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Diversity and Phylogeny of Phanerochaetoid Fungi from Eastern Ghats of Tamil Nadu, India

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ABSTRACT

Phanerochaetoid fungi are an important group of wood inhabiting fungi classified under the family Phanerochaetaceae. Twenty-five specimens of the phanerochaetoid fungi collected from Eastern Ghats of Tamil Nadu are being delimited based on phylogenetic analyses of combined dataset of ITS+nLSU and morphological attributes. These 25 specimens have been placed under 3 species belonging to 2 genera *viz. Phlebiopsis castanea* (Lloyd) Miettinen & Spirin*,* *P. griseofuscescens* (Reichardt) Nakasone & S.H. Herin, and *Oxychaete cervinogilva* (Jungh.) Miettinen. Among the described species, *Phlebiopsis griseofuscescens* is the first report to India and *P. castanea* is new addition from Tamil Nadu.

Keywords: Phanerocheataceae, *Phlebiopsis*, *Oxychaete*, Taxonomy, Phylogeny, Wood inhabiting fungi.

INTRODUCTION

The term 'Phanerochaetoid fungi' is broadly used to describe corticioid members possessing phanerochaetoid basidioma with clampless hyphae (Parmasto, 1986; Wu, 1990; Hansen and Knudsen, 1997). Jülich (1982) established the family Phanerochaetaceae to accommodate these fungi with *Phanerochaete* as the type genus. Members of this family are characterized by resupinate to effused-reflexed or pileate basidioma having monomitic to rarely dimitic hyphal system with thin- to thick-walled, simple-septate generative hyphae. The cystidia are usually present and are coarsely or apically encrusted. Basidia are usually hyaline, narrowly clavate with cylindrical to ellipsoid, thin-walled, smooth basidiospores (Jülich 1982; Parmasto, 1986; Wu, 1990). Perhaps, the phylogenetic studies on Phanerochaetaceae began with Larsson's (2007) demonstration to nest corticoid fungi within Phanerochaetaceae. Later, Binder *et al.* (2013) and Justo *et al.* (2017) contributed improved classification of the family based on multi-gene phylogenetic studies and the family is placed within the order Polyporales. Nevertheless, the systematics of Phanerochaetaceae has greatly benefitted from several molecular phylogeny-based studies and 23 genera have been accepted under this family (Hjortstam and Ryvarden, 2010; Binder *et al*., 2013; Floudas and Hibbett, 2015; Miettinen *et al*., 2016; Yuan *et al*., 2017; Justo *et al*., 2017; Chen and Wu, 2018; He *et al*., 2019; Zhao *et al*., 2021; Chen *et al*., 2021).

In India, members of this family were previously reported from Punjab (Kaur, 2017), Maharastra (Ranadive *et al*., 2011), Tamil Nadu (Natarajan and Kolandavelu, 1998) and Uttarakhand (Sharma, 2012). The present study aims to analyse the ITS and nLSU sequence based phylogenetic relationship of members of Phanerochaetaceae collected from Tamil Nadu along with their morphological characterization. Two new geographical reports - *Phlebiopsis griseofuscescens*, *P. castanea* and 1 previously reported species - *Oxychaete* PCR protocols, a guide to method and application. *cervinogilva* are described in this study.

MATERIAL AND METHODS

Collection and Morpho-taxonomical analyses of Phanerochaetoid fungi

Twenty-five specimens of phanerochaetoid fungi were collected during 2018 and 2020 in the months of January to March, from Eastern Ghats of Tamil Nadu, India. Macro-morphological characteristics of the basidiome such as nature, shape, size, texture, colour of the hymenial/abhymenial surface, type of margin (acute or obtuse), context (homogenous, duplex with or without black line), tube layer (colour, length, stratification), and pores (size and shape) were recorded in the fresh sample. Colour descriptions were based on the Methuen handbook (Kornerup and Wanscher, 1978). The micro-morphological characteristics were studied and photographed from the free-hand cut sections of the specimens that were mounted in water, 5% KOH (v/w), cotton blue (CB) (0.1%) and Melzer's reagent (IK) using a Labomed

