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SHORT COMMUNICATION

A new record of *Fulvifomes fastuosus* from Sri Lanka

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Abstract: The fungus *Fulvifomes fastuosus* has not been described from Sri Lanka to date. Morphological studies and DNA analysis were carried out to characterize the fungus. Accordingly, *F. fastuosus* produces perennial, pileate, solitary and hard basidiomes with a concentrically sulcate pileal surface and homogenous context. It consists of a dimitic hyphal system and yellowish brown acyanophilous basidiospores but lacks hymenial setae. Eventhough the same species has been recorded in China with similar characters, the size of spores and hyphae are comparatively less in the specimen described in the present study. The identity was confirmed by sequencing the internal transcribed spacer (ITS) region in the nuclear ribosomal repeat unit, using the primers ITS1F and ITS4B. The resultant DNA sequence showed the highest similarity with *Fulvifomes fastuosus* strain CBS 213.36, reported from South Korea.

Keywords: Basidiospores, dimitic, *Fulvifomes*, Hymenochaetaceae.

INTRODUCTION

The fungus *Fulvifomes* Murrill belongs to the family Hymenochaetaceae, which is considered as an important family in the phylum Basidiomycota as most species in this family are of medicinal value, while some are plant pathogens causing white rot (Dai, 2010). According to recent phylogenetic studies it has been found that the genus *Fulvifomes* is closely related to *Aurificaria* D.A. Reid and *Phylloporia* Murrill. Earlier this was not treated as an independent genus but a subgenus of *Phellinus* Quél (Zhou & Zhang, 2012). The distinctive morphological characters of *Fulvifomes* species are the dimitic hyphal system and coloured basidiospores (Zhou & Zhang, 2012). Sri Lanka being a country with a hot and humid climate harbours a rich diversity of macrofungal species, most of which have not yet been surveyed and identified. During a study on the diversity of Dry Zone macrofungi in Sri Lanka, a *Fulvifomes* sp. was discovered. The morphological characters and a molecular study showed that the species is *F. fastuosus* (Lév.) Bondartseva & S Herrera. The paper describes and illustrates the morphological and molecular biological characters of *F. fastuosus* from Sri Lanka.

METHODS AND MATERIALS

The specimens for this study were collected from a wooded area in the Institute of Fundamental Studies (IFS) Sam Popham Arboretum, Dambulla, Sri Lanka. A specimen was deposited at the herbarium of the Department of Plant Sciences, University of Colombo (UOC:DAMIA:D27b). Sections taken from the specimens were studied at magnifications up to ×1000 using an Olympus CX21FS1 microscope and phase contrast illumination. Photographs of microscopic observations were taken using a Canon, Power Shot A2600 camera. The special colour terms used follow Jordan (2004).

The genomic DNA was extracted from the fruit body using the PowerSoil® DNA Isolation Kit (MO BIO Laboratories, Inc.) according to the manufacturer's protocol. The ITS region was amplified using primers ITS1F (CTT GGT CAT TTA GAG GAA GTA A) and ITS4B (CAG GAG ACT TGT ACA CGG TCC AG) (Gardes & Bruns, 1993) in HOT FIREPol® Blend Master Mix Ready to Load (Solis BioDyne) PCR mix and Veriti 96 well Thermal Cycler (Applied Biosystems). The thermal cycler programme was as follows: initial denaturation at 95 ^{0}C for 12 mins, followed by 13 amplification cycles at 95 ^{0}C for 35 s (denaturation), at 55 ^{0}C for 55 s (annealing) and at 72 ^{0}C for 45 s (extension). Thereafter the extension time was increased up to 120 s for 14th - 26th cycle and up to 180 s for 27th

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- 35th cycle. The final extension was performed at 72 ^{0}C for 10 mins (Gardes & Bruns, 1993). The Amplified DNA was separated by electrophoresis in 1 % agarose gel and the excised fragments were purified using GFX™ PCR DNA and Gel Band Purification kit (GE Healthcare, UK) according to the manufacturer's instructions. The purified amplicons were bidirectionally sequenced using ITS1F or ITS4B primer, Big Dye Terminator v 3.1 cycle sequencing kit and 3500 DX Genetic Analyzer (Applied Biosystems) according to the manufacturer's instructions. The resultant sequences were analyzed and submitted to GenBank database (Genbank Accession No.: KJ206286). The sequence was searched over the GenBank database using BLAST at NCBI.

RESULTS

The fungus *Fulvifomes fastuosus* was grown on angiosperm wood from the IFS Sam Popham Arboretum Dambulla, Sri Lanka. The morphological characters of the fungus was as follows;

Basidiome (Figure 1): The basidiome is perennial, pileate, broadly attached, solitary, woody hard and without odour when dry. The Pilei are applanate, projecting up to 15 cm, 13.5 cm wide and 2 cm thick at the base. Pileal surface is dark brown when dry, concentrically sulcate with wide zones and the pilei margin is velvety, obtuse and rust brown. The pore surface is umber brown; margin narrow (1 mm), chestnut-brown; pores circular, 8 – 9 per mm, context chestnut-brown, concolourous with tubes, woody hard and up to 1 – 2 mm thick. Tubes are paler than pores, woody hard, up to 1.5 cm long, tube layers distinct, annual layer 1 – 2 mm in thickness.

Hyphal structure: The hyphal system is dimitic; generative hyphae simple, septate; tissues darkening in 5 % KOH.

