



First Report of *Buchwaldoboletus lignicola* (Boletaceae), a Potentially Endangered Basidiomycete Species, in South Korea

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ABSTRACT

During the 2014 survey of the mushroom flora of Gwangneung forest in South Korea, we collected two specimens of boletoid mushroom growing on a felled tree of *Pinus koraiensis*. These specimens were characterized by a light brown to reddish-brown pileus with appressed tomentum, pore surface bluing instantly when bruised, golden-yellow mycelium at the base of stipe, and lignicolous habitat. Both specimens were identified as *Buchwaldoboletus lignicola*, a rare basidiomycete, based on morphological characteristics and sequences of internal transcribed spacer (ITS; fungal barcode). Here, we describe these specimens and provide the first report of this genus in South Korea.

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Boletaceae Chevall. is a family from the order Boletales in Basidiomycota and is comprised of boletoid fruitbodies with pores. There are about 50 recognized genera and 800 species globally [1]. Most species in this family are known to form ectomycorrhizal associations with various trees. However, some of the basal genera of Boletaceae, such as *Buchwaldoboletus* Pilát, are saprophytic and lignicolous. Currently, the genus *Buchwaldoboletus* consists of 13 species and is divided into three groups, namely *Lignicola*, *Sphaerocephalus*, and *Hemichrysus* [2–5]. *Buchwaldoboletus lignicola* is the type species of *Buchwaldoboletus* and was first described by Kallenbach in 1929, as *Boletus lignicola*. Later, Pilát (1969) separated this species from *Boletus* and described a new genus *Buchwaldoboletus*, based on its decurrent and arcuate hymenophore, lack of veil, stipe with yellow mycelium, and saprophytic habit. This species is known to be distributed in South and East Asia, North America, Europe, and North Africa [3,6–10].

As part of the surveys of macrofungi in the unexploited areas of Korea, the project funded by the Korea National Arboretum, we encountered two rare boletoid mushrooms in Gwangneung Forest. They were identified as *B. lignicola* based on their morphological features and phylogenetic analysis of the internal transcribed spacer (ITS) region. It is the

first report of this species in Korea. Here, we provide detailed morphological characteristics of *B. lignicola* and present new locality (South Korea) and a host plant (*Pinus koraiensis* Siebold & Zucc.) of the species.

Two *Buchwaldoboletus* fruiting bodies were collected at Gwangneung forest, Pocheon-si, Gyeonggi Province, South Korea in 2014. They were dried and deposited at the herbarium of the Korea National Arboretum. Macro-morphological features were determined based on field notes and color photos of fresh specimens. Micro-morphological features were observed from dried specimens after sectioning and mounting in 3% KOH solution. Basidia and cystidia were stained with a solution of 1% Congo Red under a light microscope (Olympus BX53, Tokyo, Japan). Measurements of microscopic characters were obtained using ProgRes Capture Pro v.2.8.8 (Jenoptik Co., Jena, Germany). For basidiospore descriptions, the measured numbers of basidiospore, the number of basidiomata, and the number of collection sites were respectively denoted with the abbreviation [n/m/p]. In describing basidiospore dimensions, we used the notation (a–) b–c (–d). The range b–c represents 95% of the measured values and “a” and “d” are lowest and highest measured values, followed by the mean spore length and width; Q is the range of the length/width ratio for

all measured basidiospores; Q_m is the average Q value \pm sample standard deviation.

DNA was extracted from fruiting bodies using a modified CTAB procedure [11]. For the amplification of the ITS region, primer sets ITS5 and ITS4 were used in PCR mixture of 0.5 pM of each primer, 0.25 mM dNTPs, 1.5 mM MgCl₂, 10 mM Tris-HCl, 50 mM KCl, 2.5 U of Taq DNA polymerase, and 15 ng of template DNA [12]. PCR conditions for ITS were as follows: an initial denaturation step at 94 °C for 4 min, followed by 34 cycles of 94 °C for 40 s, 52 °C for 40 s, and 72 °C for 60 s; and a final elongation step at 72 °C for 8 min. PCR products were purified using an ExoSAP-IT PCR Product Cleanup Reagent (USB, Cleveland, OH). The PCR products were directly sequenced using a BigDye Terminator v. 3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) with the manufacturer's instructions. The same primers used for PCR

were employed for sequencing. Capillary electrophoresis and data collection were performed on an ABI Prism 310 Genetic Analyzer (Applied Biosystems). We edited the sequences using PHYDIT v. 3.2 [13] and deposited them to GenBank.

