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Phlebopus roseus, a new edible bolete from China, is associated with insects and plants

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ABSTRACT

Phlebopus roseus is described as new based on collections from southwest China. Phylogenetic analyses of nuclear rDNA internal transcribed spacer region ITS1-5.8S-ITS2 (ITS) and portions of nuclear 28S rDNA (28S), translation elongation factor 1- α (*tef1*), and the largest and second largest subunits of RNA polymerase II (*rpb1*, *rpb2*) support *P. roseus* as a novel species in the genus *Phlebopus* (Boletiniaceae, Boletales). The new species resembles *P. portentosus* but differs from it in that mature basidiomata have a bright rose-red-colored stipe and a radiate tubular hymenophore with nested pores. Despite extensive searching, *P. roseus* has only been found at four sites within a 24-hectare orchard dominated by *Eriobotrya japonica*, which is agriculturally important given its fruit production (loquats). Therefore, this species appears to be endemic and geographically restricted. The ecology of this bolete is also unique. In line with the trophic behavior of other species in the Boletiniaceae, our observations indicate that *P. roseus* forms a symbiotic association with the scale insect *Coccus hesperidum*, identified through sequence analysis of its mitochondrial cytochrome c oxidase subunit I (*COI*) region, to form fungus-insect galls that develop on roots of *E. japonica* trees. *Phlebopus roseus* is an edible mushroom species and is collected from the type location by farmers and sold commercially in limited quantities at local markets alongside *P. portentosus* and other fungi.

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Boletiniaceae; *Coccus hesperidum*; *Eriobotrya japonica*; soil scale insects; symbiosis; 1 new taxon

INTRODUCTION

The Boletiniaceae P.M. Kirk, P.F. Cannon & J.C. David (2001) are a distinctive family of fungi in the order Boletales (1931) that comprises two genera, *Phlebopus* (R. Heim) Singer (1936) and *Boletinellus* Murrill (1909). Species in both genera have an unusual ecology as saprotrophs but also have a complex biotrophic association with insects. In this tripartite association, the boletes form a symbiosis with soil mealy bugs of the family Pseudococcidae within a fungus-insect gall on plant roots (Gonçalves 1940; Cotter and Miller 1985; Brundrett and Kendrick 1988; Gruhn et al. 1992; Zhang et al. 2015; Nuhn 2016). Fungi within Boletiniaceae produce stout fleshy basidiomata that have a poroid hymenophore. Other characteristics of this family include the broad elliptical basidiospores and the presence of

clamp connections on hyphae composing basidioma tissues and mycelial cultures. *Phlebopus* differs from *Boletinellus* in having a ventricose, bulbous, basal, sulcate stipe.

At present, only three species have been described in *Boletinellus* and more than 12 taxa have been reported in *Phlebopus* (Murrill 1909; McNabb 1968; Heinemann and Rammeloo 1982; Watling and De Meijer 1997; Deschamps and Moreno 1999; Watling 2006; Kirk et al. 2008; Tedersoo et al. 2010; Pham et al. 2012; Nuhn 2016). Only one species, *P. portentosus*, has been previously reported from China (Zang 2006; Lei et al. 2009; Zhang et al. 2015). *Phlebopus portentosus* has been found in Yunnan, Sichuan, Guangxi, and Hainan provinces in China, northern Thailand, and Sri Lanka (Sanmee et al. 2010; Pham et al. 2012; Zhang et al. 2015). This species has also been reported from Laos,

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Cambodia, Vietnam, Indonesia, and Malaysia (Watling 2006; Kirk et al. 2008). Here, we provide a complete morphological description and multigene phylogenetic analysis of a new species of *Phlebopus*, which occurs intermixed with *P. portentosus* under trees of *Eriobotrya japonica* (loquat) in Miyi County, Panzhihua, Sichuan, China. Further, we report on the unique ecology of this bolete, which grows in association with scale insects to form fungus-insect galls on roots of *E. japonica*.

MATERIALS AND METHODS

Collection and morphological analysis.—Basidiomata of *P. roseus* were collected from Miyi County in Sichuan, China, in 2015. The holotype (HKAS 102569) and three other specimens were deposited in the KUN Cryptogamic Herbarium (TABLE 1). The samples were brought back fresh for isolation and morphological examination. For tissue isolation, surfaces of basidiomata were wiped with 75% ethanol, cuts made with forceps, and then the basidioma was split in half by hand. A small piece of flesh was excised from the junction between the pileus and the stipe and plated onto potato dextrose agar (PDA) and incubated at 24 C for 2 wk.

Macroscopic and microscopic characteristics of basidiomata were described from fresh specimens following the methods of Yang and Zhang (2003). Briefly, sections were made by hand with a razor blade and mounted in water. For the best sections, water was replaced with lactophenol solution by suction before taking micrographs. Observations and photographs were made with an Olympus BH-2 microscope (Tokyo, Japan). Scanning electron microscopy (SEM) images were made at KUN. The pileus and stipe dimensions were inferred from 10 basidiomata. Measurements of spores, basidia, and cystidia were made from five basidiomata. Since we suspected the presence of a new species in 2013, we recommended that farmers weigh *P. roseus* basidiomata

separately from those of *P. portentosus* after each harvest to estimate relative yield of the two species annually.