(Optic-CX BINO) microscope. The taxonomic illustrations were prepared using LABOMED CxL2 compound microscope. Microscopic measurements and illustrations were made in 5% KOH solution. An average of dimensions of 50 basidiospores was considered for basidiospores size measurement (minimum-mean-maximum) and Q value based on the length/width ratio was also calculated. The specimens described were deposited in the Madras University Botany Laboratory (MUBL), Centre for Advanced Study in Botany, University of Madras, Chennai, India.

The following abbreviations have been used during the course of present studies.

IKI^- - inamyloid; IKI^+ - amyloid; CB^- - acyanophilous; CB^+ - cyanophilous; L - mean basidiospore length (arithmetic average of the basidiospores observed); W - mean basidiospore width (arithmetic average of the basidiospores observed); Q - variation in the L/W ratio; basidium length excludes the length of the sterigmata; n - number of basidiospores measured.

Genomic DNA extraction, PCR amplification, and sequencing

The extraction of total genomic DNA was made from the mycelia and/or dried basidiomes following the protocol of Doyle and Doyle (1987) modified by Góes-Neto *et al*. (2005). The ITS region was amplified with the primer pairs ITS5 and ITS4 (White *et al*., 1990). The nLSU region was amplified with the primer pairs LROR and LR7 (Vilgalys and Hester, 1990). The cycling parameter for the amplification of ITS region included initial denaturation at 95°C for 3 min, followed by 32 cycles at 95°C for 30 s, 52°C for 30 s, and 72°C for 1 min, and a final extension of 72°C for 3 min. The PCR cycling parameter for nLSU involved initial denaturation at 94°C for 1 min, followed by 34 cycles at 94°C for 30 s, 45°C for 30 s, and 72°C for 1.5 min, and final extension at 72°C for 10 min. The PCR products were purified and sequenced at Eurofins Genomics India Pvt Ltd, Karnataka, India. All the sequences generated in this study were deposited to GenBank.

Phylogenetic analyses

The combined sequence dataset comprising of ITS and nLSU sequences generated from the present study and relevant sequences retrieved from GenBank (NCBI) were used for the molecular phylogenetic analysis with *Fomitopsis pinicola* AFTOL ID 770 and *Trametes versicolor* FP135156sp as the outgroup. The sequences of were aligned using MEGA X v10.0.2 configured for windows and edited manually to increase the alignment similarity (Kumar *et al*., 2018). The best-fit evolutionary model was selected by hierarchical likelihood ratio test (hLRT) and Akaike information criterion (AIC) in MrModeltest v.2.3 (Nylander, 2004). The maximum likelihood (ML) and Bayesian Inference (BI) analyses were performed with the combined dataset. The ML tree was constructed using raxmlGUI 2.0 (Edler *et al*., 2021) by 1000 rapid bootstrap replicates with GTRGAMMA and GTRCAT model to access best tree from all searches and ML bootstrap values of the nodes, respectively (Guindon and Gascuel, 2003; Darriba *et al*., 2012). Bayesian Inference (BI) was performed using MrBayes 3.2.7a with four Markov chains, starting trees for 80,000,000 generations until the split deviation frequency < 0.01 and the trees were sampled every 100 generations (Ronquist *et al*., 2012). The first 25 % of all trees were discarded as burn-in and the remaining were used to calculate Bayesian Posterior Probabilities (BPP) of the clade. All trees were viewed in FigTree v. 1.4.2.

