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New record of *Thysanorea papuana* from India

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Abstract

Studies on litter degrading microfungi from forests of Western Ghats is discovering many fungi, some of which are very rare in nature. This paper illustrates *Thysanorea papuana* and is the first report of its occurrence in India, extending distribution from its originally described locality of Papua New Guinea. The genus *Thysanorea* is monotypic and is reported for the first time from India. The identity of the fungus is confirmed based on morphological characters and molecular phylogeny of ITS and LSU regions. Isolation of this fungus is an important distributional record for this rare fungal species.

Key words – Biodiversity – Western Ghats

Introduction

Many fungi were collected during studies on litter degrading microfungi from forests of Goa. This paper illustrates *Thysanorea papuana* (Aptroot) Arzanlou, W. Gams & Crous and is the first report of its occurrence in India, extending the distribution from originally described locality of Papua New Guinea. The species, which belong to a monotypic genus, is described and illustrated with micro-photographs. The culture obtained from single spore isolation was used to generate ITS and LSU sequence-data.

Materials & Methods

Thysanorea papuana was isolated from an unidentified dead twig from the forests of Valpoi, Goa, India. Samples were taken to the laboratory in zip lock polythene bags, and examined under a stereoscope. The fungus was picked up with a sterile needle, mounted in lactophenol and observed under a light microscope. The culture was obtained by single spore isolation (Choi et al. 1999). A drop of sterile distilled water was placed on a flame-sterilized slide and the sporulating fungal mass was aseptically transferred into the water and teased with flame-sterilised needle in order to obtain a spore suspension. The suspension was spread onto malt extract agar (MEA) plates with antibiotics incorporated (20 mg/L each streptomycin and penicillin). The developing colonies, arising from individual conidia, were aseptically transferred onto fresh plates. After confirming the identity of the culture, molecular sequencing was done at Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram, Kerala, India.

DNA isolation and PCR analysis

Fresh fungal mycelia (20 mg) were scraped from the growing culture incubated at 28°C for 7 days. DNA isolation and PCR analysis was done according to Prabhugaonkar & Bhat (2011). The

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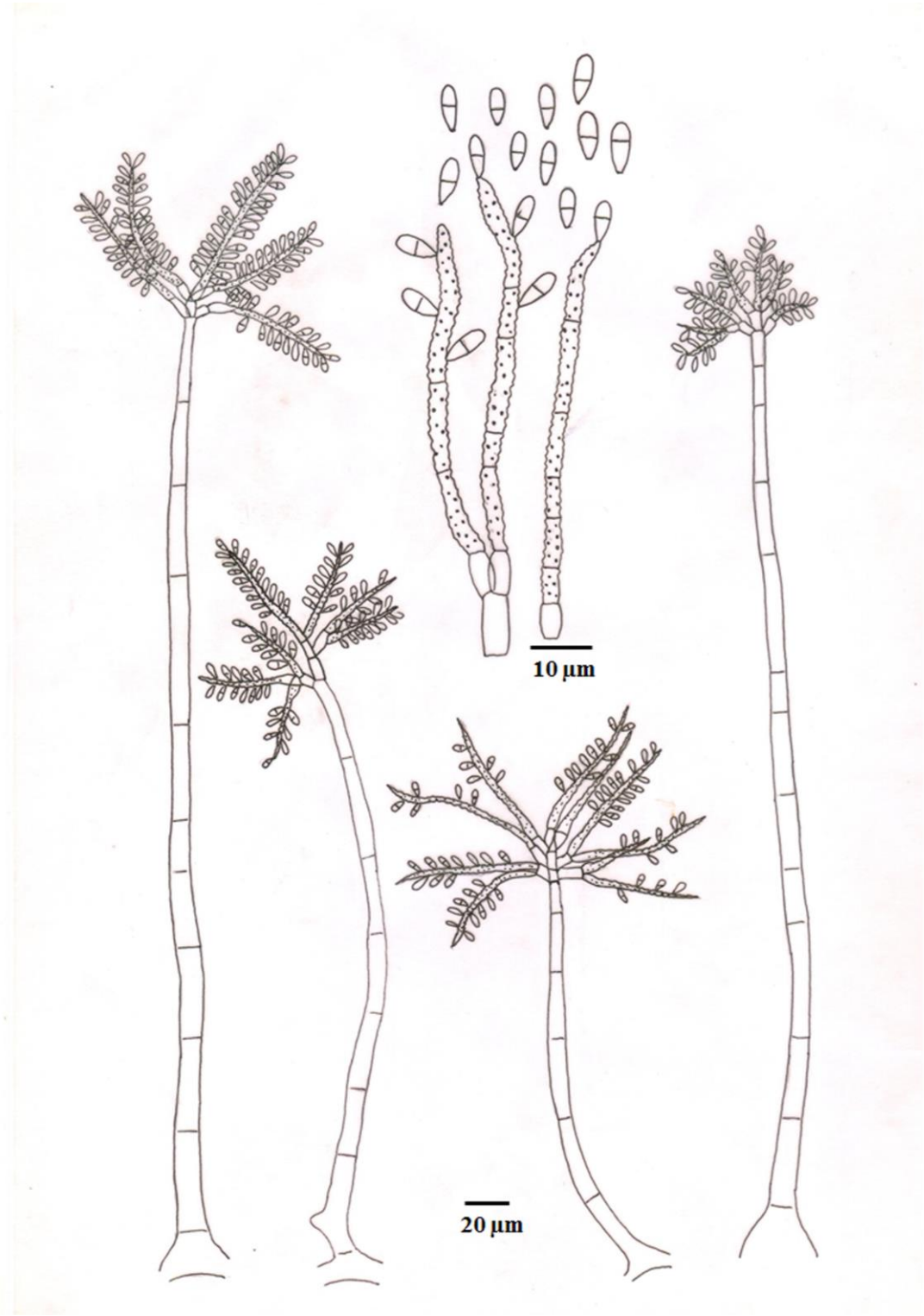