Insect analysis.—Roots of *Eriobotrya japonica* were collected beneath *P. roseus* basidiomata. The fungus-insect galls were found on some of the collected roots. The fungus-insect galls were washed under tap water and then cut open to reveal scale bug-like larvae inside that appeared similar within and between galls. Three insect larvae were collected from one gall, stored at 4 C in 75% ethanol, and their DNA was analyzed separately.

Molecular methods.—Four specimens, each collected from a different fruiting site within the loquat orchard (see SUPPLEMENTARY FIG. 1), were analyzed (TABLE 1). Total DNA was extracted from silica-dried basidiomata tissues with a modified cetyltrimethylammonium bromide (CTAB) methodology (Hofstetter et al. 2002; Li et al. 2011). Nuc rDNA internal transcribed spacer region ITS1-5.8S-ITS2 (ITS barcode) and portions of nuc 28S rDNA (28S), single-copy protein genes that encode RNA polymerase II largest (*rpb1*) and second largest (*rpb2*) subunits, and translation elongation factor 1- α (*tef1*) were amplified by polymerase chain reaction (PCR) following the protocol provided by Li et al. (2011). Primers used to amplify each locus are presented in TABLE 2. Purification and bidirectional Sanger sequencing of PCR products were conducted by Beijing Genomics Institute on an ABI3130 automated sequencer (Beijing, China).

Insect DNA was extracted from larvae using an animal tissue genomic DNA kit (Tiangen Bio-Chem Technology, Beijing, China) following the manufacturer's protocol and was stored at -20°C until use. The mitochondrial cytochrome *c* oxidase subunit I (*COI*) region was amplified by PCR with the forward primer PcoF1 and reverse primer LepR1 (TABLE 2). PCR reactions were performed in a final volume of 25 μL containing 12.5 μL Premix

Table 1. Specimens, pure cultures, and sequences obtained in this study.

Taxon	Collection number	GenBank accessions	
		ITS/28S rDNA	<i>rpb1/rpb2/tef1</i>
<i>Phlebopus roseus</i>	MY2017273	MK734170/MK734301	MK736300/MK747251/MK736294
<i>Phlebopus roseus</i>	MY2017274	MK734175/MK734306	—/MK747256/MK736299
<i>Phlebopus roseus</i>	MY2017275 ^a	MK734171/MK734302	MK736301/MK747252/MK736295
<i>Phlebopus roseus</i>	MY2017275 _a ^b	MK734173/MK734304	MK736303/MK747254/MK736297
<i>Phlebopus roseus</i>	MY2017277	MK734172/MK734303	MK736302/MK747253/MK736296
<i>Phlebopus roseus</i>	MY2017277 _a ^b	MK734174/MK734305	MK736304/MK747255/MK736298

Note. All specimens were harvested in Miyi County, Sichuan, China, $26^{\circ}55'19''\text{N}$, $102^{\circ}06'44''\text{E}$, alt. 1220–1300 m, in an orchard of *Eriobotrya japonica* on 9 Jan 2015.

^aMY2017275 is the holotype and corresponds to specimen HKAS 102569 kept in the Cryptogamic Herbarium, Kunming Institute of Botany (KUN), Academia Sinica, Kunming, Yunnan, China. Paratypes MY2017273, MY2017274, and MY2017277 correspond to specimens HKAS 106938, HKAS 106939, and HKAS 106940, respectively.

^bPure cultures MY2017275_a and MY2017277_a were obtained from corresponding basidiomata. No sequence is available at RPB1 for MY2017274.

Table 2. Fungal and insect primer sequences used in this study.

Locus	Primer name	Primer sequence	Reference
ITS	ITS1F	CTTGGTCATTTAGATGAAGTAA	Gardes and Bruns 1993
	ITS4	TCCTCCGCTTATTGATATGC	White et al. 1990
28S	LROR	ACCCGCTGAACCTTAAGC	Vilgalys and Hester 1990
	LR5	TCCTGAGGGAACCTTCG	Vilgalys and Hester 1990
<i>rpb1</i>	RPB1-Af	GTCCGGGWCATTTTGGTC	Matheny et al. 2002
	fRPB1-Cr	TTGTCCATGTANGTRGCRACA	Matheny et al. 2002
<i>rpb2</i>	bRPB2-6F	TGGGGYATGGTNTGYCCYGC	Matheny 2005
	bRPB2-7IR	CCCATRGCYTGYTMCCTATDGC	Matheny 2005
<i>tef1</i>	983F	GCYCCYGGHCAYCGTGATYAT	Rehner 2001
	1567R	ACHGTRCCRATACCACCRATCTT	Rehner 2001
<i>COI</i>	PcoF1	CCTTCAACTAATCATAAAATATYA	Park et al. 2011
	LepR1	TAAACTCTGGATGTCCAAAAATCA	Park et al. 2011

Note. ITS = nuclear ribosomal DNA internal transcribed spacer ITS1-5.8S-ITS2; 28S = large subunit of the nuclear ribosomal repeat; *rpb1* = RNA polymerase II largest subunit; *rpb2* = RNA polymerase II second largest subunit; *tef1* = translation elongation factor 1- α ; *COI* = mitochondrial cytochrome c oxidase subunit I.