RESULTS

Molecular phylogeny

In this study, a total 107 specimens representing 61 taxa were used along with 2 outgroups namely, *Fomitopsis pinicola* (AFTOL ID 770) and *Trametes versicolor* (FP135156sp). The combined dataset consisting a total of 200 sequences (106 ITS and 94 LSU) including 12 sequences (8 ITS and 4 LSU) were used to reconstruct phylogenetic trees of Phanerochaetaceae. The dataset had an aligned length of 1,606 characters (ITS= 685 and nLSU= 921characters), of which 922 characters were constant, 667 were variable and parsimony-uninformative and 537 were parsimony-informative. The best model of nucleotide evolution for the ITS+nLSU dataset estimated and applied in the Bayesian analysis was found to be GTR+I+G. Bayesian analysis and ML analysis resulted in a similar topology. The Bayesian analysis had an average standard deviation of split frequencies = 0.001492 (BI). The phylogenetic tree, inferred from the ITS and nLSU sequences, highlighted that the sequences generated from this study clustered within 2 species of *Phlebiopsis* (*P. castanea* and *P. griseofuscescens*) and *Oxychaete cervinogilva* **(Figure 1)**.

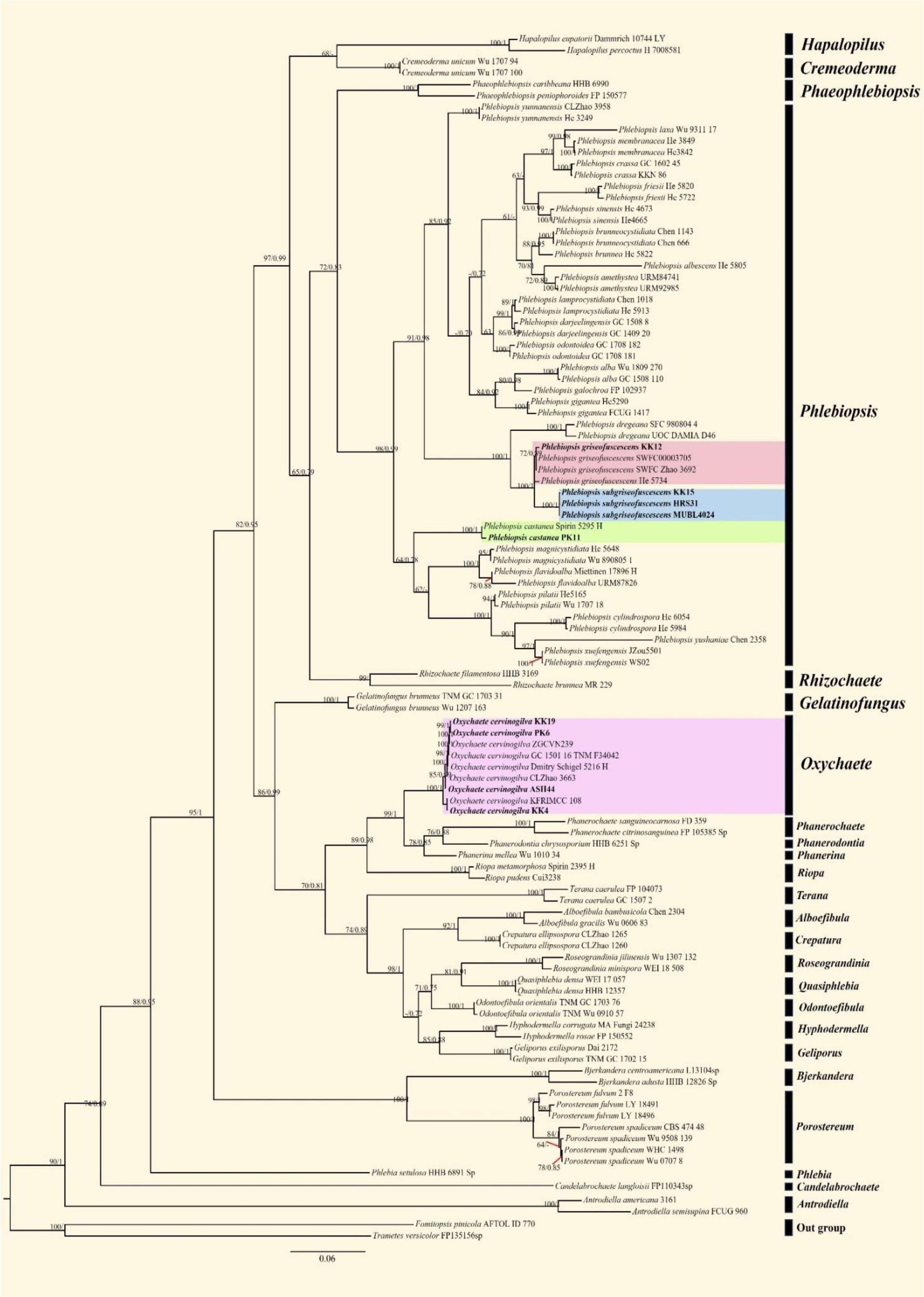

Figure 1: Phylogram of Phanerochaetaceae inferred from ML analyses using the combined ITS + nLSU dataset. Branches are labelled with ML bootstrap ≥ 70% and BPPs ≥ 0.75 from Bayesian analyses with the ougroups *Fomitopsis pinicola* AFTOL ID 770 and *Trametes versicolor* FP135156sp. Newly generated sequences are in bold and highlighted.

Taxonomy

Phlebiopsis castanea (Lloyd) Miettinen & Spirin, MycoKeys **17**:25, 2016.

≡ *Irpex castaneus* Lloyd, Mycological Writings **6(65)**: 1060, 1920. **Figure 2**

(ITS: PQ425915; LSU: PQ425917)