Fig. 1 – *Thysanorea papuana*, conidiophores, conidiogenous cells and conidia.

5.8S nuclear ribosomal gene with the two flanking internal transcribed spacers (ITS) and 28S nrDNA sequence (LSU) genes were amplified and sequenced using the primer pairs ITS-1F + ITS-4R (White et al. 1990) and LR5 + LROR (Crous et al. 2009), respectively. The sequence quality was checked using Sequence Scanner Software v1 (Applied Biosystems). Sequence alignment and required editing of the obtained sequences were carried out using Geneious Pro v5.1 (Drummond et al. 2010).

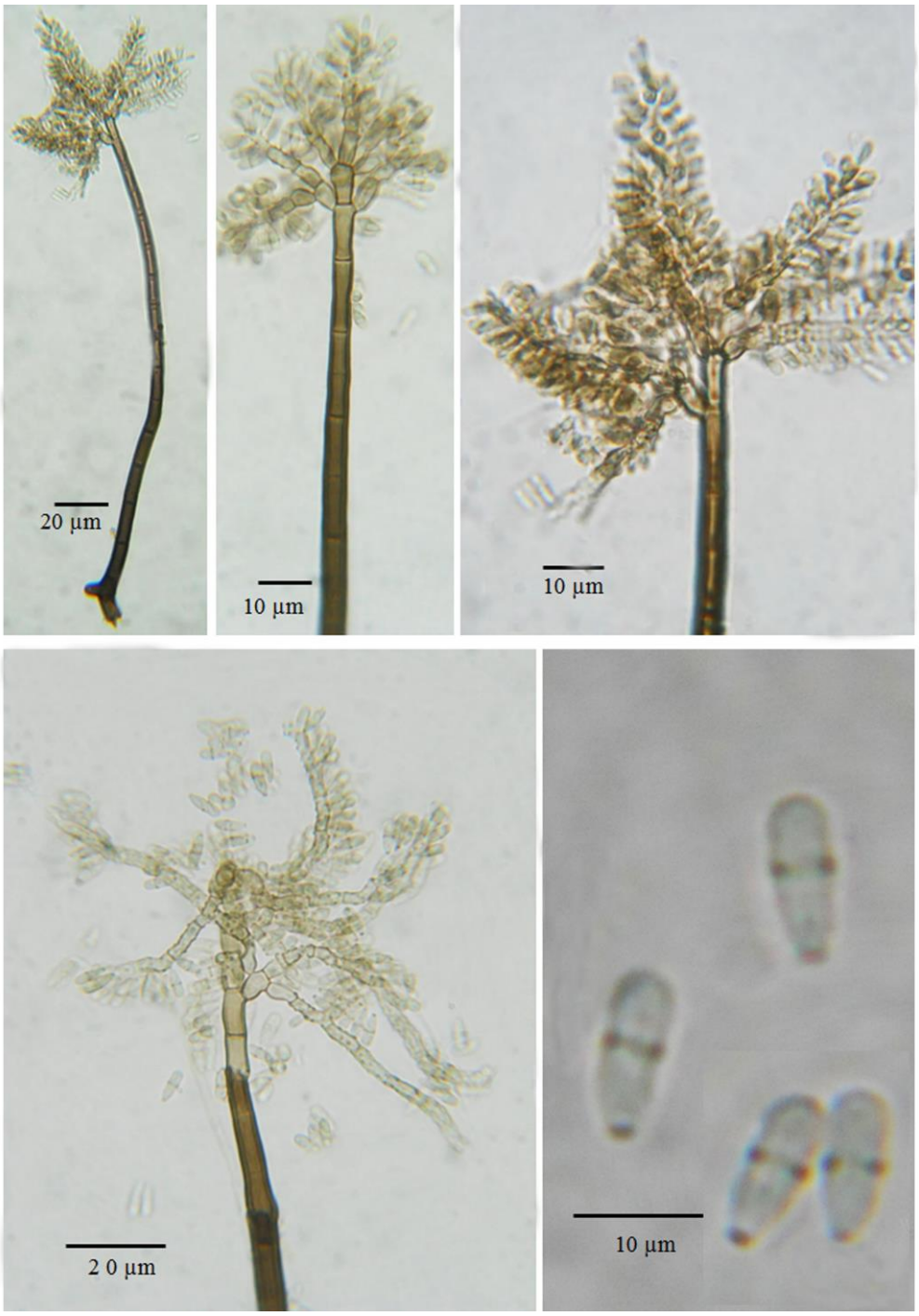

Fig. 2 – *Thysanorea papuana*, conidiophores, conidiogenous cells and conidia.

Sequence alignment and phylogenetic analysis

The sequences were blasted in GenBank with Blastn. LSU and ITS data sets were analysed. Based on the blasts, further related sequences were assembled for each