(Beijing Qingke, Chengdu, China), 1 μ L of each primer (5 μ M; Sangon Biotech, Shanghai, China), 1 μ L DNA template, 9.5 μ L double-distilled water (ddH₂O). Amplifications were performed with the following cycling parameters: 94 C for 5 min, followed by 35 cycles of 94 C for 30 s, 46.7 C for 45 s, and 72 C for 1 min, and with a final extension at 72 C for 10 min. PCR products were run on a 1% agarose gel and stained with GelRed (Biotium, Hayward, California). PCR products were purified and sequenced bidirectionally by the Qingke Biological Company, Beijing, China.

Fungal sequences were edited and assembled using SeqMan II (Larsson and Sundberg 2011). The BLASTn algorithm was used to compare sequences (TABLE 1) with those on GenBank, and with those belonging to reference taxa for phylogenetic analyses. All sequences used for comparison are presented in SUPPLEMENTARY TABLE 1. Sequence alignments for each locus were performed individually in MUSCLE 3.7 (Edgar 2004). Alignments were visually inspected and ambiguous regions excluded in Mesquite 3.51 (Maddison and Maddison 2018). Given that different reference taxa were available for each locus, with most reference sequences belonging to ITS and 28S, we split and sorted the data into two concatenated data matrices using SequenceMatrix (Vaidya et al. 2011). The first data matrix consisted of concatenated ITS and 28S alignments for 32 taxa and 1864 characters. The second data matrix consisted of a concatenated alignment of *rpb1*, *rpb2*, and *tef1* and included 21 taxa and 3762 characters. Outgroup taxa in the Scleroder-matineae were chosen based on recent analyses of the Boletales and published data available on GenBank (Sato and Toju 2019).

Maximum likelihood (ML) analyses were conducted with RAxML 8.2.12 (Stamatakis 2014). Data partitions for protein-coding gene exons were determined with PartitionFinder (Lanfear et al. 2012) based on codon and locus using the Akaike information criterion. Intron

regions were excluded. A GTRGAMMA nucleotide substitution model was used for each of the five partitions that were found. One thousand ML bootstrap replicates were executed in RAxML under a GTRGAMMA model. For Bayesian inference (BI) estimations, independent analyses were conducted in MrBayes 3.2.7a (Ronquist et al. 2012). Two independent Markov chain Monte Carlo (MCMC) runs with four chains were conducted for 10 million generations sampling every 100 generation. MCMC convergence was assessed by visualizing with Tracer (Rambaut et al. 2018) and determining that effective sampling size (ESS) was above 400. The first 25% of trees were discarded as burn-in, and posterior probabilities (PPs) were calculated from the remaining distribution of trees. MrBayes and RAxML analyses were computed through the CIPRES Web portal (www.phylo.org) and visualized with FigTree (<http://tree.bio.ed.ac.uk/software/figtree>).

Insect sequences were assembled and edited manually using Sequencher 4.1.4 (Gene Codes, Ann Arbor, Michigan). The consensus sequence was queried against the National Center for Biotechnology Information (NCBI) public database GenBank with the BLASTn algorithm to aid identification. Similar sequences were downloaded from GenBank to conduct a phylogenetic analysis for verification. Reference insect sequences and the *COI* sequence derived from the fungus-root gall were aligned in MUSCLE 3.7 (Edgar 2004). The alignment was visually inspected and ambiguous regions excluded in Mesquite 3.51 (Maddison and Maddison 2018). Maximum likelihood (ML) analyses were conducted as above following with 1000 ML bootstrap replicates. The consensus insect *COI* sequence was deposited at GenBank (MT482789).

RESULTS

Pure culture isolation.—Pure culture isolates of *P. roseus* were obtained from basidioma tissues sampled from the junction between the pileus and the stipe and

