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Truncospora ochroleuca (Polyporales) from North East India based on Scanning Electron Microscopy and Molecular Data Analysis

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ABSTRACT

This study reports the ultrastructural study of basidiocarp and molecular base identification of a poroid wood-inhabiting fungus, *Truncospora ochroleuca* from Meghalaya, North East India. Scanning Electron Micrographs of LS and VS of the fruiting body provided a much clear three-dimensional image and stereoscopic view of the internal structures of the fruiting body. The fungal tissue was considered for extraction of genomic nuclear DNA and was amplified by two primers, ITS1F and ITS4R. Based on nucleotide homology and phylogenetic analysis, the studied fungus is identified as *T. ochroleuca*. This research work is the first to report *T. ochroleuca* from Meghalaya, India.

Keywords: Context layer, ITS, Molecular Barcode, SEM, Truncated spores, Tube layer.

INTRODUCTION

The diversity of the wood rotting fungi in Meghalaya had been investigated by Lyngdoh *et al*., (2014), Sailo *et al.,* (2019), and Chuzho *et al*., (2023). They reported 42 species wood rotting fungi. During our investigations on wood-inhabiting fungi in East Khasi Hills, Meghalaya, an unreported species of wood rotting fungi was found. Identification of the fungal specimen was carried out by compiling morphological study data and phylogenetic analysis (based on ITS sequences) data.

The genus *Truncospora*Pilát (1953) is characterized by relatively small, pileate basidiomata (about 1.5–3 cm long, 2.5–3.5 cm wide, and 1–4 cm thick), non-dextrinoid to dextrinoid skeletal hyphae, and truncate, strongly dextrinoid basidiospores (Pilát, 1953; Corner, 1989;Decock, 2011; Zhao and Cui, 2013;Spirin*et al.*,2015). The type species of *Truncospora* is *T. ochroleuca* (Berk.) Pilát.

The genus *Truncospora* has been often treated as a synonym of *Perenniporia* (Ryvarden, 1972, 1991; Ryvarden and Melo, 2014).*Perenniporia detrita* (Berk.) Ryvarden, *P. ochroleuca* (Berk.) Pilát and *P. ohiensis* (Berk.) Ryvarden formed a morphologically homogeneous alliance that should be recognized at the genus level for which the name *Truncospora* was available (Decock and Ryvarden 1999). These taxa differ from *Perenniporia* by having relatively small, pileate basidiomata, and ellipsoid, large, apically truncate and dextrinoid basidiospores. Molecular phylogenetic analysis based on the IST+LSU rDNA gene regions supported *Truncospora*as monophyletic and places the genus as a clade distinct from the *Perenniporia* sensu stricto clade (Zhao and Cui, 2013).

Truncospora ochroleuca is a polypore that grows out from wood. In vertical cross-section, it is roughly triangular with a horizontal lower side, sloping upper side and the third side is vertical and flush against the wood. The upper surface is smooth, lacking even the velvety hairiness of many polypores. It may be creamy or yellowish- to orange-brown and usually is concentrically banding in different shades and it is also likely that there is concentric and radial ridging. The creamy lower surface is composed of numerous pores (2-4 per millimetre). The fruit body may extend to 5 or more centimetres out from the wood and may be up to 2 centimetres thick at the wood surface. It has a firm texture.

T. ochroleuca is a rare species in East Khasi Hills, Meghalaya and are therefore, very uncommon and scarce. This research work is the first to report *T. ochroleuca* from Meghalaya, India.

METHODS AND MATERIALS

Field work and Morphological studies

The specimens of the rare wood-rotting fungus (bearing catalogue no.ZLS-SHG15-01, ZLS-SHG15-02 &ZLS-SHG15-03) were collected fromdead fallen branches of *Picea*sp.tree in East Khasi Hills of Meghalaya and preserved at the Department of Microbiology, Shillong College. All

the morphological data including the size, shape, odour, colour of the basidiocarps and spores were considered (Atri *et al.;* 2005, Largent, 1977). Spores were measured from sections cut from the tubes.

Optical Microscopy of Spores

Freshly collected spores were observed directly (without staining) as well as after staining with Lactophenol cotton blue. Spores were studied at magnification up to × 400 using a compound microscope (Olympus CH20i, Olympus Corporation, Japan). The staining of the spores with acidic Lactophenol Cotton blue was classified into two types of reactions as follows: positive cyanophilic reaction (a dark blue staining of the spores) and negative cyanophilic reaction (where the spores do not change color) [Kuo, 2006].

