

Buchwaldoboletus xylophilus, a boletoid fungus new to India

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Abstract

Buchwaldoboletus xylophilus, a saprobic, non-ectomycorrhizal bolete is reported for the first time from Kerala State, India. The fungus was collected from soil around *Bambusa bambos*. Morphological and molecular characters were employed for taxonomic identification. Comprehensive description, maximum likelihood tree generated using nrITS sequence data and photographs are presented. This forms the first report of the species from India.

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INTRODUCTION

Boletoid members are generally ectomycorrhizal, found associated with members of the plant families, Betulaceae, Casuarinaceae, Dipterocarpaceae, Ericaceae, Fabaceae, Mimosaceae, Myrtaceae, Pinaceae and Salicaceae^[1-4]. Members of the boletoid genus *Buchwaldoboletus* are known to be lignicolous^[4]. The genus was erected by Pilát in 1969, with *B. lignicola* as the type species, and was considered in the family Boletaceae^[5]. *Buchwaldoboletus* is presently included in the subfamily Chalciporoidae of Boletaceae based on molecular studies^[6,7]. The genus has a worldwide distribution, with 12 described species to date^[8-10]. Members of the genus are characterized by lignicolous habit, yellow to brownish basidiomata, turning blue when bruised, small spores, absence of clamp connections, and yellow basal mycelium. Only one species of the genus, *B. parvulus*, has been reported from India so far^[11,12]. *Buchwaldoboletus xylophilus*, originally described from Sri Lanka^[13], and later reported from Malaysia, China and Philippines, has a tropical Asian distribution^[12-15]. However, *B. xylophilus* has not been collected from India so far. We report the occurrence of *B. xylophilus* in India, based on molecular and morphological studies carried out on a specimen collected from Kerala State, India.

MATERIALS AND METHODS

Morphological studies

Fresh basidiomata were collected from Palode, Thiruvananthapuram district, Kerala State, India. Basidiomata were photographed in the field using SONY DSC-HX400V camera, and macroscopic features were noted. Collected specimens were dried using a hot air oven at 60 °C, and were used for microscopic studies. Microscopic examination was carried out using light microscope (Magnus MX211led). Sections were taken from different parts of the dried fruit bodies for microscopic study. One percent congo red and 1% phloxine were used for staining. Excess stain was washed off with 5% KOH. Pigmentation of

the microscopic structures was noted in water. Dextrinoid or amyloid reactions of different tissues in basidioma were observed using Melzer's reagent. Length and width of 20 basidiospores were noted, and Q-value was calculated. Basidiospores were also observed in cotton blue for checking cyanophilic nature. Measurements of basidia and cystidia are presented as length × width. The microscopic photographs taken with Amscope (MU300) microscope camera. The specimens are deposited in the Herbarium of The Zamorin's Guruvayurappan College, Kozhikode, Kerala, India (ZGC).

DNA extraction and PCR amplification

The nrITS gene region was used for phylogenetic analysis. For DNA extraction, REExtract-N-Amp kit by Sigma-Aldrich Company was used. ITS1F and ITS4R were the two primers for the amplification of nrITS gene region^[16]. After PCR amplification, quality check for the samples was carried out by gel electrophoresis (2% agarose gel). Sequencing reaction was set-up in Applied Biosystems™ MiniAmp™ Plus Thermal cycler using Big Dye™ Terminator V3.1 kit. Forward and reverse DNA sequencing reaction of PCR amplicon was carried out with ITS1 and ITS4 primers using BDT v3.1 Cycle sequencing kit on ABI 3730xl Genetic Analyzer.

Phylogenetic analyses of sequence data

The newly retrieved sequences were compared with the other available sequences on GenBank using BLASTN software. The newly generated nrITS sequences of *Buchwaldoboletus xylophilus*, along with those retrieved from blast searches in GenBank were used for the phylogenetic analysis. The sequences were aligned using MAFFT web tool (www.ebi.ac.uk/Tools/msa/mafft/) with default settings. The MAFFT aligned sequences were aligned manually by using MEGA X64^[17]. The nrITS sequence dataset consist of best matched species in BLASTN search and the newly retrieved sequence. The sequences of morphologically or geographically related species were also included in the dataset followed by the recent publications on the phylogeny of *Buchwaldoboletus*^[7,9,15]. The final aligned ITS dataset consisted of 17 sequences, including two sequences from *Gyrodon lividus* (Bull.) Sacc. in the family

Paxillaceae set as the out group taxon^[15]. A phylogenetic tree was constructed using the maximum likelihood (ML) method with 1000 bootstrap replications in MEGA X64^[18].