Basidiome annual, resupinate, adnate, leathery when fresh, corky when dry, up to 16 cm long, 4 cm wide, and 2 mm thick. Pore surface yellowish brown (5C5), pores angular to irregular or irpicoid, 1–2 per mm. Tubes brown (5E5), up to 1 mm deep. Subiculum homogenous, concolorous with the tube layers, up to 1 mm thick. Hyphal system monomitic, generative hyphae simple-septate. Subicular generative hyphae thin- to thick-walled, occasionally branched, 4–7.5 µm in diam. Tramal generative hyphae thin- to thick-walled, frequently branched, 2.4–7 µm in diam. Cystidia conical to subulate, originating from tramal layer, thick-walled, apically heavily encrusted for over half of their length, encrustation dissolving in KOH over time, 45–58 × 8–12 µm (encrustation included). Basidia clavate, with 4 sterigmata, 25–32 × 6–8 µm. Basidiospores broadly allantoid, hyaline, thin-walled, smooth, sometimes with oily contents, (5–)5.2–6.8(–7.1) × (2–)2.2–2.6(–3.1) µm, Q=2.8 (n = 30/2) CB−, IKI−.

Specimens examined: India, Tamil Nadu, Thiruvannamalai district, Jawadhu Hills, 12°29'01"N 78°55'07"E, on dead wood, 27 March 2018, Kezhocuyi Kezo (MUBL1178, PK20). Trichy district, Pachaimalai Hills, 11º17'52"N 78º34'12"E, on dead wood, 15 February 2018, Kezhocuyi Kezo (SRF9). Salem district, Yercaud, 11°47'41"N 78°12'44"E, on dead angiospermous wood, 23 January 2018, Kezhocuyi Kezo (HRS9, HRSII3). Kallakurichi district, Kalvarayan Hills, 11°51'36.5"N 78°42'35.5"E, on dead wood, 26 January 2020, Kezhocuyi Kezo (IN16).

Note: *Phlebiopsis castanea* is characterized by poroid to irpicoid hymenophore, encrusted cystidia and broadly allantoid basidiospores. Phylogenetically our isolate (MUBL1178) clustered with *P. castanea* with well support ML (100) and BYPP (1) value. Morphologically the present specimen is consistent except for a slightly larger cystidia (50–60 × 10–11 µm) (Núñez and Ryvarden, 2001; Chen *et al*., 2018).