DNA sequences of other reported Boletaceae species were obtained from GenBank for phylogenetic analyses (Table 1). They were aligned with DNA sequences generated for this study using ClustalX v. 1.81 [14]. Ambiguously aligned positions were adjusted manually using PHYDIT. The constructed datasets were analyzed with the algorithms of maximum parsimony (MP) using PAUP* v. 4.0 b10 and Bayesian inference using MrBayes v. 3.1.2 [15,16]. Parsimony analysis was performed with a heuristic search of 1000 random addition replicates and tree bisection-reconnection branch-swapping. MP bootstrap support values (MPBS) were assessed to evaluate the supports for internal nodes from 1000

Table 1. Information of ITS sequences used in this study.

Species	Voucher	Locality	GenBank accession
<i>Aureoboletus tenuis</i>	GDGM:32601	China	KF265358
<i>Aureoboletus venustus</i>	HKAS:77700	China	KU321702 ^a
<i>Boletus hiratsukae</i>	TMI 18352	Japan	NR_119672 ^a
<i>Boletus nobilissimus</i>	BUF Both4244	USA	NR_119671 ^a
<i>Boletus rubriceps</i>	SFSU Arora11340	USA	NR_137806 ^a
<i>Borofutus dhakanus</i>	HKAS 73785	Bangladesh	NR_120117 ^a
<i>Borofutus dhakanus</i>	CMU-ST58-001	Thailand	KU168045
<i>Buchwaldoboletus lignicola</i>	KM157323	England	GQ981493
<i>Buchwaldoboletus lignicola</i>	–	Italy	HM003619
<i>Buchwaldoboletus lignicola</i>	–	Sweden	HM003618
<i>Buchwaldoboletus lignicola</i>	–	Scotland	HM003617
<i>Buchwaldoboletus lignicola</i>	3533	Canada	KM248950
<i>Buchwaldoboletus lignicola</i>	KA14-0711	South Korea	MH170896
<i>Buchwaldoboletus lignicola</i>	KA14-0907	South Korea	MH170897
<i>Butyriboletus roseogriseus</i>	PRM:923483	Czech	NR_151842 ^a
<i>Butyriboletus yicibus</i>	SFSU Arora9727	China	NR_137796 ^a
<i>Chalciporus piperatus</i>	K80S25b	New Zealand	GQ267470
<i>Chalciporus piperatus</i>	2591	Canada	KM248949
<i>Chalciporus radiatus</i>	GDGM50080	China	KP871806
<i>Chalciporus radiatus</i>	GDGM43285	China	KP871804 ^a
<i>Chalciporus rubinellus</i>	2626	Canada	KM248951
<i>Chalciporus rubinellus</i>	191/81	USA	EU685111
<i>Rubinoletus rubinus</i>	18508	UK	JF908793
<i>Rubinoletus rubinus</i>	KW 50674F	Ukraine	KJ562360
<i>Chalciporus trinitensis</i>	18465	Guatemala	JF908790
<i>Gyrodon lividus</i>	17191	Italy	JF908786
<i>Gyrodon lividus</i>	REG G11	Germany	DQ534568
<i>Harrya chromapes</i>	–	Canada	KM248941
<i>Harrya chromapes</i>	ITS199	Japan	KC552019
<i>Porphyrellus porphyrosporus</i>	DJM1332	USA	JN021085
<i>Pseudoboletus parasiticus</i>	18898	Italy	JF908801
<i>Pseudoboletus parasiticus</i>	2164-QFB-25840	Canada	KM248932
<i>Pulveroboletus flaviscabrosus</i>	HKAS83190	China	KX453802 ^a
<i>Pulveroboletus rubroscabrosus</i>	HKAS75537	China	KX453816 ^a
<i>Rubroboletus rhodoxanthus</i>	MA-Fungi 47703	Portugal	AJ419189
<i>Rubroboletus satanas</i>	Bs2	Germany	DQ534567
<i>Strobilomyces confusus</i>	BRNM 766848	South Korea	KT121567
<i>Strobilomyces pteroreticulosporus</i>	BRNM 718716	South Korea	KT121565 ^a
<i>Strobilomyces strobilaceus</i>	LE253886	Russia	JQ318985
<i>Tylophilus microsporus</i>	HMAS 84730	China	NR_137924 ^a
<i>Tylophilus neofelleus</i>	YT20090720	Japan	KM975489
<i>Tylophilus porphyrosporus</i>	GO-2009-237	Mexico	KC152268
<i>Xanthoconium affine</i>	3735	USA	KM248938
<i>Paxillus ammoniavirescens</i>	IK-00554	Poland	KX610700
<i>Paxillus rubicundulus</i>	Orton:2905	United Kingdom	NR_147640 ^a