Scanning Electron Microscopy of Fruiting Body

LS and VS of the internal layer of the fruiting bodies were prepared and mounted on aluminium stubs using double-sided adhesive tape and thereafter covered with a fine layer of gold by sputtering in a vacuum sputterer (JFC-1100, JEOL, Japan) for 30 minutes. The samples were then observed in a Scanning Electron Microscope (JSM-6360 JEOL, Japan) at an accelerating voltage of 20 kV. The descriptions and measurement were based on the guidelines given by Brundrett *et al*., 1996.

DNA Isolation and Sequencing

The internal structures of the fruiting body (compact mycelial mass that constitutes the tube layer and context) were considered for the extraction of genomic nuclear DNA using AllPrep® Fungal DNA/RNA/Protein Kit (Qiagen, Germany) and were amplified by two primers ITS1F (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4R (5'-TCCTCCGCTTATTGATATGC-3') [White *et al.* 1990]. The quality of the DNA was evaluated on a 1.0% Agarose Gel and a single discrete PCR amplicon band of high-molecular-weight DNA was observed. Amplification was carried out under the following parameters: initial denaturation at 95° C for 5 min; 35cycles of denaturation at 95° C for 1min, annealing at 52° C for 30 s, extension at 72° C for 1 min; and a final extension at 72° C for 10 min. The PCR amplicon was purified by column purification to remove contaminants. DNA sequencing reaction of PCR amplicon was carried out using BDT v3.1 Cycle Sequencing Kit on ABI 3500xl Genetic Analyzer (Genexplore, India).

Phylogenetic Analysis

The ITS sequence of the fungal specimen was deposited to NCBI and PQ098043 is the assigned accession number. A searched for its appropriate homologous hits was done using BLASTn in NCBI database. The analogous sequences which showed close proximity to the query sequence were considered as a hit. Neighbour - joining (NJ) was used to analyze the evolutionary distance matrix between the query sequence and its homologous hits. NJ tree was computed using MEGA 11 software (Tamura *et al.,* 2021).On the basis of the maximum identity score, the first ten sequences were selected and aligned using multiple sequence alignment software programs. The phylogenetic tree generated with the amplified sequence of the Internal Transcribed Spacer (ITS) regions of the specimens of the studied wood rotting fungus of Meghalaya is shown in **Figure 1**.

RESULTS

Phylogenetic Analysis

Molecular phylogenetic analysis of the ITS sequences indicated that the studied fungus is closely related to, but distinct from the *T. macrospora* clade. *T. macrospora* was the sister group to the studied fungal species.

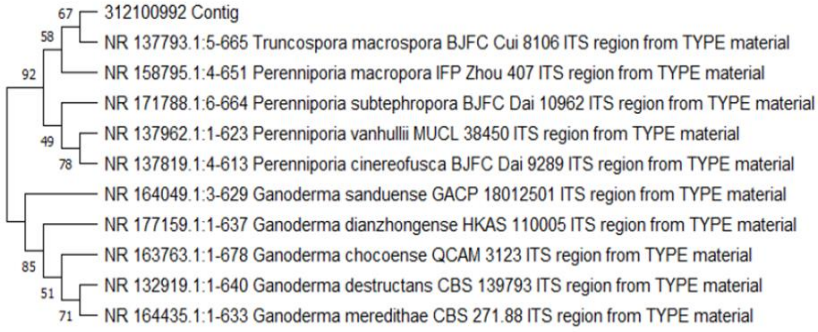


Figure 1: Phylogenetic Tree - Molecular Phylogenetic analysis by Neighbor-Joining method (Note: 312100992 Contig = *Truncospora ochroleuca* ZLS-SHG ITS region)

Scanning Electron Microscopy

Scanning Electron Micrographs of LS and TS of the fruiting body, pores, pore tubes and spores provided a much clearer three-dimensional image and stereoscopic view of the internal structures of the fruiting body. Size and shape of the pores and pore tube; pore tubes that are empty and those that are filled with interconnected hyphal filaments; compact mycelial mass that constitutes the pores tube wall; size, shape and ornamentation on the spore surface, the hilar appendix (point of attachment to the basidium before separation) and the spore apex (opposite end to the hilar appendix) were observed clearly (Figure 2 (a f).

