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The phylogeny of selected *Phylloporus* species, inferred from NUC-LSU and ITS sequences, and descriptions of new species from the Old World

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Abstract The phylogeny of *Phylloporus* (Boletaceae) has not been well studied, and the taxonomic relationships of this genus have varied considerably among authors. The following study presents phylogenetic relationships of *Phylloporus* based on two nuclear ribosomal DNA regions, ITS and LSU. The ITS dataset includes 39 collections and the LSU dataset contains 50 collections of *Phylloporus*. A combined analysis of both genes did not resolve the deeper nodes in the phylogeny, but the results suggest that *Phylloporus* is monophyletic and a sister group of the *Xerocomus subtomentosus* group. The lamellate hymenophore configuration is a synapomorphy that distinguishes *Phylloporus* from the other genera in the family. The placement of a lamellate genus within Boletaceae suggests that hymenophore evolution is not well understood in the family. This is the first phylogeny of *Phylloporus* and includes 20 species from different geographic regions. Six taxa of *Phylloporus* from the Old World are here presented. *Phylloporus cyanescens* is a new combination for an Australasian taxon formerly named as a variety of *P. bellus* (Massee) Corner. *Phylloporus pumilus* is described from Indonesia, and 4 species are described from Thailand: *P. castanopsidis*, *P. dimorphus*, *P. infuscatus*, and *P. rubiginosus*.

Keywords Boletaceae · Agaricomycotina · Taxonomy · Distribution

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Introduction

Phylloporus is a relatively small genus in the Boletaceae and species in this genus are represented in tropical forests worldwide (Corner 1970; Heinemann and Rammeloo 1986; Montoya and Bandala 1991; Neves and Halling 2010; Singer and Gómez 1984; Singer et al. 1990; Watling 2008). This genus is considered to be best represented in Malaysia and Australia, where probably most of the described species are distributed (Corner 1970; 1974; Watling 2008). Fourteen other species in the genus have been recorded in Africa (Heinemann and Rammeloo 1987a, b). Five species are also found in North America (Bessette et al 2000; Neves and Halling 2010; Singer 1945; Smith and Thiers 1971) and the type species, *Phylloporus pelletieri*, is from Europe (Ladurner and Simonini 2003). However, most regions have not been well studied regarding this genus or other boletes, and new records are frequently reported (Li et al. 2011; Zeng and Yang 2011; Zeng et al. 2011).

The genus contains 70 named species; however, many parts of the neotropics and the paleotropics have not been

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extensively studied. Surveyed tropical collections suggest that some of these regions are hot spots for *Phylloporus* diversity, for example Malaysia (Corner 1970), Africa (Heinemann and Rammeloo 1986), Costa Rica (Neves and Halling 2010; Singer and Gómez 1984), and Colombia (Singer et al. 1990).

Most of the *Phylloporus* species are known to form mycorrhizae with various trees, including species of Fabaceae (Caesalpinioideae, Mimosoideae), Casuarinaceae, Dipterocarpaceae, Fagaceae, Myrtaceae, and Pinaceae (Halling and Mueller 2002; Heinemann and Rammeloo 1986).

Phylloporus was considered by Corner (1972) to be a primitive member of the Boletaceae due to the presence of a lamellate hymenophore. Pegler and Young (1981) defined *Phylloporus* as a derived genus for the same reason, but included smooth spores as a supporting feature. However, the spores of the type species, *P. pelletieri*, have a bacillate ornamentation just like some species in *Xerocomus* (Sutara 2008). It has been reported that *Phylloporus*, as in *Xerocomus*, has both subglobose and fusoid spored species (Neves and Halling 2010; Heinemann and Rammeloo 1986), supporting Pegler & Young's hypothesis (1981). Robust molecular phylogenies of the Boletales have shown *Phylloporus* as a derived genus placed next to *Xerocomus* (the *Xerocomus subtomentosus* group) (Binder 1999; Binder and Hibbett 2006).