RESULTS

Molecular phylogeny

Phylogenetic study by using nrITS data showed the identity of the Kerala collection as *Buchwaldoboletus xylophilus* (Fig. 1). BLAST search of the nrITS sequence obtained from the Indian collection showed 99.71% identity with *B. xylophilus* reported from China (GenBank accession no: MW783443). Our specimen cluster together with Chinese collections with 100% ML Bootstrap support in phylogenetic tree.

Taxonomy

Buchwaldoboletus xylophilus (Petch) Both & B. Ortiz, *Bull. Buffalo Soc. Nat. Sci.* 40: 3 (2011) Fig. 2

Index Fungorum number: IF545884; Faces of Fungi number: FoF11793

Basidiomata medium to large sized. Pileus 6–10 cm in diam., convex to applanate; surface yellow to brownish yellow, submentose, dry. Pileal context light yellow to yellow, up to 3 cm thick in mature specimens, staining light blue to bluish black on touch or bruising. Hymenophore adnate to slightly decurrent; pores and tubes concolourous, pale yellow, yellow or olive yellow, staining dark blue when cut or bruised; pores small, 2–3 per mm, oblong, nearly radially arranged; tubes 0.6–1.2 cm long. Stipe 3.5–6 × 0.6–2 cm, central, subcylindrical, slightly broader at bottom, surface yellowish to olive yellow or brownish yellow or reddish brown, nearly glabrous; context concolourous with that of pileus, tinged with brownish yellow colour, staining bluish when cut. Basal mycelium pale yellow to sulphur yellow.

Basidiospores 4.5–5.5 × 3–3.5 μm, globose to subglobose, thin walled, uniguttulate, pale yellow in water. Basidia 13–18 × 5–7 μm, clavate, 4 spored, thin walled, hyaline; sterigmata up to

4.5 μm long. Hymenophoral trama boletoid; hyphae 2.5–10 μm wide, thin walled. Pleurocystidia 17–48 × 6–11 μm, scattered, fusoid ventricose with long beak, thin walled, hyaline. Cheilocystidia 17–30 × 4–6.5 μm, numerous, fusoid with long beak in many, clavate with acute tip in some, thin walled, hyaline. Pileipellis a trichoderm of 100–205 μm wide; outer pellis 2.5–5 μm wide, with interwoven, frequently septate thin to slightly thick walled hyphae; inner layer composed of interwoven thin walled, loosely packed filamentous hyphae, 3–6 μm wide. Pileal trama parallel to interwoven; hyphae 4–15 μm wide, thin to slightly thick walled, hyaline to pale yellow. Stipitipellis a cutis interrupted by caulohyemenium; caulobasidia same as hymenial basidia in size and shape. Stipe trama parallel to interwoven; hyphae 3–8 μm wide, thin walled, hyaline. Caulocystidia 15–25 × 4–7 μm, clavate to broadly clavate, thin walled, pale yellow. Clamp connections absent in all tissues.

Habitat: Solitary, scattered on soil, under *Bambusa bambos* (L.) Voss.

Materials examined: India, Kerala State, Thiruvananthapuram district, Palode, 24 Sep 2021, Salna N., ZGCSN110; 1 Oct 2021, Salna N., ZGCSN111.

DISCUSSION

The distinguishing characters of *B. xylophilus* are the dry to subviscid, minutely velvety, ferruginous-brown pileus, yellowish context, very small pores that change to bluish on bruising, reddish brown stipe with sulphur yellow mycelium at the base, small basidiospores (4.5–5.5 × 3.2–4 μm) and trichodermial type of pileipellis^[12–14]. The species originally described as *Boletus xylophilus* from Sri Lanka^[19]. The holotype was collected from decaying stumps and rotting logs of dicotyledonous trees^[12,19]. Ortiz-Santana & Both^[12] published a preliminary world-wide survey on the genus *Buchwaldoboletus* and grouped 12 species under three stirps with new combinations. *B. xylophilus* included in stirp 1 (*Lignicola* group) along with *B. lignicola* (Kallenb.) Pilát, *B. kivuensis* (Heinem. & Gooss.-Font.)