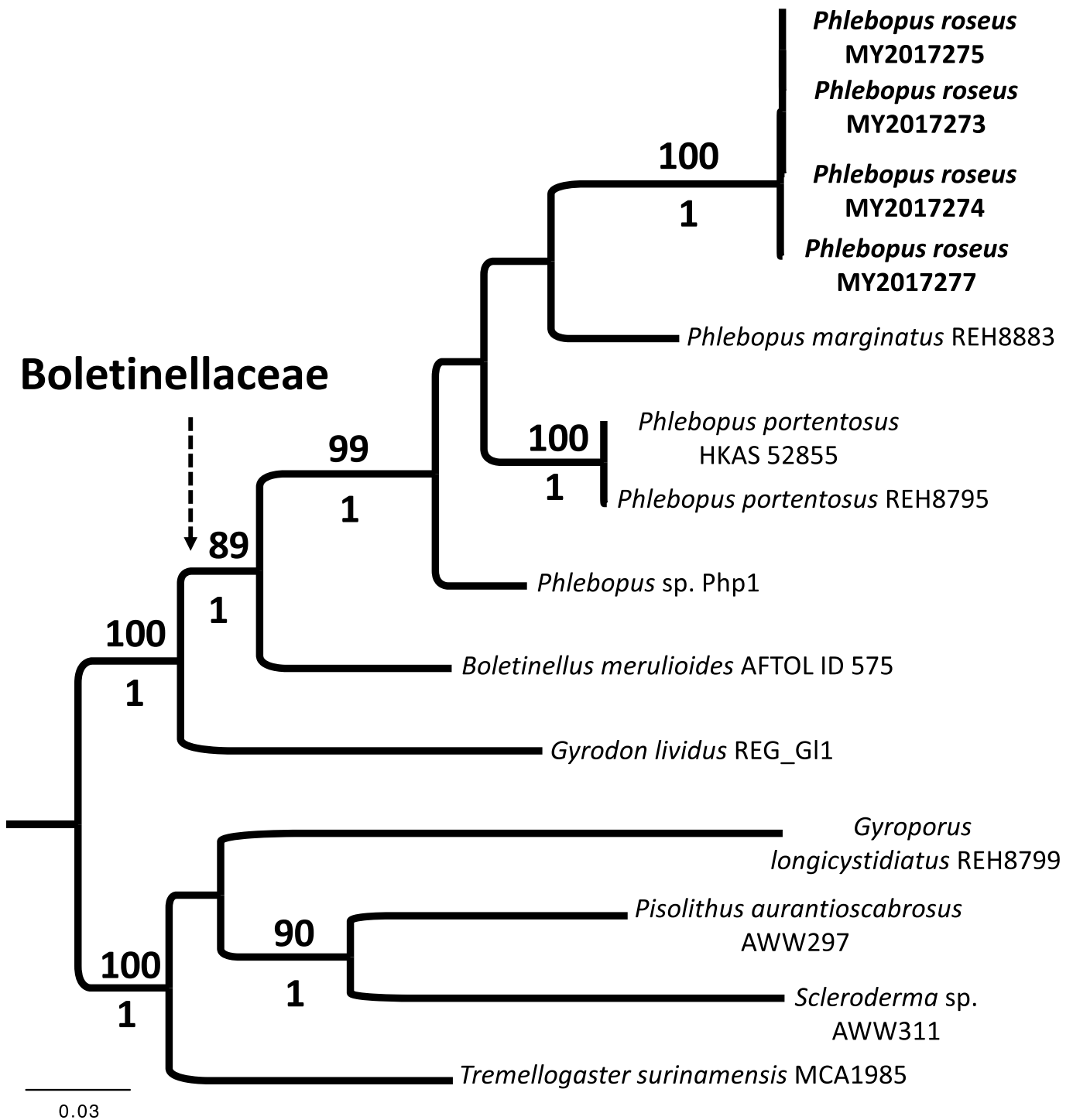


Figure 1. Maximum likelihood (ML) phylogeny based on the combined data set of *rpb1*, *rpb2*, and *tef1* nucleotide sequences showing the placement of *Phlebopus roseus* (labeled and in bold) within *Phlebopus* and the Boletinellaceae. The node comprising Boletinellaceae is labeled with a dotted arrow. Taxa within Paxillaceae (*Gyrodon*) and Sclerodermatineae (*Gyroporus*, *Pisolithus*, *Scleroderma*, *Tremellogaster*) were used as outgroups to root the phylogeny. ML bootstrap support values >70% are shown above nodes. Bayesian posterior probabilities (0–1) are shown below nodes.

plated onto PDA. A total of 73 homogeneous mycelial colonies were obtained out of 86 attempts (85%). Microscopic observation revealed that subcultures

contained clamp connections, indicating that they were dikaryotic. Isolates completely colonized 7.5-cm-diam plates after 25 d of incubation at room temperature (24 °C).

Fungal phylogenetic analyses.—Independent assessments of the phylogenetic placement of *P. roseus* based on protein-coding (FIG. 1) and rDNA (FIG. 2) data provided multiple levels of resolution and sampling. The phylogenies were similar in topology, in that *Phlebopus* formed a monophyletic lineage derived from a shared ancestor with *Gyrodon* (Paxillaceae). rDNA data indicated that *Boletinellus* is weakly supported monophyletic group, but only one taxon was sampled in the single-copy protein-coding gene matrix, so no firm conclusions can be made about the monophyletic status of this genus. In both sets of analyses, *P. roseus* was resolved within the Boletinellaceae (70% bootstrap support and 1.0 PP) (FIGS. 1, 2). ITS sequences of *P. roseus* are >10% different from those of other sampled *Phlebopus* species.

Insect phylogenetic analyses.—The COI sequence generated from the insect larvae showed 99.52% similarity with *Coccus hesperidum* (GenBank KY085328; see Amouroux et al. 2017). Phylogenetic analysis of COI further supports the identification of the larvae as *C. hesperidum* (SUPPLEMENTARY FIG. 2). Similarly, the morphology of the insect larvae found in the galls is *Coccus*-like and consistent with that of *C. hesperidum*.

TAXONOMY

Phlebopus roseus M. Yang, C.-Y. Liu & Y. Wang, sp. nov. FIGS. 3, 4
MycoBank MB818925

Type: CHINA. SICHUAN: Miyi County, 26°55' 19"N, 102°06'44"E, alt. 1220–1300 m, on soil in understory of an *Eriobotrya japonica* (loquat) orchard, 9 Jan 2015, Mei Yang MY2017275 (**holotype** HKAS 102569). GenBank: ITS = MK734171; 28S = MK734302; *rpb1* = MK736301; *rpb2* = MK747252; *tef1* = MK736295.