The phylogenetic relationships in *Phylloporus* remain unclear despite several broad-scale studies of the Boletales (Binder and Bresinsky 2002; Binder and Hibbett 2006; Grubisha et al. 2001). A phylogenetic study by Binder (1999) based on molecular data of the nrLSU that included *Phylloporus rhodoxanthus* and *P. pelletieri*, placed *Phylloporus* within *Xerocomus* Quél. sensu stricto; however, Binder (1999) maintained *Phylloporus* and *Xerocomus* as independent genera because of morphological differences in the hymenophore configuration. He also noted the sister group relationship of *Phylloporus* with the *Xerocomus subtomentosus* complex (*X. illudens*, *X. lanatus*, and *X. subtomentosus*) and suggested that, based on his analyses, these taxa could be treated as a single genus.

Based solely on DNA sequences of two *Phylloporus* species, Bresinsky and Besl (2003) reduced the genus to synonymy with *Xerocomus* and suggested that the non-European species, with the exception of the North American *P. rhodoxanthus*, should be placed in a new genus. No new name was suggested and no tropical taxa were included in the analyses. Nevertheless, this synonymy exacerbates the systematic problems of *Phylloporus* especially since *Xerocomus* is not considered a monophyletic genus (Binder 1999; Binder and Hibbett 2006), reflected by some recent splits into satellite genera by Sutara (2008), including *Pseudoboletus* (*X. parasiticus*), *Xerocomellus* (the *X. chrysenteron* group) and *Hemileccinum* (*X. depilatus, X. impolitus*). The main difference between Xerocomus s. str. and *Phylloporus* is the lamellate hymenophore produced by *Phylloporus* species in contrast to the wide tubular hymenophore seen in *Xerocomus*. Even though the hymenophore of some species in *Phylloporus* shows a high degree of anastomosis, it is very rarely tubular as in *Xerocomus*, and this lamellate hymenophore is only one characteristic that typically distinguishes *Phylloporus* from other genera in the family.

The current study presents the most inclusive phylogeny of *Phylloporus* to date, and provides data necessary for studies of character evolution in the genus. One hypothesis that can be tested is: Are lamellate hymenophores in the Boletaceae evolved from tubular hymenophores, or vice versa? If the former hypothesis is correct, then lamellae in *Phylloporus* species has evolved secondarily via morphological reduction and would be convergent with lamellate hymenophores in the Agaricales.

In this work, six taxa are described; four are new species from Thailand and one is new from Indonesia. A new combination is also proposed from Malaya and Australia. Until now, no species of *Phylloporus* have been described from Thailand or Indonesia, and this study reports the first records for these countries. The results include color photographs of the described species, line drawings, and SEM micrographs of the spores.

Materials and methods

Specimens

The specimens were studied macro- and microscopically following traditional mycological methods (Largent 1986; Largent et al. 1977), and were tested for Imler's reaction (also known as *fleeting amyloid*) (Ladurner and Simonini 2003; Watling and Gregory 1991).

Color terms and codes (e.g., 5D3) are those of Kornerup and Wanscher (1978). The scanning electron microscopy (SEM) studies of the spores were made by mounting fragments of the hymenophore on aluminum stubs (EMS#75610) using carbon adhesive tabs (EMS#77825-12), and coating them with 10 nm of gold using a Hummer II sputter coater. The basidiospores were examined with a Hitachi S-2700 scanning electron microscope operating at 10KeV.

The descriptions were generated from a Delta database (Dallwitz 1980; Dallwitz et al. 1993 onwards). Herbarium acronyms are from Thiers (2012). Voucher material was studied and identified to morphological species.

The phylogenetic analyses include *Phylloporus* specimens from Australia, Belize, Costa Rica, Germany, Indonesia,

Malaysia, Mexico, Panama, Slovakia, Thailand, and the United States. These collections are listed in Table 1. In total, 20 species of *Phylloporus* and seven species of *Xerocomus* are included in the analyses. Sequences of the nuclear ribosomal DNA large subunit (nrDNA-LSU) were generated for 47 *Phylloporus* collections. An additional three LSU sequences were retrieved from GenBank. The LSU dataset also includes 14 sequences from *Xerocomus*, *Tylopilus*, *Boletus*, and *Aureoboletus*. Ribosomal DNA internal transcribed spacer (nrDNA-ITS) sequences were generated for 39 *Phylloporus* collections. Four ITS sequences were generated for *Xerocomus* and one was retrieved from GenBank.