^aType specimen.

replicates of the MP analysis. Posterior probabilities (PPs) were calculated using the Metropolis-coupled Markov Chain Monte Carlo method. Two parallel runs were performed with one cold and three heated chains for 3 million generations, starting with a random tree with sampling every 100th generation. We assessed the convergence of two independent runs to remove the trees which were not in convergence with a criteria of the average standard deviation of the split frequencies being below 0.01 using the burn-in command. The remaining trees which were converged used in calculating a 50% majority

consensus tree and estimating PP. The distance matrix was calculated using PHYDIT with Kimura-2-Parameter distance method.

Taxonomic description

Buchwaldoboletus lignicola (Kallenb.) Pilát, *Friesia* 9(1–2): 217 (1969) (Figure 1).

Pileus 4–10 cm in diameter, convex with in rolled margin and covered with soft appressed tomentum, light brown to reddish-brown. Hymenophore tubulose, tubes detersible, decurrent, yellow to golden at

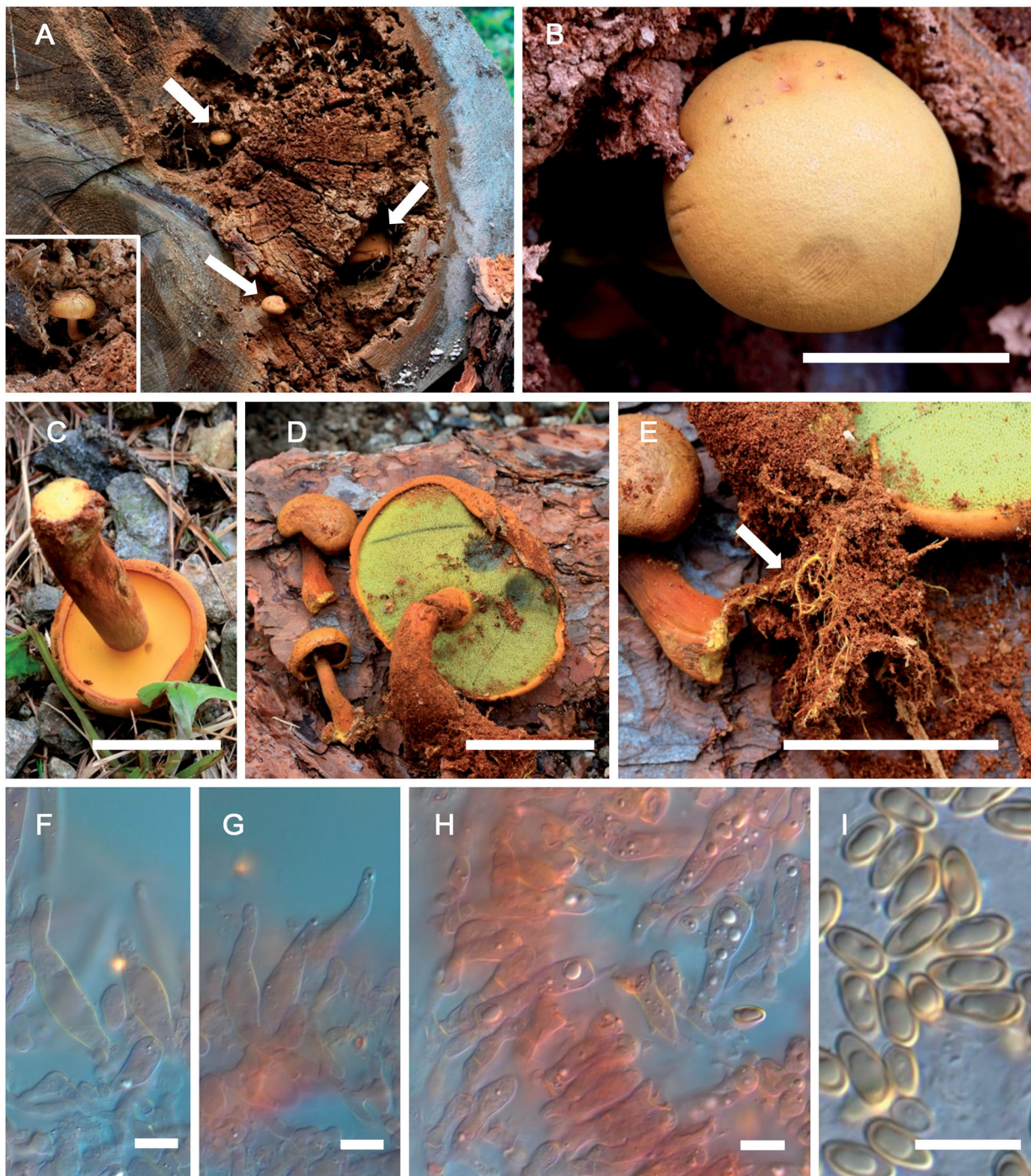


Figure 1. Morphological characters of *Buchwaldoboletus lignicola* KA14-0711 and KA14-0907. (A–D) Fruiting bodies of *B. lignicola*; (E) golden-yellow mycelia at the base of stipe; (F, G) Pleurocystidia (stained with 1% Congo red solution); (H) Basidia and basidioles (stained with 1% Congo red solution); (I) Basidiospores in 3% KOH. (Scales bars: B–E = 5 cm, F–I = 10 μ m).