Context: Generative hyphae are yellowish, thin walled, simple septate, negative in Melzer's reagent (IKI^-), acyanophilous (CB^-), 2.5 – 3.8 µm in diameter (n = 10/1); skeletal hyphae (Figure 2) dominant, brown, thick walled with wide lumen, unbranched, negative in Melzer's reagent, acyanophilous, 3.8 – 5.0 µm in diameter (n = 10/1) (n = number of spores, hypae measured from given number of specimans).

Tubes: Generative hyphae are thin walled, simple, septate, negative in Melzer's reagent, acyanophilous, 1.9 – 2.5 µm in diameter (n = 10/1); skeletal hyphae (Figure 3) dominant, brown, thick walled with narrow

Figure 1: Basidiocarp of *Fulvifomes fastuosus* (holotype)

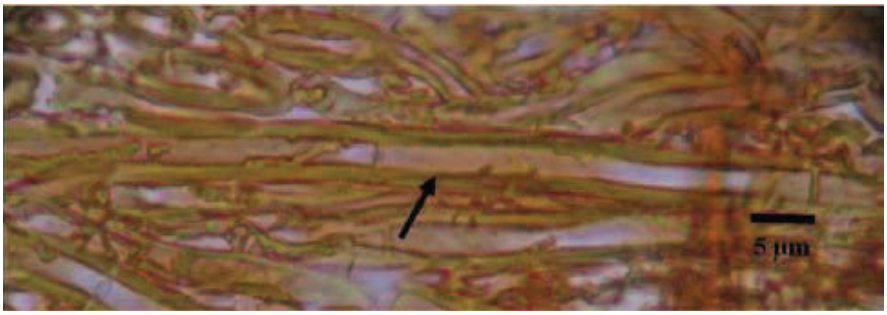

Figure 2: Skeletal hyphae from context (microscopic observation from holotype)

lumen, unbranched, negative in Melzer's reagent, acyanophilous, 2.5 – 3.1 µm in diameter (n = 10/1). Setae absent. Hymenium collapsed, basidia and basidioles not seen. (n = number of spores, hypae measured from given number of specimans)

Spores: Basidiospores subglobose, thick walled, yellowish brown, smooth, negative in Melzer's reagent, acyanophilous, mean spore length, L (arithmetic average of all spores) = 4.49 µm, mean spore width, W (arithmetic average of all spores) = 4.01 µm, variation in the L/W ratios within the specimen studied = 1.12 (n = 20/1) (n = number of spores, hypae measured from given number of specimans) (Figure 4).

DNA analysis: Searching the resultant sequence over the GenBank database using BLAST programme showed the highest similarity with *Fulvifomes fastuosus* strain CBS 213.36 (accession No.: AY558615.1), with a max score of 1238, 98 % identity and an E value of 0.0.

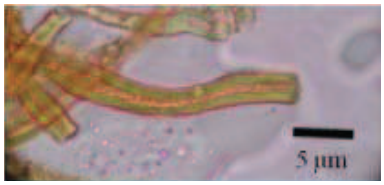

Figure 3: Skeletal hyphae from tubes (microscopic observation from holotype)

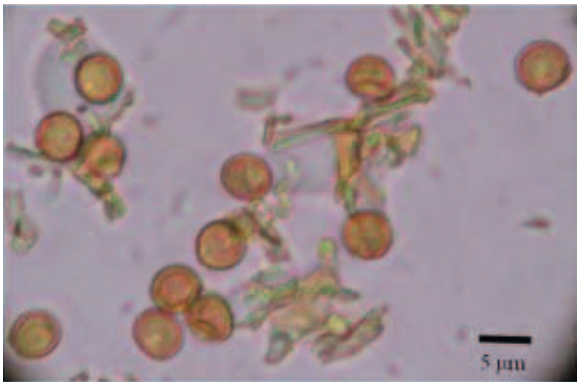

Figure 4: Basidiospores of *F. fastuosus* (microscopic observation from holotype)

DISCUSSION

The morphology and DNA analysis confirm that the fungus is *Fulvifomes fastuosus*. A specimen collected from Peradeniya and identified as *Fomes endotheius* (Berk.) Cooko (Petch & Bisby, 1950) is a heterotypic synonym of *F. fastuosus*.

According to Dai (2010), species in the genus *Fulvifomes* are somewhat heterogeneous in certain characters such as the presence or absence of setae and spores being cyanophilous (CB^+) or acyanophilous (CB^-). Yet all the species are united by their yellowish brown basidiospores. The species studied in this investigation do not have setae. The basidiospores are CB^-, which is in agreement with the characters recorded for the same species by Dai (2010). However, the sizes of basidiospores and hyphae recorded in this study are much less than the ones reported previously. For basidiospores, the mean spore length (L) and the mean spore width (W) recorded by Dai (2010) were, 5.43 and 4.85 µm, respectively whereas in the present study it was 4.49 and 4.01 µm, respectively. In both studies the variation in the L/W ratios within the specimen studied (Q) is similar. The difference could be due to the two specimens recorded being from different geographical areas.

According to literature *F. macgregorii* (Bres.) Y.C. Dai is another species, which is similar to *F. fastuosus*, also lacking setae but with relatively large pores (4 - 5 per mm) (Dai, 2010). *F. cambodiensis* Zhou and Zhang, which was described and illustrated recently from Preah Vihear, Cambodia (Zhou & Zhang, 2012), vary from the specimen examined in the present study due to the presence of hymenial setae.

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