Molecular methods

Ninety-eight new sequences were generated for this work, including 55 LSU sequences (47 from *Phylloporus*) and 43 ITS sequences (39 from *Phylloporus*).

The DNA was isolated from recent collections and from herbarium specimens. In the field, a small portion of the material to be used for DNA extraction was preserved in a tube with silica desiccant. For the herbarium specimens, a piece of the pileus was used in the extraction. In both cases DNA was extracted from samples using the E.Z.N.A.™ Fungal Miniprep Kit (Qiagen) after the sample of the specimen had been subjected to treatment in a Mini Beadbeater-8 cell disrupter (Biospec Products) to pulverize the material. The nrDNA-LSU sequences were amplified using primers LR5 and LR0R (Moncalvo et al. 2000) and cycle sequenced with these same primers plus the internal primers LR3R and LR3 (Vilgalys and Hester 1990). The primers ITS1-F (Gardes and Bruns 1993) and ITS4 (White et al. 1990) plus 5.8S and 5.8SR (Vilgalys and Hester 1990) were used for amplification and sequencing of the ITS regions (ITS1-5.8S-ITS2). PCR reactions were performed in 25 µl using *Taq* DNA polymerase under the following thermocycling conditions: initial denaturation at 94°C for 2 min followed by 30 cycles of 94°C for 30 sec, 55°C for 1 min, and 72°C for 1 min, followed by a final extension step of 72°C for 5 min. Amplification products were purified using Pellet Paint (Novagen) or the QIAquick PCR Purification Kit (QIAGEN Inc.) following the manufacturers' instructions. Sequencing was performed using Big Dye 3.1 chemistry on an Applied Biosystems (3730xl) automated DNA sequencer.

The combined dataset included 58 *Phylloporus* terminals and 15 non-*Phylloporus* collections. LSU was not amplified successfully in 9 out of the 73 collections included in the analyses. The ITS sequences were not successfully amplified for 27 collections included in this dataset and were then treated as missing data.

The failure to extract amplifiable DNA was probably because of the following: (1) the specimens were not properly dried and (2) the humidity was too high where the specimens were stored. First, if the temperature while drying a specimen is too high, the quality of the DNA will be affected. Second, fungal collections must be stored in a dry location, which discourages insect and mold attacks. In tropical regions, preservation of fungi collections has been difficult for many herbaria because they lack the proper facilities. In many cases, to avoid damage by insects and mold, the herbaria have placed mothballs with the specimens. It is possible that the mothballs also influence DNA extraction.

Analytical methods

Sequence chromatograms were edited and contigs assembled using Sequencher 4.5 (GeneCodes, Ann Arbor, Michigan). Nucleotide sequences were aligned using Clustal X 1.83.1 (Thompson et al. 1997) and adjusted by eye using MacClade 4.08 (Maddison and Maddison 2005) where necessary.

The datasets were analyzed using parsimony (MP), maximum likelihood (ML), and Bayesian methods (MB) as described below.

Parsimony analyses were performed in PAUP* 4.0b4 (Swofford 2002). All characters were treated as unordered and equally weighted, with gaps treated as missing data. A heuristic search was performed using 1000 random-addition sequences with one tree held at each step, tree bisection-reconnection (TBR) branch swapping, Multrees option enabled, and branches collapsed when maximum branch length equaled zero. Maxtrees settings were 1000 for the LSU and the ITS analyses (results not shown) and 2000 for the combined analyses. Branch support was assessed using 1000 bootstrap replicates with full heuristic searches, one random addition sequence per bootstrap replicate, and saving one tree per random addition sequence. *Aureoboletus auriporus* was defined as the outgroup for the LSU and the combined dataset because it is a clade sister to the *Xerocomus subtomentosus* complex clade, where *Phylloporus* is included in previous phylogenetic analyses (Binder 1999; Binder and Hibbett 2006). For the ITS dataset, where *Aureoboletus* was not included due to difficulties in the alignment, *Xerocomus illudens*, a taxon basal to *X. subtomentosus* according to the same phylogenetic works (Binder 1999; Binder and Hibbett 2006), was used as the outgroup.