first, then olivaceous yellow, turning greenish-blue above tubes when cut or bruised; pores circular to angular or irregular, 1–3 per mm. **Stipe** 3–7 cm long, 0.6–1.7 cm thick, central or somewhat eccentric, cylindrical, sometimes base somewhat thickened, rust-yellow to reddish-brown with a golden-yellow mycelia at the base, context yellow. **Partial veil** absent.

Basidiospores [100/2/2] (7.0–) 7.2–8.8 (–11.2) × (3–) 3.1– 4(–5.4) μm, Q=(1.9–) 2–2.5 (–2.6), Qm = 2.22 ± 0.15, elliptic, smooth, thick-walled, pale yellow. **Basidia** [n = 40] 20.5–34 × 6–9 μm, clavate or broadly clavate, 4-spored, sterigmata 2–4 μm long. **Pleuroystidia** 28–51 × 6.3–10.2 μm, fusiform, ventricose-lageniform, often with a long rostrum, hyaline to yellowish in 3% KOH, thin-walled, smooth. **Caulocystidia** 31.4–42.5 × 5.5–8.5 μm, fusiform, fusiform to clavate, ventricose-lageniform, hyaline to yellowish in 3% KOH, thin-walled, smooth. **Caulobasidia** [n = 30] 22–30 × 5–9 μm, clavate, 4-spored, sterigmata 2.5–3.6 μm long. Clamp connections absent in all tissues.

Habitat: Solitary or scattered on dead conifer stump (*Pinus koraiensis* Siebold & Zucc, *Pinaceae*).

Edibility: unknown

Examined specimens: KA14-0711, Gwangneung Forest, Pocheon-si, Gyeonggi Province, Korea, July 28 2014, coll. Jo et al., KA14-0907 same place, August 13 2014, coll. Jo et al.