Remarks: This is the first report of *P. castanea* from Tamil Nadu with molecular phylogenetic evidences.

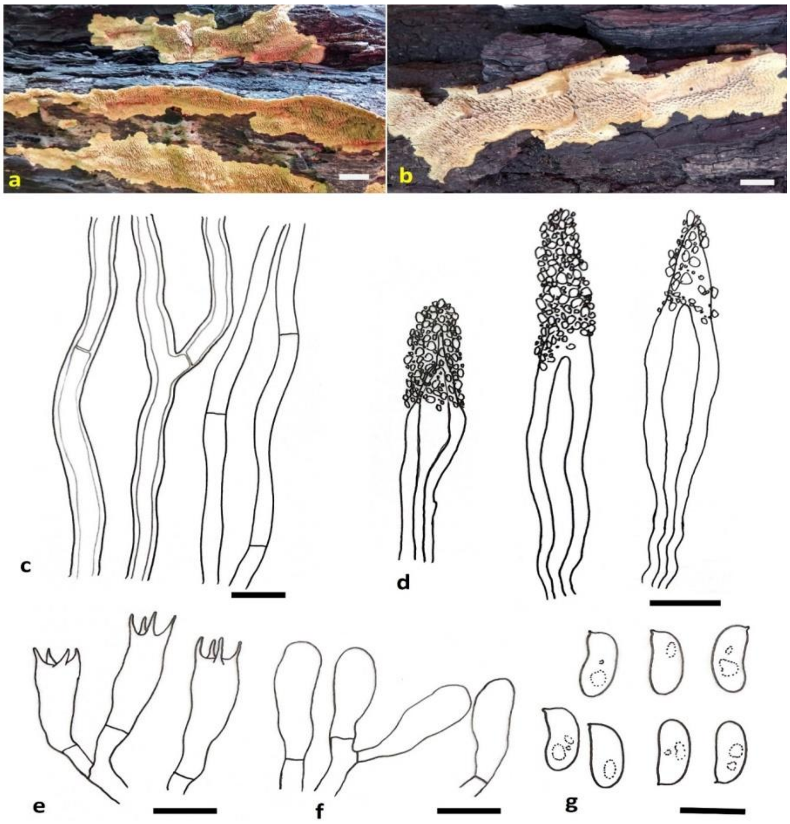

Figure 2: *Phlebiopsis castanea*. a-b. Basidiome showing hymenial surface; c. Generative hyphae; d. Encrusted cystidia; e. Basidia; f. Basidioles; g. Basidiospores. Scales bar a-b = 1 cm, c-f = 10 µm, g = 5 µm.

Phlebiopsis griseofuscescens (Reichardt) Nakasone & S.H. He, Frontiers in Microbiology, **12(622460)**:15, 2021.

≡ *Hydnum griseofuscescens* Reichardt, Verhandlungen der Zoologisch-Botanischen Gesellschaft Wien **16**: 374, 1866. **Figure 3**

(ITS: PQ425916; LSU: PQ425918)

Basidiome annual, resupinate to effused-reflexed, subceraceous when fresh, turning to leathery upon drying, up to 13 cm long, 7 cm wide, 3 mm thick. Pilei often laterally fused. Pilear surface tomentose, white (6A1) when fresh, turning orange white (6A2) on drying. Context duplex, greyish orange (6B3) towards tomentum and greyish drown (6E4) towards tube, up to 1 mm in thickness. Hymenial surface poroid when young becoming lacerated and splitting to spines or odontioid with age, brownish violet (11D7) when fresh, turning greyish brown (7D3) on drying, pores 1–2 per mm. Tubes concolorous with the hymenial surface, up to1 mm deep. Margin sterile, acute, white (6A1). Hyphal system monomitic, generative hyphae simple-septate. Context generative hyphae thin- to thick-walled, occasionally branched, 3–6 µm in diam. Tramal generative hyphae thin- to thick-walled, frequently branched, 2.4–5 µm in diam. Cystidia conical, thick-walled, apically encrusted with crystalline material, 55–78 × 5–8.5 µm, cystidioles absent. Basidia narrowly clavate, with 4 sterigmata, simple-septate, 18–28 × 3.5–5.5 µm. Basidioles dominant, narrowly clavate, with 4 sterigmata, simple septate, 15–24 × 3.5–5 µm. Basidiospores ellipsoid, hyaline, thin-walled, smooth, (4–)4.5–6(–6.2) × (2.5-)3–3.3(-3.5) µm (n = 30/2), Q = 1.33, IKI–, CB–.