Prior to ML and MB searches, an optimal model of sequence evolution was selected with the program Model Test 3.7 (Posada and Crandall 1998), using one of the most parsimonious trees to evaluate the models. Maximum likelihood analyses were implemented in PAUP* 4.0b10 (Swofford 2002) and Bayesian analyses were implemented

Table 1 Material included in the phylogenetic analysis of *Phylloporus* with the country of provenance, the respective GenBank accession numbers for LSU and ITS sequences, and the herbarium and collection numbers. Some sequences were retrieved from GenBank

Species	Origin	GenBank accession #		Collection
		LSU	ITS	number
Aureoboletus auriporus (Peck) Pouzar	USAeast	DQ534636		35.97
Aureoboletus auriporus (Peck) Pouzar	Costa Rica	JQ003659		MAN020
Boletus bicolor Peck	USAeast	AY612800		TH6933
Boletus leptospermi McNabb	New Zealand	DQ534632		NZ23
Phylloporus alborufus M.A. Neves & Halling	Costa Rica	JQ003678	JQ003624	MAN022
Phylloporus arenicola A.H. Sm. & Trappe	USA	JQ003704		JT27954
Phylloporus arenicola A.H. Sm. & Trappe	USAwest	JQ003660		DED6622
Phylloporus bellus (Massee) Corner	USAeast	JQ003686	JQ003618	REH8710
Phylloporus bellus (Massee) Corner	Japan	AY612817		MCA559
Phylloporus bellus (Massee) Corner	Costa Rica	JQ003661		REH7733
Phylloporus cyanescens (Corner) M.A. Neves & Halling	Australia	JQ003684	JQ003621	REH8681
Phylloporus bogoriensis Höhn.	Indonesia	JQ003680	JQ003625	DED7785
Phylloporus bogoriensis Höhn.	Malaysia		JQ003619	REH8691
Phylloporus caballeroi Singer	Panama	JQ003662	JQ003638	REH7906
Phylloporus castanopsidis M.A. Neves & Halling	Thailand	JQ003689	JQ003642	MAN104
Phylloporus castanopsidis M.A. Neves & Halling	Thailand	JQ003691	JQ003643	MAN107
Phylloporus castanopsidis M.A. Neves & Halling	Thailand	JQ003693	JQ003646	MAN118
Phylloporus castanopsidis M.A. Neves & Halling	Thailand	JQ003696		MAN124
Phylloporus centroamericanus Singer & L.D. Gómez	Costa Rica	JQ003663	JQ003637	MAN016
Phylloporus centroamericanus Singer & L.D. Gómez	Costa Rica		JQ003636	MAN018
Phylloporus centroamericanus Singer & L.D. Gómez	Costa Rica		JQ003635	MAN030
Phylloporus centroamericanus Singer & L.D. Gómez	Costa Rica	JQ003664	JQ003634	MAN037
Phylloporus centroamericanus Singer & L.D. Gómez	Costa Rica		JQ003633	MAN043
Phylloporus centroamericanus Singer & L.D. Gómez	Costa Rica		JQ003632	MAN057
Phylloporus centroamericanus Singer & L.D. Gómez	Costa Rica		JQ003631	MAN059
Phylloporus dimorphus M.A. Neves & Halling	Thailand		JQ003644	MAN111
Phylloporus dimorphus M.A. Neves & Halling	Thailand	JQ003697	JQ003648	MAN128
Phylloporus foliiporus (Murrill) Singer	USAeast	JQ003687	JQ003641	JLM1677
Phylloporus infuscatus M.A. Neves & Halling	Thailand	JQ003695		MAN123
Phylloporus leucomycelinus (Singer & M.H. Ivory) Singer	USAeast	JQ003677	JQ003628	MB00-043
Phylloporus leucomycelinus (Singer & M.H. Ivory) Singer	USAeast	JQ003667		REH8705
Phylloporus leucomycelinus (Singer & M.H. Ivory) Singer	USAeast	JQ003666	JQ003653	MB05-007
Phylloporus leucomycelinus (Singer & M.H. Ivory) Singer	USAeast	JQ003665		MB03-65
Phylloporus leucomycelinus (Singer & M.H. Ivory) Singer	USAeast	JQ003679		REH4582
Phylloporus leucomycelinus (Singer & M.H. Ivory) Singer	USAeast	JQ003673		PRL5805
Phylloporus leucomycelinus (Singer & M.H. Ivory) Singer	USAeast	JQ003710		MB03-038
Phylloporus orientalis Corner	Australia	JQ003700		REH8731
Phylloporus orientalis Corner	Australia	JQ003701	JQ003651	REH8755
Phylloporus orientalis Corner	Australia	JQ003709	JQ003652	REH8756
Phylloporus pelletieri (Lév.) Quél.	Germany	AF456818		Pp1
Phylloporus pelletieri (Lév.) Quél.	Slovakia	JQ003668	JQ003639	Q7199c
Phylloporus phaeoxanthus Singer & L.D. Gómez	Costa Rica	JQ003669		MAN017
Phylloporus phaeoxanthus Singer & L.D. Gómez	Costa Rica	JQ003670		MAN064
Phylloporus phaeoxanthus ssp simplex Singer & L.D. Gómez	Costa Rica	JQ003671		REH7388
Phylloporus pumilus M.A. Neves & Halling	Indonesia	JQ003682	JQ003626	REH8063
Phylloporus pumilus M.A. Neves & Halling	Indonesia	JQ003681	JQ003627	REH8062