Remarks: Morphologically, *B. lignicola* is similar to *B. hemichrysus*, *B. pseudolignicola*, *B. pontevedrensis*, and *B. xylophilus*. However, *B. hemichrysus* has a golden-yellow pileus, red-brown to reddish-brown pore, and a ventricose stipe, and *B. lignicola* does not share those features [4,17,18]. Similarly, *B. pseudolignicola*, a member of *Sphaerocephalus* group, has a yellow to cinnamon-brown pileus, unlike *B. lignicola*, and has a smaller basidiospore than *B. lignicola* [4]. *B. pontevedrensis* is a recently described member of a group of *Lignicola*; it differs from *B. lignicola* by having a bigger pileus, shorter stipe, and slightly longer basidiospores [3]. *Buchwaldoboletus xylophilus* differs from *B. lignicola* by having shorter basidiospores [19].

The ITS dataset included 45 taxa and 795 characters, of which 364 were parsimony-informative. The MP tree was 1329 steps long with a consistency index of 0.5813, retention index of 0.6765, and homoplasy index of 0.4698. The Bayesian analyses were conducted with a model of GTR+I+G and the first 9000 trees were discarded as burn-in (burninfrac = 0.30). The phylogenetic trees of the ITS dataset in this study showed a similar main branch topology as presented in previous studies [20–24].

Boletaceae was monophyletic (Figure 2). Among the Boletaceae clade, subfamily *Chalciporoideae* Wu

& Yang (including *Buchwaldoboletus* and *Chalciporus*) formed a basal group. This group appears to be parasitic or saprophytic. Although *Chalciporoideae* was only supported by MPBS (87%), the clade grouping *Buchwaldoboletus* and *Chalciporus* were strongly supported in both MPBS and PP (Figure 2). The ITS sequences of our two *B. lignicola* specimens (KA14-0711 and KA14-0907) formed a group with five previously reported European and Canadian *B. lignicola* specimens (HM003617, HM003618, HM003619, GQ981493 and KM248950) with strong support values (MPBS = 99%, PP = 0.99) (Figure 2).

Buchwaldoboletus lignicola is a basidiomycete fungus that is distributed mainly in Europe and North America [4,25]. However, this species is rarely observed in Asia. For this reason, only a few studies and sequences have been published for *B. lignicola*. In Japan, three species of *Buchwaldoboletus* (*B. xylophilus*, *B. pseudolignicola*, and *B. sphaerocephalus*) have been recorded [26] excluding *B. lignicola*. However, there has not been a report of genus *Buchwaldoboletus* species in Korea. *B. lignicola* has been found on conifer stumps, including *Larix decidua* Mill., *Picea abies* (L.) H. Karst., *Pinus sylvestris* L., *Pinus strobus* L., and rarely on *Prunus avium* L. [4,7,27]. In addition to the report of the species in Korea, this study presents a new host for *B. lignicola*, Korean pine (*Pinus koraiensis*).

Some considered *B. lignicola* to be saprotrophic based on its lignicolous habitat [28]. Other studies, however, present that the species is often found with *Phaeolus schweinitzii* (Fr.) Pat., a brown-rot tree pathogen [8,29]. Additionally, Nuhn et al. [21] suggest that *B. lignicola* has a mycoparasitic nutritional mode, based on the confrontation assay between the hyphae of *B. lignicola* and *P. schweinitzii*. Although *P. schweinitzii* was not observed at the habitat of *B. lignicola* in this study, previous research has shown that *B. lignicola* can occur where *P. schweinitzii* occurs.

Although *B. lignicola* is not recorded in the IUCN Red List, the species is listed as a critically endangered species in Bulgaria [30], endangered species in the Czech Republic [31], and vulnerable in Great Britain [32]. In South Korea, nine species of macrofungi (*Albatrellus dispansus*, *Amanita hemibapha* subsp. *javanica*, *Ganoderma neojaponicum*, *Grifola frondosa*, *Hericium coralloides*, *Inonotus obliquus*, *Lyophyllum fumosum*, *Oudemansiella brunneomarginata*, and *Phellinus linteus*) are registered as protected forest species by the Korea Forest Service. Considering its rarity, *B. lignicola* may need to be considered as a protected forest species of South Korea in the future.