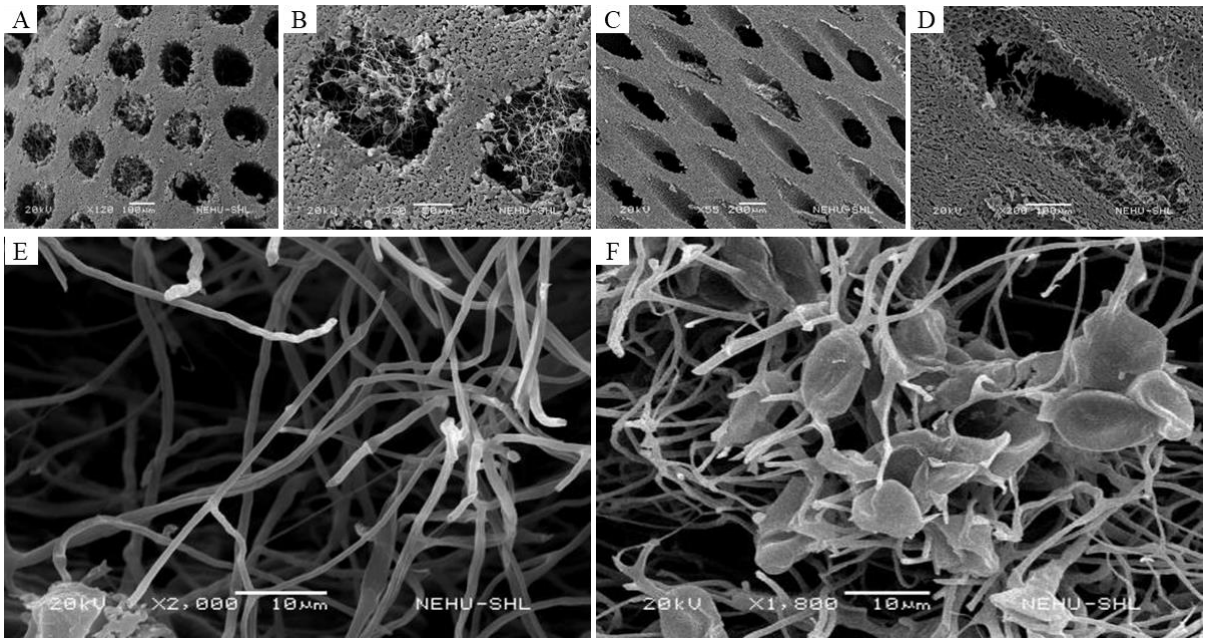


Figure 2: A and **B,** SEM Image of Basidiocarp (VS) showing pores; **C** and **D,** SEM Image of Basidiocarp (LS) showing pore tubes; **E** and **F,** SEM Image of Interwoven mycelium and basidiospores inside the pore tubes.

TAXONOMY

Truncospora ochroleuca (Berk.) Pilát, Sb. Nár. Mus. v Praze, Rada B, Prír. Vedy 9(2): 108(1953)

Classification: *Polyporaceae, Polyporales, Agaricomycetes, Basidiomycota, Fungi*

Materials Examined: INDIA, MEGHALAYA: East Khasi Hills District, Shillong, Nongrim Hills, on dead fallen branches of *Picea sp.* tree, 15 Dec 2024, 25°34′8”N 91°53′56”E, leg. Zoliansanga, ZLS-SHG15-01; *ibid.*, Nongrim Hills, on dead fallen branches of *Picea sp*. tree, 15 Dec 2024, 25°34′49” N 91°54′27”E, leg. Zoliansanga, ZLS-SHG15-02; *ibid.*, Nongrim Hills, on dead fallen branches of *Picea sp*. tree, 15Dec 2024, N 25°34′4”N 91°53′27” E, leg. Zoliansanga, ZLS-SHG15-03.

Macromorphological characters (Figure 3 and **4):** *Habitat* - Appear solitarily on dead fallenbranches of *Picea* sp. tree. *Colour*- Cream to ochraceous pileus. *Odour* - Distinctive. *Texture*- Corky to wood corky upon drying.

Fruiting body- Basidiocarps annual, pileate, sessile, solitary corky to wood corky upon drying. *Cap* - Pileus usually semicircular, projecting up to 1.5 - 2.0 cm, 2.2 -3.5 cm wide, and 0.5 - 1.5 cm thick at base. *Pileal surface* - cream white to cream buff at young, with a distinct dark brownish crust, glabrous. Sterile margin narrow, up to 1 mm wide.