Table 1 (continued)

Species	Origin	GenBank accession #		Collection
		LSU	ITS	number
Phylloporus purpurellus Singer	Costa Rica	JQ003672	JQ003630	MAN050
Phylloporus Quél.	Australia	JQ003685	JQ003620	REH8682
Phylloporus Quél.	Thailand	JQ003690		MAN105
Phylloporus Quél.	Thailand	JQ003698	JQ003649	MAN131
Phylloporus Quél.	China		JQ003640	48854
Phylloporus rhodoxanthus (Schwein.) Bres.	USAeast	JQ003688	JQ003654	JLM1808
Phylloporus rhodoxanthus (Schwein.) Bres.	USAeast	U11925		SAR 89.457
Phylloporus rhodoxanthus (Schwein.) Bres.	USAeast	JQ003674		MAN075
Phylloporus rhodoxanthus (Schwein.) Bres.	USAeast	JQ003676		MAN099
Phylloporus rhodoxanthus (Schwein.) Bres.	USAeast	JQ003675	JQ003629	REH8714
Phylloporus rhodoxanthus (Schwein.) Bres.	USAeast	x	x	MAN098
Phylloporus rubiginosus M.A. Neves & Halling	Thailand	JQ003692	JQ003645	MAN117
Phylloporus rubiginosus M.A. Neves & Halling	Thailand	JQ003694	JQ003647	MAN119
Phylloporus scabripes Ortiz, T.J. Baroni & Neves	Belize	JQ003683	JQ003623	REH8531
Phylloporus scabripes Ortiz, T.J. Baroni & Neves	Belize		JQ003622	REH8558
Phylloporus sp.1 *sensu* Watling	Australia	JQ003699	JQ003650	REH8729
Tylopilus P. Karst.	Australia	x		REH6808
Xerocomus spadiceus var. *gracilis* (A.H. Sm. & Thiers) L.D. Gómez	USAeast	JQ003703		MB04-022
Xerocomus hortonii (Sm. & Thiers) Binder & Besl	USAeast	AF139713		84.94
Xerocomus illudens (Peck) Singer	USAeast	AF139714		64.98
Xerocomus illudens (Peck) Singer	USAeast	JQ003705	JQ003658	MB03-055
Xerocomus illudens (Peck) Singer	USAeast	JQ003706		MB04-016
Xerocomus perplexus A.H. Sm. & Thiers	USAeast	JQ003702	JQ003657	MB00-005
Xerocomus Quél.			AY372285	not known
Xerocomus Quél.	Costa Rica	JQ003707	JQ003656	MAN061
Xerocomus Quél.	Costa Rica	JQ003708	JQ003655	MAN063
Xerocomus subtomentosus (L.) Fr.	Germany	AF139716		Xs1