fungus. The combined data matrix was aligned using MAFFT v.7 (http://mafft.cbrc.jp/alignment/software) and manually adjusted using MEGA 6.06 to allow maximum alignment and maximum sequence similarity. A phylogenetic analysis was conducted using maximum likelihood (ML) in MEGA 6.06 (Kumar et al. 2008) with 1000 bootstrap replicates. The most suitable substitution models for the respective datasets were selected by using MEGA6.06. Tamura 3-parameter model with Gamma distribution was used in analysis. Gaps were treated as a pairwise deletion and trees were viewed with MEGA6.06. All newly generated ITS and LSU sequences used in this study are deposited in GenBank.

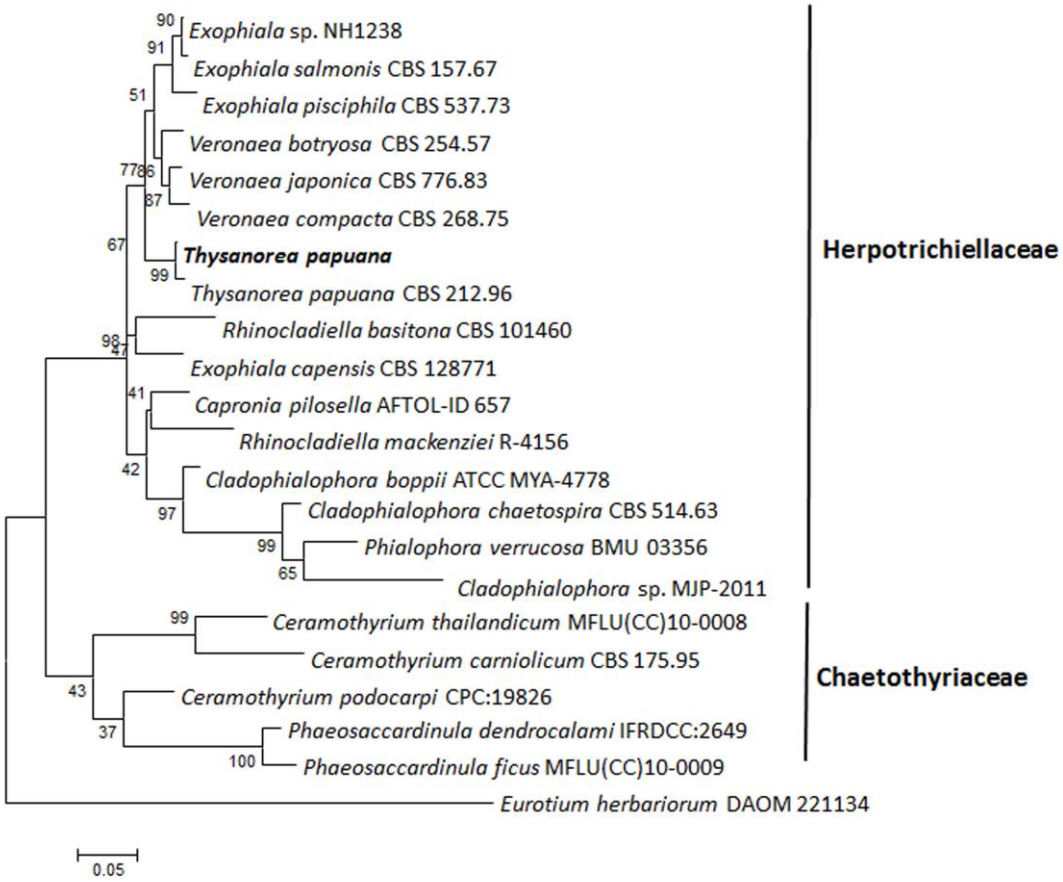

Fig. 3 – Maximum likelihood tree of *Thysanorea papuana* and related taxa based on combined analysis of ITS and LSU sequences. Species described in this study is in bold.