Diagnosis: *Phlebopus roseus* differs from other *Phlebopus* species by its reddish stipe and its tubular hymenophore with nested angular pores of multiple sizes.

Etymology: *roseus* (Latin), referring to the rose-red stipe.

Description: Pileus up to 170 mm wide, convex or plano-convex when young, becoming applanate with a reflexed margin and often grooved in age, margin often extending beyond the pores; surface dry, velutinate; dark brown when young, becoming yellowish brown with age, often tinted reddish brown; flesh thick, bright yellow, reddish underneath the pileus cuticle, blue where touched; 5% KOH negative on pileus surface and context. Tubes very thin, radiating from the stipe, slightly adnexed; bright yellow when young, sordid yellow at maturity; pores concolorous with tubes, pores and tubes blue

where bruised; pores pentagonal or hexagonal, often nested with a larger single pore (1–2 × 3–4 mm) having 2–5 smaller pores (0.5–2 mm) with shallower ridges within (FIG. 3E). Stipe up to 150 mm long, ventricose-bulbous, 23 mm wide at the apex, enlarged below and up to 85 mm wide at the base; dry, coarsely sulcate basally, velutinate; roseus to reddish brown, usually yellowish at the basal end of the stipe; flesh sordid, yellow, becoming blue where handled. Odor not distinctive; taste meaty, slightly earthy.

Basidiospores 6.5–8 × 5–6 µm, smooth, broadly elliptic or obovate, thin-walled, deep yellow in water, apical pore absent, dark brown in deposit. Basidia 15–25 × 10–12 µm, hyaline, clavate, 4-spored. Pleurocystidia 20–50 × 7–15 µm, clavate or heteromorphous. Hymenophoral trama bilateral, hyaline, hyphae to 11 µm wide. Pileipellis a trichoderm, with sparse erect terminal cells 28–30 × 3–6 µm, these cylindric with rounded apices and brown pigment. Stipitipellis consisting of interwoven hyphae with dense, erect, terminal cells, 2–5 µm wide, up to 90 µm long, thin-walled, with brownish intracellular pigment. Clamp connections present (FIG. 4A).

Ecology and distribution: solitary or in groups on soil of sandy loam with pH 5.7–7.3, under trees of *Eriobotrya japonica*, alt. 1220–1300 m, forming fungus-insect galls with *Coccus hesperidum* on the roots (FIG. 4D–E), occurring summer to autumn. Known only from an abandoned loquat plantation of about 24 ha located in Miyi, Sichuan, China. Known only from the type locality.

Other specimens examined: CHINA. SICHUAN: Miyi County, same locality as the holotype MY2017275, 9 Jan 2015, Mei Yang MY2017273 (HKAS 106938); 9 Jan 2015, Mei Yang MY2017274 (HKAS 106939); 9 Jan 2015, Mei Yang MY2017277 (HKAS 106940).

DISCUSSION

Boletinellaceae is a small family within the Boletales. Our data confirm that this family consists of two genera: *Phlebopus* and *Boletinellus*, although the placement of *B. rompelii* in the genus was poorly resolved as previously shown (FIG. 2; Louzan et al. 2007). *Gyrodon* was the only representative of Paxillaceae that was included, and it fell outside of Boletinellaceae in our phylogenetic analysis of protein sequences. The phylogenetic placement of Boletinellaceae within the Boletales was poorly resolved in Wilson et al. (2012); however, a more recent phylogenetic study (Sato and Toju 2019) suggested that Boletinellaceae is the sister group of Boletineae (the suborder comprising Boletaceae and Paxillaceae).

Within Boletinellaceae, only one species, *Phlebovirus portentosus* (type from Sri Lanka), had been documented from subtropical and tropical China (Zang 2006; Lei