Specimens examined: India, Tamil Nadu, Trichy district, Pachaimalai Hills, 11°17'21.2"N 78°36'12.8"E on dead wood, 14th Feb. 2018, Kezhocuyi Kezo (MUBL1179).

Note: *Phlebiopsis griseofuscescens* is characterized by resupinate to effused-reflexed basidiome, brownish violet hymenophore, encrusted cystidia and small ellipsoid basidiospores. Phylogenetically our isolate (MUBL1179) clustered with *P. griseofuscescens* with well support ML (72) and BYPP (0.89) value. *P. griseofuscescens* was originally described as *P. lacerata* by Xu *et al*. (2020) from China. However, Zhao *et al*. (2021) merged it with *P. griseofuscescens* based on morphological and phylogenetic analyses.

Remarks: This is first report from India with molecular evidence.

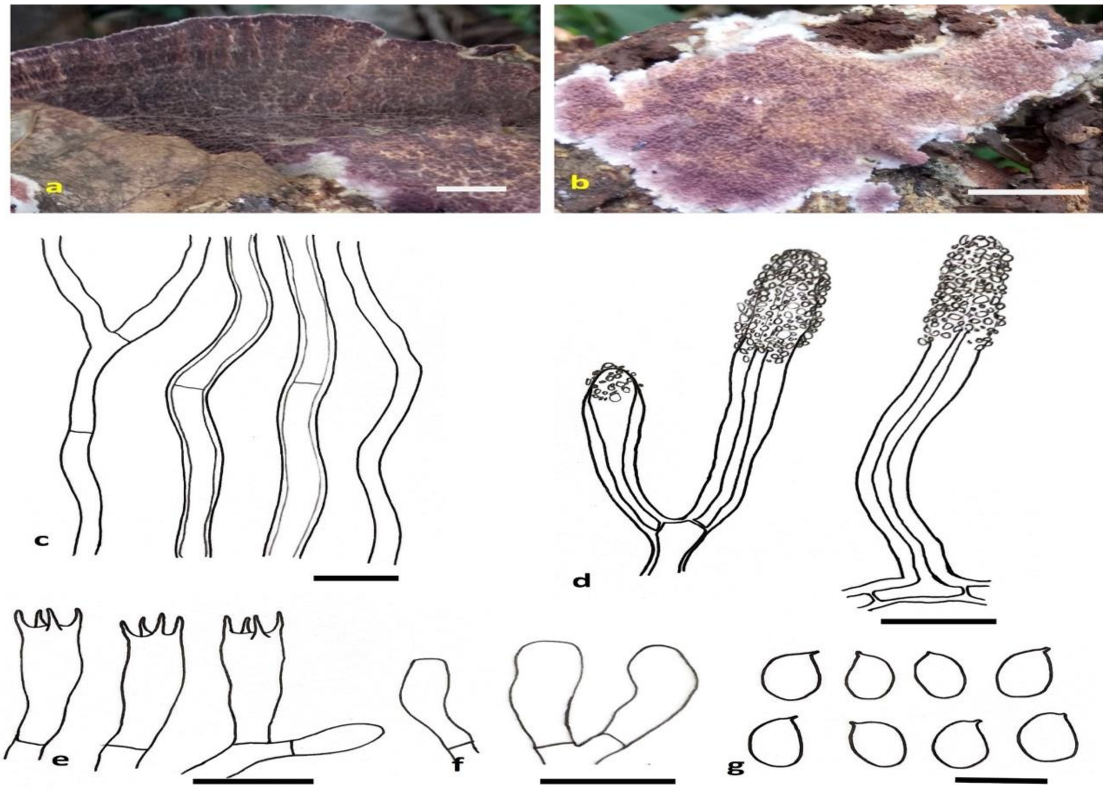

Figure 3: *Phlebiopsis griseofuscescens*. a-b. Basidiome; c. Generative hyphae; d. Encrusted cystidia; e. Basidia; f. Basidioles; g. Basidiospores. Scales bar a-b = 1 cm, c-f = 10 µm, g = 5 µm.

Oxychaete cervinogilva (Jungh.) Miettinen, MycoKeys **17**:20, 2016.

≡ *Polyporus cervinogilvus* Jungh., Praemissa in floram cryptogamicam Javae insulae, **45**:1838.

Figure 4

(ITS: PQ425913, PQ425914, PQ425911, PQ425912)

Basidiome annual, resupinate to effused-reflexed, leathery when fresh, corky when dry, up to 18 cm long, 6 cm wide, and 2 mm thick. Pilear surface yellowish brown (5D5), with a lighter margin (5D4), pilei projecting up to 4 cm (5D4) and 1–2 mm thick. Hymenial surface poroid, pores regular, brownish yellow (6C3), 2–3 per mm. Tubes concolorous with the hymenial surface, up to 1 mm. Context homogenous concolourous with the pore surface, up to 1 mm thick. Hyphal system monomitic; Contextual generative hyphae, thin- to