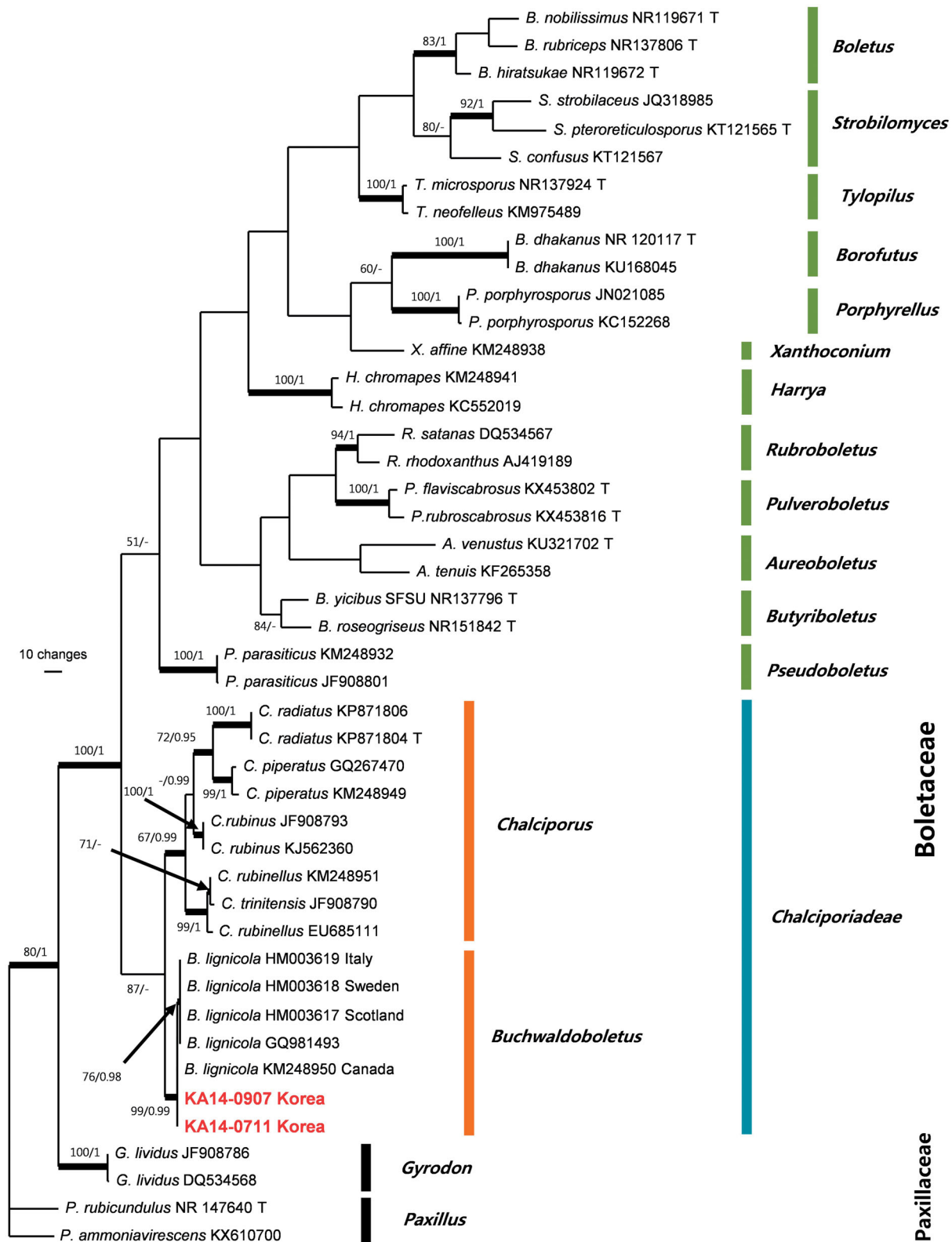


Figure 2. One of 104 most parsimonious trees from a heuristic analysis of ITS sequences. Broad black branches indicate maximum parsimony bootstrap value (MPBS) >60% and Bayesian posterior probabilities >0.95. Only MPBS values >50% are shown above or below branches. The symbol "T" indicated the type materials.

Disclosure statement

No potential conflict of interest was reported by the authors.

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