Context - distinctly thinner than the tube layer. *Pores* - Pores regular, more or less round, 3 – 4 per mm; dissepiments thick, entire. *Pore tubes* - woody hard, 200 - 240 µm in diameter, up to 2 - 4.5 mm depth. *Basidiospores* - Hyaline, ellipsoid, truncate, thick-walled, rough surface with longitudinal ridges from anterior to posterior end, strongly cyanophilous, size ranges between 13.5 – 16.0 × 7.0 – 8.5µm. *Stem* - Basidiocarp has no stem and is directly attached to the dead wood surface. Therefore, they are sessile. *Hyphal structure* - Hyphal system dimitic; generative hyphae with clamp connections; skeletal hyphae cyanophilous, branched and interwoven.

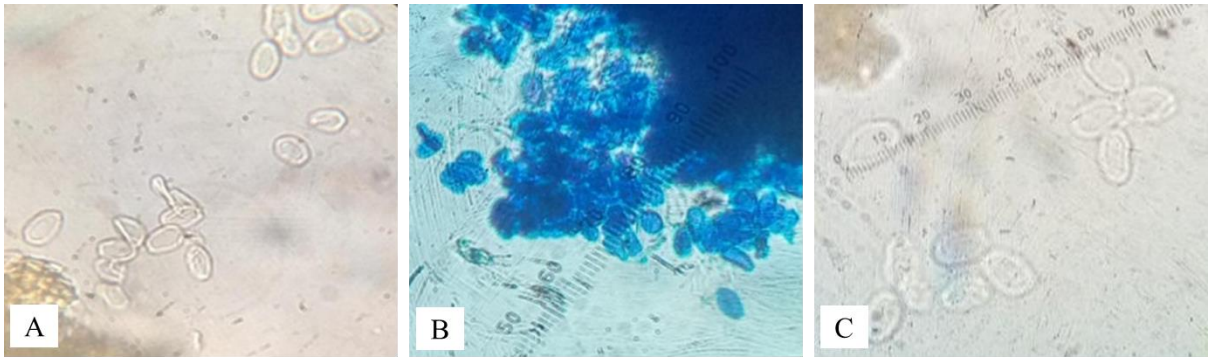


Figure 3: A, *Truncospora ochroleuca* basidiospores (no staining) under 40X; **B,** *T. ochroleuca* basidiospores (stained with Lactophenol) under 40X; **C,** *T. ochroleuca* basidiospores (no staining) under 100X. The space between two bars is equal to 1.0µm.



Figure 4: **A-C,** *Truncospora ochroleuca* in its natural habitat: ZLS-SHG15-01, ZLS-SHG15-02 & ZLS-SHG15-03; **D,** Pileus showing pores arrangement; **E,** Pileus showing its measurement; **F,** Pileus depicting the number of pores per mm; **G,** Transverse section of Basidiocarp; **H,** Lateral section of Basidiocarp (*Scale bars: Distance between two vertical bars = 1 mm.*)

CONCLUSION

Both morphology and rDNA data confirmed that the specimens of the fungus studied were *Truncospora ochroleuca*. The fruit-body of the fungal specimens are similar to *T. ochroleuca* by being pileate, annual, pileus up to 3-5 cm wide, 5-6 cm long and 1 cm thick at the base, soft when fresh, rigid and hard when dry, upper surface ochraceous to pale brown, slightly zonate, glabrous, margin sharp and deflexed in dry specimens, pore surface ochraceous to pale leather coloured, pores round, 3-4 pores per mm, pore tubes up to 5 mm deep, pale ochraceous;however, basidiospores are smaller (13.5–16.0 × 7.0–8.5 µm).

Utilizing molecular markers such as the ITS fungal DNA barcode and its corresponding phylogenetic analyses is crucial in accurately identifying wood rotting fungal species. In the present study, molecular characterization based on ITS was preferred to other loci molecular characterization since the ITS region relatively serve as the most reliable barcode for identifying wild mushrooms as the species identity generated by the ITS marker matched more accurately with the morphological characteristics / appearance of the specimens. 18S rRNA gene have been used for fungal identification. However, the 18S rRNA gene in fungi has very few hypervariable domains, which could limit its effectiveness in proper resolution of fungi identity (Khaund and Joshi, 2014)

T. ochroleuca may be confused with *T. macrospora* by sharing pileate basidiocarps, similar pore size (2–4 per mm), dextrinoid skeletal hyphae and ellipsoid, truncate, strongly dextrinoid basidiospores; however, *T. ochroleuca* is distinguished from *T. macrospora* by having cream to ochraceous pileus, and smaller basidiospores (14.0–17.0 × 7.0–9.5 µm). [Decock, 2011]

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