thick-walled, simple- septate, occasionally branched, 4–5.5 µm in diameter; traumal generative hyphae, thin- to thick-walled, frequently branched, simple-septate 3–5 µm in diameter. Cystidia abundant, thick-walled, apically encrusted, 23–53 × 4.5–9 µm. Basidia narrowly clavate, with 4 sterigmata, 16–25 × 5–8 µm. Basidiospores cylindrical, curved, thin-walled, smooth, (6-)6.2–8.4(-8.8) × 2.8–3.5(-3.8) µm, Q=2.2, CB−, IKI−.

Specimens examined: India, Tamil Nadu, Trichy district, Pachaimalai Hills, 11°17'22.3"N 78°36'13.1"E, on dead wood, 14 February, 2018, Kezhocuyi Kezo (MUBL1177; KK4; SRFII4; KS1). Thiruvannamalai district, 12°29'13.5"N 78°55'07.0"E, on dead angiospermous wood, 27 March, 2018, Kezhocuyi Kezo (PK6, TM2, TM23, TM51). Namakkal district, Kolli Hills, 11°19'17"N 78°23'09"E, on dead wood, 29 January, 2020, Kezhocuyi Kezo (ASII44, ITS; AS35). Namakkal district, Kolli Hills, 11°19'42.7"N 78°21'19.3"E, on dead wood, 29 January, 2020, Kezhocuyi Kezo (KRVK14). Salem district, Yercaud, 11°48'18.2"N 78°11'59.5"E, on dead angiospermous wood, 23 January, 2018, Kezhocuyi Kezo (SMIIZ).

Notes: *Oxychaete cervinogilva* is characterized by its effused-reflexed basidiome, regular poroid hymenial surface, monomitic hyphal system with simple-septate generative hyphae, curved, comparatively larger, cylindrical basidiospores (Miettinen *et al.*, 2016). Phylogenetically our isolates (ASII44, KK4, MUBL1177, PK6) clustered with *Oxychaete cervinogilva* clade.

Remarks: Natarajan and Kolandavelu (1998) reported it as *Oxyporus cervinogilvus* from Kolli Hills, Tamil Nadu. In the present study, specimens of *Oxychaete cervinogilva* were collected from Kolli Hills, Yercaud Hills and Pachaimalai Hills.