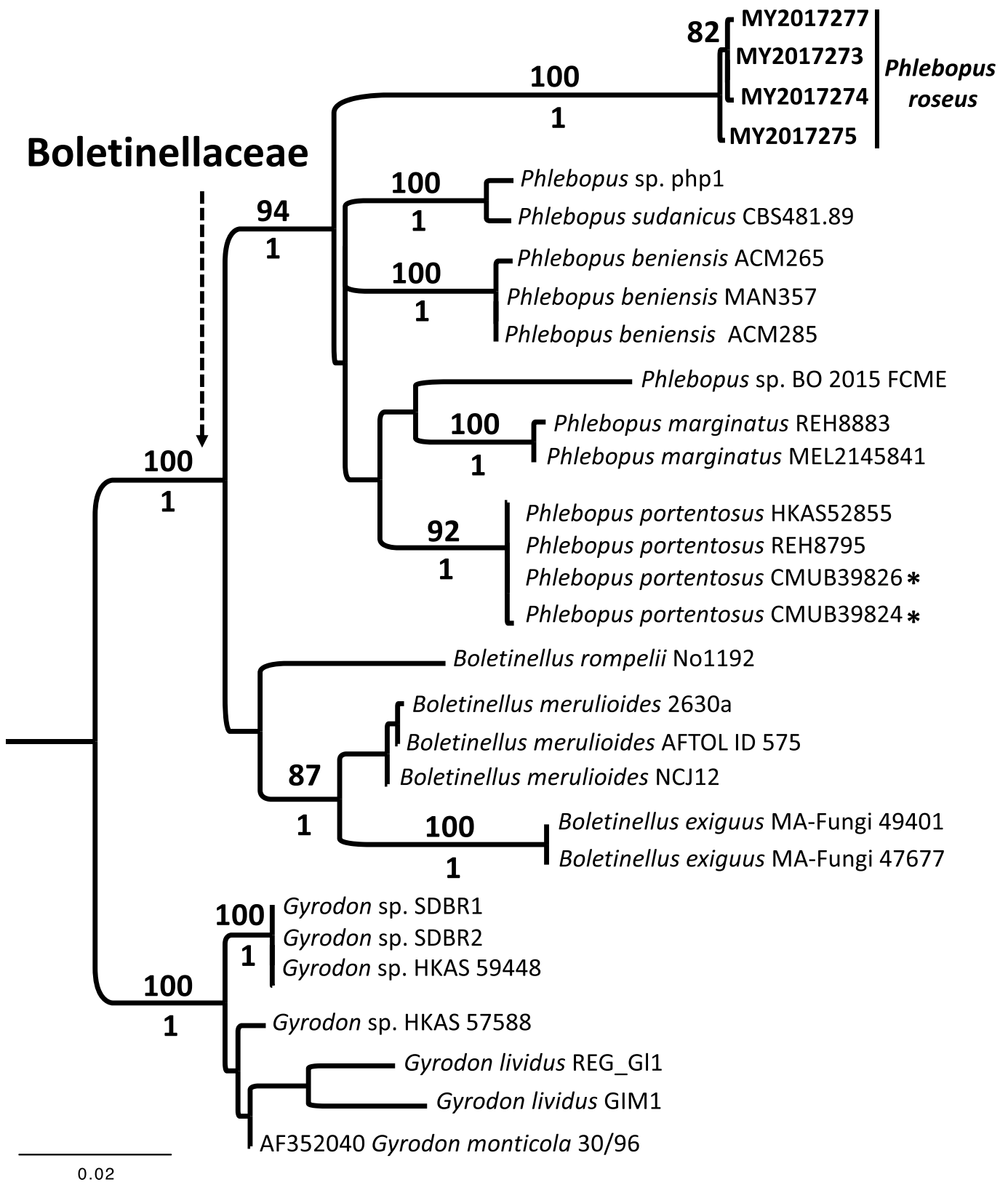


Figure 2. Maximum likelihood (ML) phylogeny based on the combined data set of ITS and 28S showing the placement of *Phlebopus roseus* (bold) within *Phlebopus* and the Boletinellaceae. The node comprising Boletellaceae is labeled with a dotted arrow. Taxa within *Gyrodon* (Paxillaceae) were used as outgroups to root the phylogeny. ML bootstrap support values >70% are shown above nodes. Bayesian posterior probabilities (0–1) are shown below nodes. An asterisk (*) denotes that the taxonomy of this collection provided in GenBank has been reassigned based on the molecular analysis.