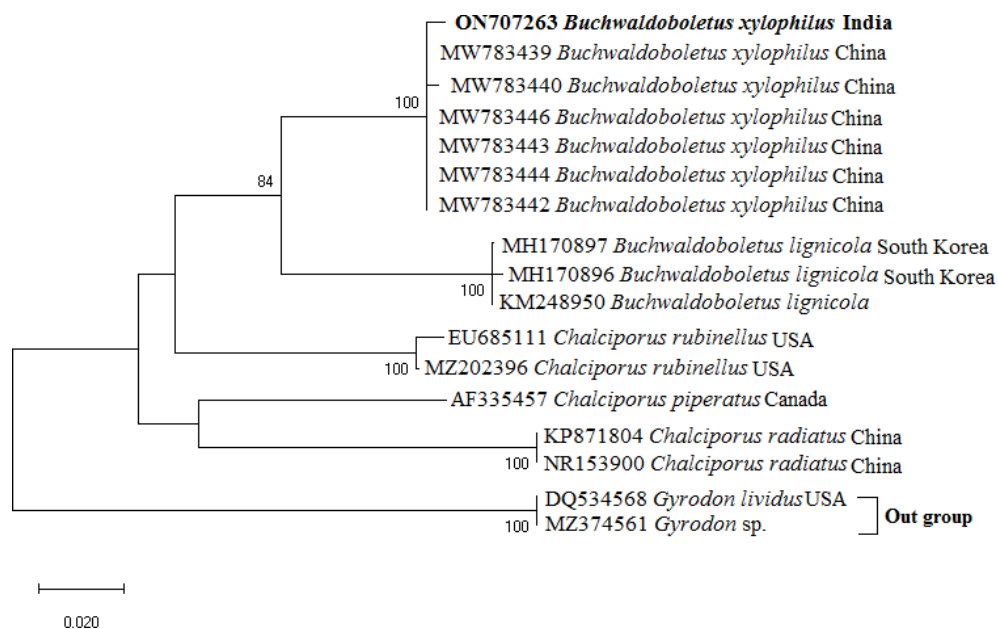


Fig. 1 Maximum likelihood tree generated from ITS sequence data. ML values greater than 50% are indicated above/below branches. The newly generated ITS sequence of the strain from this study is shown in bold letters. GenBank accession numbers of all taxa are shown.