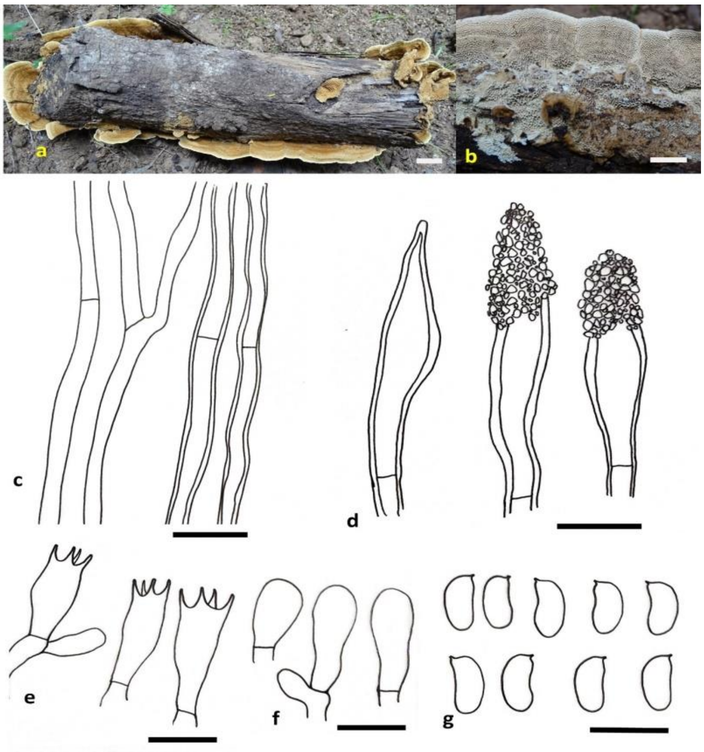

Figure 4: *Oxychaete cervinogilva*. a-b. Basidiome; c. Generative hyphae; d. Encrusted cystidia; e. Basidia; f. Basidioles; g. Basidiospores. Scales bar: a-b = 1 cm, c-g = 10 µm

DISCUSSION

The current study provides significant insights into the diversity of phanerochaetoid fungi within the Eastern Ghats of Tamil Nadu, India, specifically focusing on the Phanerochaetaceae family. The findings detail the identification of 25 isolates of phanerochaetoid fungi across 5 different study areas (Jawadhu Hills, Kalvarayan Hills, Kolli Hills, Pachaimalai Hills, and Yercaud) in the districts of Thiruvannamalai, Kallakurichi, Namakkal, Trichy, and Salem. These fungi were classified under the Phanerochaetaceae family, and the study provided both taxonomic and phylogenetic perspectives on these species, which contribute to the global understanding of phanerochaetoid fungi.

The most abundant species, *Oxychaete cervinogilva*, was found to be present at all the collection sites, signifying its wide ecological distribution across the regions studied. This species, originally identified as *Polyporus cervinogilvus*, was later reclassified by Miettinen *et al*. (2016) based on both morphological and phylogenetic analyses, this represents a crucial case of taxonomic revision based on both morphological and phylogenetic data. Its identification as the lone species of the genus *Oxychaete* holds significance for future ecological and evolutionary studies. Nevertheless, its predominant presence across various hills indicates its adaptability and ecological significance in these habitats. The other species, *Phlebiopsis castanea* and *P. griseofuscescens* are also notable for their distinct taxonomic histories, with *P. castanea* and *P. griseofuscescens* being re-established based on phylogenetic studies (Miettinen *et al*., 2016; Xu *et al*., 2020; Zhao et al., 2021).

This study not only enriches the fungal diversity of Tamil Nadu but also contributes to the broader scientific community's understanding of Phanerochaetaceae diversity, classification, and ecological roles. Future research could focus on further taxonomic studies, ecological assessments, and the exploration of the full potential of these fungi in forest management, biotechnology, and conservation efforts.

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