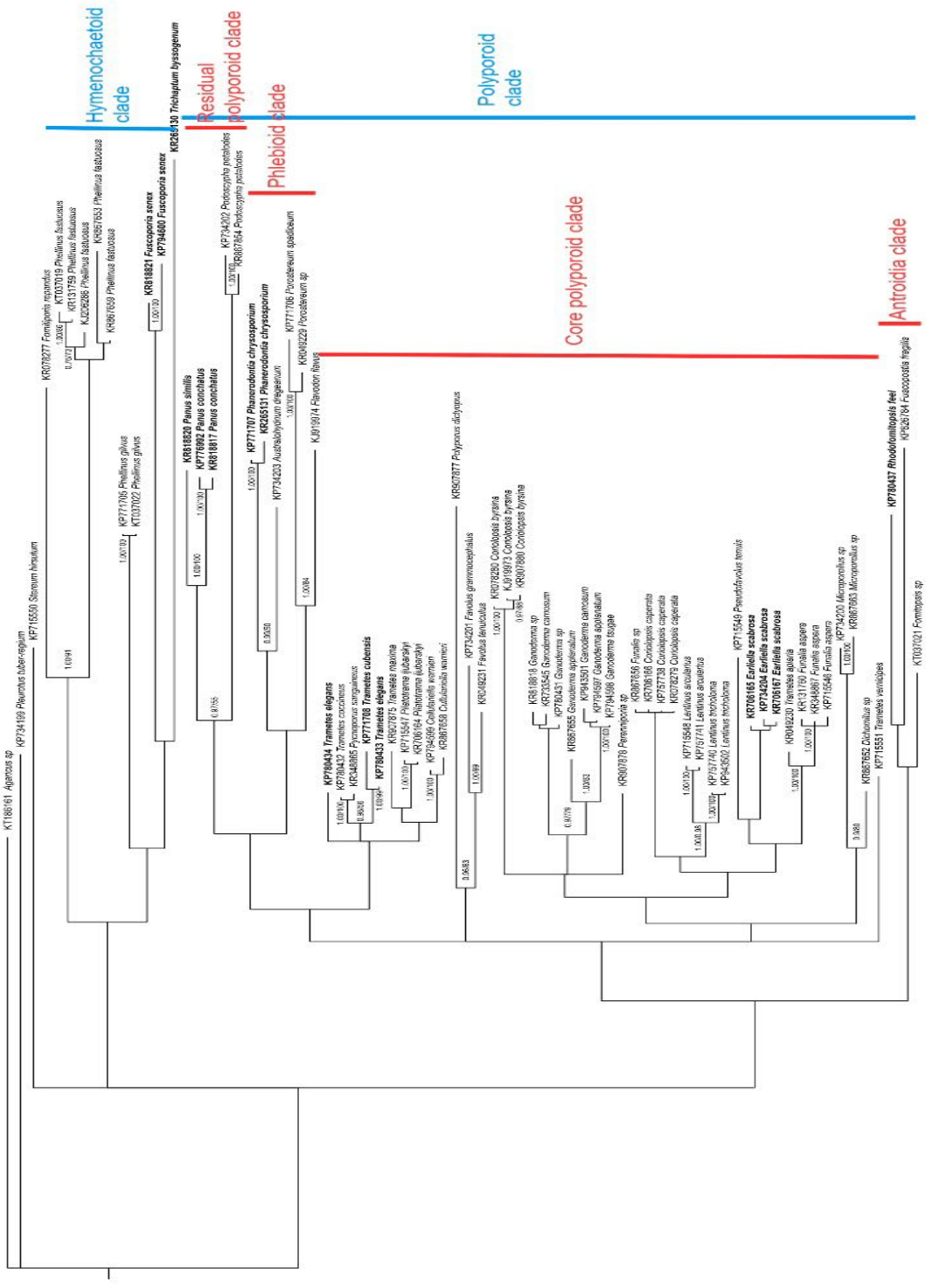

Figure 10. Bayesian analysis and maximum parsimony of the ITS rDNA region of polypores encountered in the study, where the tree was generated from Bayesian analysis; bootstrap values ≥50% are shown above. New records are highlighted.

as in the analysis reported in the reference [27]. Diversity studies and monitoring fungi are important in detecting influence of climate change, anthropogenic disturbances such as air pollution and quantifying their impacts [09]. However, the diversity data are mostly incomplete in many of the regions worldwide due to taxonomic obstacles, paucity of trained mycologists, low number of published, rigorous, long term studies [29].

Available information on Sri Lankan macrofungi is also lacking specifically on polypores and other aphyllophorales. Sri Lanka being a tropical country and a biodiversity hotspot, the absence of information on Sri Lankan macrofungal biodiversity makes it a difficult task to assess the wealth of biodiversity present in Sri Lanka as well as track the changes over the time. Therefore, the knowledge gained on macrofungi species biodiversity at the community and species level will enable the respective authorities to monitor the effectiveness of or the need for conservation and follow up natural and artificial disturbances. Even though PCR and other molecular based methods have provided new tools for the enumeration of fungal species, combining the new technology with more conventional methods (morphological characters & identification guides) are also important, to gain a fuller understanding of interactions occurring in the environment [30].

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Conflict of interest statement

The authors declare that they have no conflicts of interest.

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