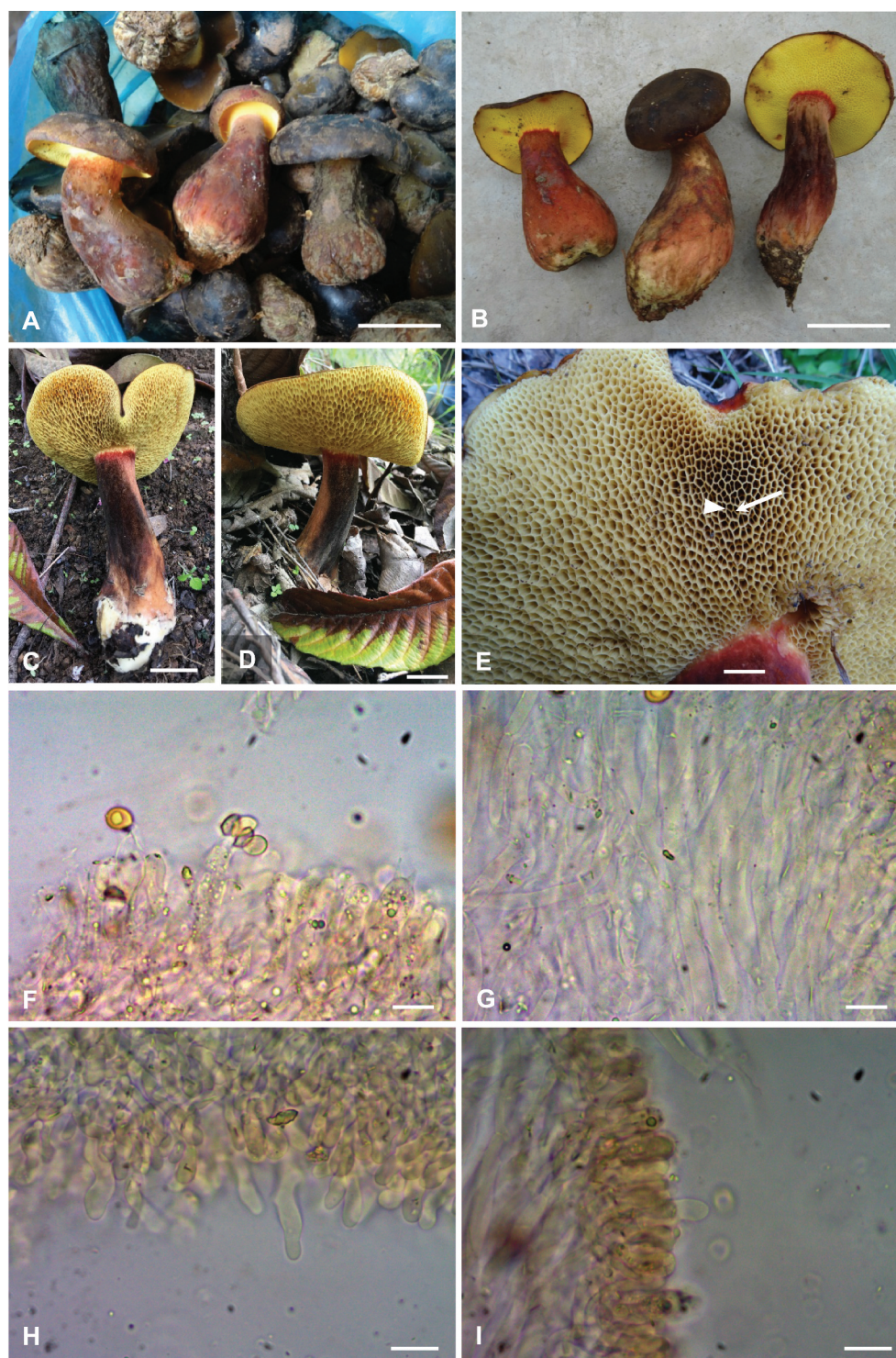


Figure 3. *Phlebopus roseus*. A. Mushrooms sold at a farmer's market, Miyi, Sichuan, China, include a mixture of basidiomata of *Phlebopus roseus* and *P. portentosus*. B. Three young basidiomata. C–D. Mature basidiomata occurring in an orchard of *Eriobotrya japonica*. E. Pores organised in a nested pattern with 2–5 small pores (thin walls; arrow) inside single larger pores (thick walls; arrow head). F. Basidia and basidiospores. G. Bilateral hymenophoral trama. H–I. Cystidia. Bars: A, B = 5 cm; C, D = 2.5 cm; E = 10 mm; F, G = 10 µm; H, I = 20 µm.

et al. 2009; Zhang et al. 2015). In 2013, we first found *P. roseus* within an orchard of *Eriobotrya japonica* (loquat) in Sichuan Province, China. Fruiting bodies occurred in close proximity to *P. portentosus* but were

distinguishable by their reddish stipes. Morphologically, the radiate tubular hymenophores with nested pores on mature mushrooms are more similar to *Boletinus* than to *Phlebopus*. Aside from this, and the rose-colored stipe,