Results

Taxonomy

Thysanorea papuana (Aptroot) Arzanlou, W. Gams & Crous, in Arzanlou, Groenewald, Gams, Braun, Shin & Crous, *Stud. Mycol.* 58: 80 (2007)

Colonies on MEA effuse, flat, dark brown, reverse dark brown, with irregular margin, attaining 1 cm diam. in 7 days at 28°C. *Colonies* on natural substrate effuse, dark brown to black, hairy. *Mycelium* partly immersed, partly superficial, composed of light brown, smooth, branched, thin-walled, 1.5–2 µm wide hyphae. *Conidiophores* macronematous, mononematous, thick-walled, septate, dark brown, smooth, 220 – 490 × 4–10 µm, apically branched, forming a head with up to 3 levels of 30–50 µm long branchlets. *Conidiogenous cells* terminal and intercalary, thin-walled, smooth, brown at the base, paler towards the apex, 8–20 × 2–4 µm, denticulate; denticles numerous, thickened, lightly pigmented, about 1 µm diam. *Conidia* solitary, thin-walled, smooth, pale brown, obovoid, always 1-septate, septa darkened and slightly raised at sides, 6–9 × 2–3 µm, with a truncate base and darkened hilum, 1 µm diam.

Specimen examined – India, Goa, Valpoi on unidentified dead twig, J. Pratibha, 26 Jan 2013, Herb. No. VTL-14. (Fig. 1, Fig. 2)

Notes – Arzanlou et al. (2007) introduced the genus *Thysanorea* to accommodate *Periconiella papuana* based on partial sequences of the 28S (LSU) rRNA gene and the ITS region (ITS1, 5.8S rDNA and ITS2) and morphological characters like complex head consisting of up to six levels of branches and having dimorphic conidiophores with more or less prominent denticle-like conidiogenous loci. This fungus was collected in current study from terrestrial litter samples.

Phylogenetic analyses

Twenty-two taxa are included in the phylogenetic analysis (Table 1, Fig. 3). Preliminary phylogenetic analysis showed that the fungus has affinities with the genus *Thysanorea*. *Thysanorea* was introduced to accommodate *Periconiella papuana* (Herpotrichiellaceae), which is unrelated to *P. velutina*, the type species of *Periconiella* (Mycosphaerellaceae) (Arzanlou et al. 2007). Thus, a dataset of two families Herpotrichiellaceae and Chaetothyriaceae from order Chaetothyriales was assembled. *Eurotium herbariorum* (Eurotiales) was selected as the outgroup taxon. Molecularphylogeny showed that the Indian isolate is similar to the type species *Thysanorea papuana* with 99% bootstrap support, thus confirming its identity. This forms an interesting new record to India of a fungal genus originally reported from Papua New Guinea (Arzanlou et al. 2007).

Table 1 Origin of DNA sequences used in combined analyses of ITS and LSU. Newly deposited sequences are in bold

Taxon	Accession no.	ITS	LSU
Capronia pilosella	AFTOL-ID 657	DQ826737	DQ823099
Ceramothyrium carniolicum	CBS 175.95	KC978733	KC455251
Ceramothyrium podocarpi	CPC:19826	KC455251	KC005795
Ceramothyrium thailandicum	MFLU(CC)10-0008	HQ895838	HQ895835
Cladophialophora boppii	ATCC MYA-4778	JN882312	JN874491
Cladophialophora chaetospira	CBS 514.63	KF928449	KF928513
Cladophialophora sp.	MJP-2011	JF263533	JF263534
Eurotium herbariorum	DAOM 221134	JN942870	JN938918
Exophiala capensis	CBS 128771	JF499841	JF499861
Exophiala pisciphila	CBS 537.73	AF050272	AF361052
Exophiala salmonis	CBS 157.67	JF747137	AY213702
Exophiala sp.	NH1238	AB488490	AB488490
Phaeosaccardinula dendrocalami	IFRDCC:2649	KF667242	KF667245
Phaeosaccardinula ficus	MFLU(CC)10-0009	HQ895840	HQ895837
Phialophora verrucosa	BMU 03356	KF881928	KJ930100
Rhinocladiella basitona	CBS 101460	EU041806	EU041863
Rhinocladiella mackenziei	R-4156	FJ427211	FJ427212
Thysanorea papuana	**GUFCC 18020**	**KR259881**	**KR259882**
Thysanorea papuana	CBS 212.96	EU041814	EU041871
Veronaea botryosa	CBS 254.57	EU041816	EU041873
Veronaea compacta	CBS 268.75	EU041819	EU041876
Veronaea japonica	CBS 776.83	EU041818	EU041875

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