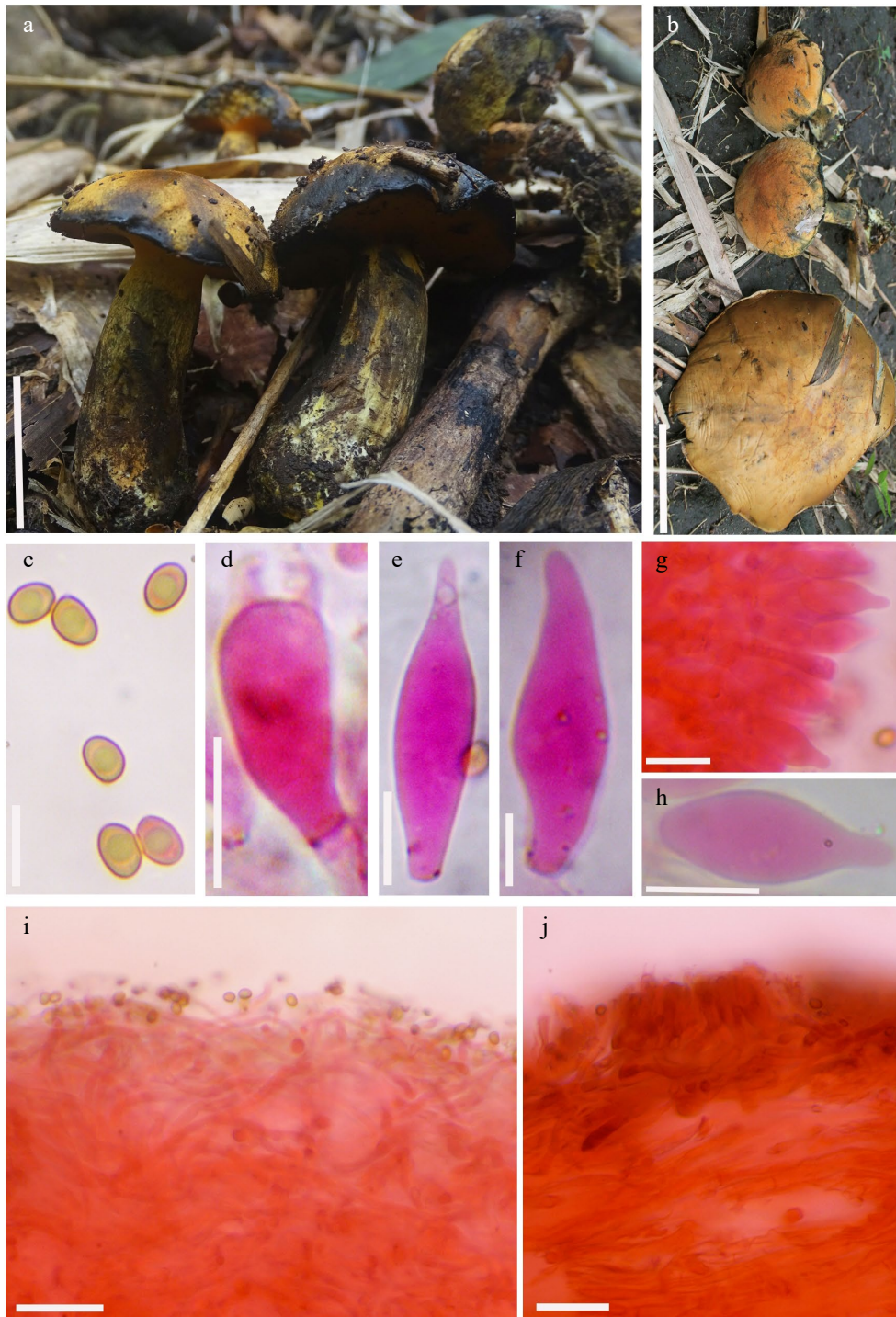


Fig. 2 *Buchwaldoboletus xylophilus*. (a), (b) Basidiomata. (c) Basidiospores. (d) Basidium. (e), (f) Pleurocystidia. (g), (h) Cheilocystidia. (i) Pileipellis. (j) Stipitipellis. Scale bars: (a) = 3 cm, (b) = 5 cm, (c)-(h) = 10 μ m, (i), (j) = 20 μ m.

Both & B. Ortiz, *B. brachyspermus* (Pegler) Both & B. Ortiz and *B. duckeanus* (Singer) Both & B. Ortiz.

B. xylophilus look like *B. lignicola* in yellowish brown pileus and bluing of the pileal context when cut. However, *B. lignicola* have large basidiospores (6–9 (–12) \times 3–4 μ m) and cystidia (29–80 \times 4–9 μ m). *B. brachyspermus*, originally described from Central America as *Pulveroboletus brachyspermus* and it closely resembles *B. xylophilus* except very small pores, russet-colored stipe, and large sized cystidia (40–55 \times 11–14 μ m)^[12]. *B.*

parvulus was described from Karnataka State, India as *Boletus parvulus*. This species were reported in groups from the dead bamboo stumps^[11,12]. *B. xylophilus* shares some similarities with *B. parvulus* such as bluish color change on the pileal context when cut, spore range, size of cystidia and presence of trichodermal pileipellis. But *B. parvulus* possess small basidiomata (0.7–1.3 cm), reddish yellow, pulverulent pileus, short eccentric stipe, and 2 to 4 spored basidia. The Chinese collections of *B. xylophilus* are very much similar to those presented here by

having large basidiomata with yellowish brown pileus, yellowish pileal context changes to blue when injured, small basidiospores, and growing under *Bambusa remotiflora* Kuntze^[15]. The present collection from India was obtained from soil around *Bambusa bambos*, and represents a new record from India.

The Chinese collection seen as most similar to that reported here in the phylogenetic tree as well. Our collection is grouped with other ITS sequences of *B. xylophilus* with 100% BootStrap support. *B. lignicola* formed as sister taxon in the analysis.

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Conflict of interest

The authors declare that they have no conflict of interest.

Dates

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