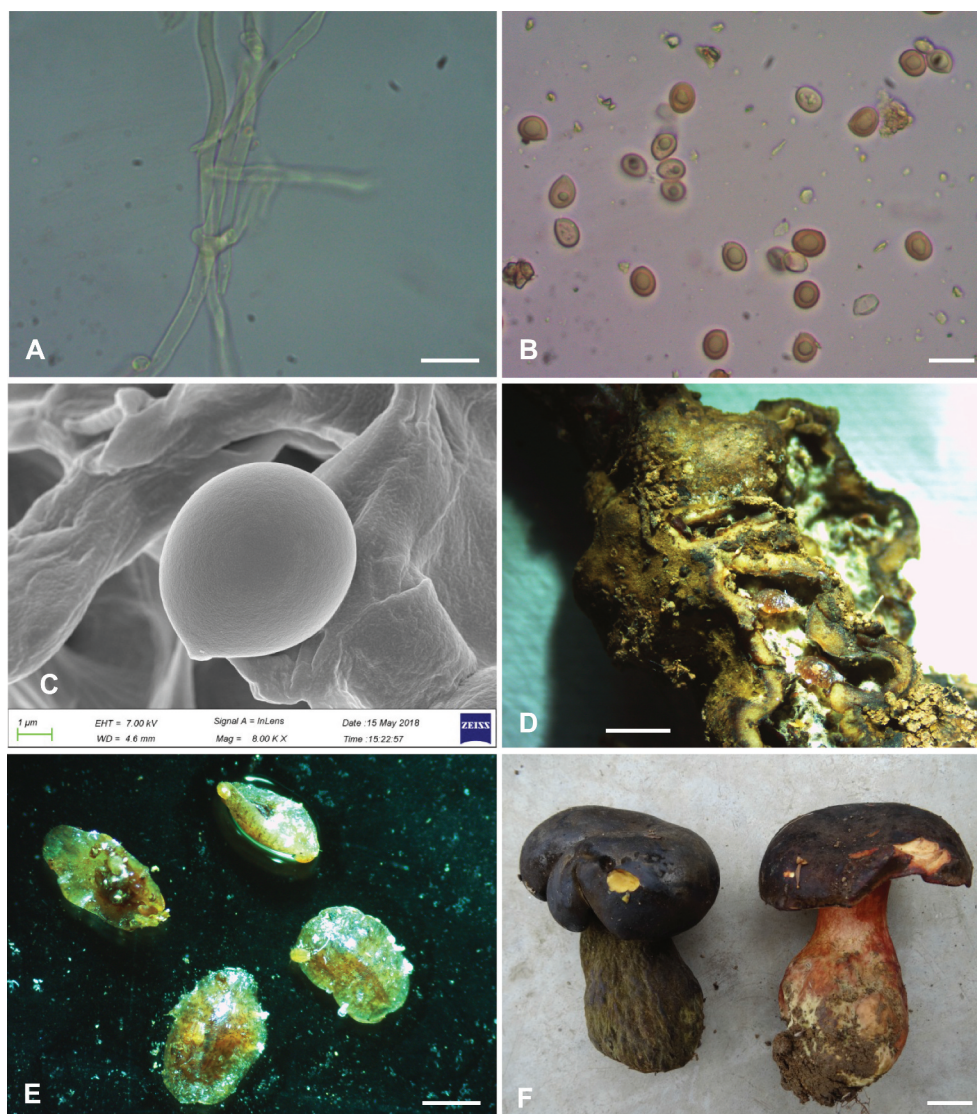


Figure 4. *Phlebopus roseus*. A. Clamp connections. B. Basidiospores. C. Scanning electron microscope photograph of basidiospore. D. Insect-fungal galls with scale insect (*Coccus hesperidum*) larvae. E. Scale bug larvae. F. *Phlebopus roseus* (right) and *Phlebopus portentosus* (left). Bars: A = 20 μm; B = 10 μm; D, E = 1 mm; F = 2.5 cm.

P. roseus morphologically resembles *P. portentosus* in terms of stature and shape. The reddish stipe of *P. roseus* (FIG. 3A–E) also distinguishes this bolete from other species of *Phlebopus*. Taken together, this set of characters is unique to any described bolete species. Phylogenetic analyses of three single-copy protein genes (*tef1*, *rpb1*, *rpb2*; FIG. 1) and rDNA (ITS, 28S; FIG. 2) also support *P. roseus* as a new species in the genus *Phlebopus*.

Over the last six years, we have been monitoring the only known presumed population of *P. roseus*. The yields have been very low compared with those of *P. portentosus*, which occurs in the same orchard (SUPPLEMENTARY TABLE 2). About 5 tons of *P. portentosus* are harvested each year from the orchard, whereas yields of *P. roseus* fruiting body production barely reaches 95–150 kg per year. Despite visiting nearby markets and having

many discussions with local farmers, we have found no evidence of *P. roseus* occurring in other locations in the region or elsewhere in China. In fact, *P. roseus* has only been found in four sites within a 24-ha orchard of *E. japonica* (SUPPLEMENTARY FIG. 1). Even though *P. roseus* may fruit in abundance locally, *P. roseus* appears to be endemic and geographically restricted in its distribution. Until additional data become available from herbarium materials or other reports, intense commercial harvesting of *P. roseus* from the type locality would be imprudent. Local efforts to conserve natural populations of this edible mushroom species and to protect its habitat have become urgent issues in Miyi County, China. Proposals are underway to the local authority to turn these sites into a natural reserve, but several legal issues remain to be addressed before any protective actions are

taken. For the time being, careful monitoring and management of the commercial harvesting of *P. roseus* are recommended. Pressing scientific questions that remain relate to whether the distribution of *P. roseus* depends on that of its plant host and insect associate, and whether collections of *P. roseus* exist in fungaria as an unclassified or misclassified collection.

It is noteworthy that dikaryotic mycelial cultures were obtained from *P. roseus* fruiting bodies. Not only does this provide genetic and biological resources for this species, but it also may indicate that this species has a certain degree of saprotrophic capacity. Fungi in the Boletiniaceae are interesting in that they occupy a trophic niche that includes insect symbionts (Brundrett and Kendrick 1987). When excavating beneath basidiomata in the field, we discovered that scale bugs were associated with *P. roseus* mycelial mats and formed fungus-insect gall structures around plant roots (FIG. 4D–E). Whether these fungi are able to utilize *C. hesperidum* honeydew, other metabolites, or necromass remains an open question. A related species, *P. portentosus*, is often associated with soil mealy bugs in the field (Fang et al. 2020). *Phlebopus portentosus* has been reported to associate with up to six species of insects in the family Pseudococcidae, and they are known to produce fungus-insect galls on plant roots (Zhang et al. 2015). Yet, *P. portentosus* can be cultivated as a saprotroph on dead organic substrates independent of mealy bugs (Ji et al. 2011). *Phlebopus roseus* appears to have a similar mode of nutrition as *P. portentosus*; however, the two bolete taxa associate with different species of insects. More work is required to determine the ecology of *P. roseus* and how scale bugs interact with this boletoid fungus and plant host. Understanding the ecological and physiological interactions between these organisms deserves further investigation. Future research to conserve and cultivate this new edible bolete species is strongly recommended.

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