in MrBayes 3.1.2 (Huelsenbeck and Ronquist 2001). Posterior probabilities (PP) for the combined dataset were determined by running one cold and three heated chains for 2 million generations in parallel mode, saving trees every 100th generation, and 2000 suboptimal trees at the beginning of the runs were discarded (burn-in phase).

Results

Phylogenetic analysis of combined LSU and ITS sequences

The combined data set for 73 sequences included 2795 characters with 1588 constant characters, 357 parsimony-uninformative characters, and 850 parsimony-informative characters. The parsimony analysis resulted in 2000 most parsimonious trees (L=3354 steps, CI=0.6035, RI=0.6903, RC=0.4166). The phylogenetic analysis using ML resulted in three trees (xln likelihood=19894.71357), the combined analysis is presented in Fig. 8.

Taxonomy

Five new species of *Phylloporus* and one new combination are here presented.

Phylloporus castanopsidis M.A. Neves & Halling **sp. nov.** (Figs. 1, 2)

MYCOBANK: MB 563611

ETYMOLOGY: castanopsidis, for the host tree *Castanopsis*

Pileus convex to plano-depressed to infundibuliform, dry, 0.8–3.5 cm broad, with NH_4 pale brown with pinkish tints. Context pale yellow, cyanescent. Lamellae adnexed, yellow, staining absent or light blue. Stipe equal, whitish, longitudinally ribbed, orangish towards the base. Context pale yellow. Basal mycelium pale yellow. Spores 7.7–10.5×3.5–4.2 μm. Clamp connections absent.


a
b
c
d
e
f
3.75 µm
g

◀ **Fig. 1** **a**. *Phylloporus castanopsidis*; **b**. *Phylloporus dimorphus*; **c** & **d**. *Phylloporus infuscatus*; **e**. *Phylloporus pumilus*; **f**. SEM of *P. pumilus* spores; **g**. *Phylloporus rubiginosus*

Pileus 0.8–3.5 cm broad, at first convex or uplifted, with age plano-depressed to infundibuliform, dry, even; at first brownish orange or pale red brown (8F6), then ochraceous brown (8E5, 8D5); margin smooth; becoming subsquamulose, with NH_4 pale brown with pinkish tints. *Flesh* pale yellow, staining pale blue; odor absent; taste inconspicuous; with NH_4 light pink. *Hymenophore* lamellate, adnexed. *Lamellae* subdistant, not anastomosing, sometimes inconspicuously intervenose, mostly simple, when young yellow (3A4), staining absent or light blue; edges even. *Stipe* 1–2 cm long, 2–5 mm wide, equal, curved, dry; upper half when young finely squamulose, whitish cream (4B2), with age longitudinally ribbed; on lower half when young orangish ochraceous; base pale yellow; interior solid; flesh when young pale yellow. *Basal mycelium* yellow, or pale yellow. *Fleeting-amyloid reaction* positive.

Basidiospores 7.7–10.5 μm long, 3.5–4.2 μm wide, mean Q=2.36, subfusoid, smooth, slightly dextrinoid, in KOH light brown melleous. *Basidia* 21.7–28 μm long, 7–8.4 μm wide, clavate, hyaline, 4 -sterigmate. *Hymenial cystidia* 47.6–59.5 μm long, 9.8–12.6 μm wide, numerous on sides and edges of lamellae, thin walled, hyaline, fusoid or subcylindrical, encrusting pigment absent. *Hymenophoral trama* bilateral or divergent (at the edge of the lamellae); hyphae cylindric, (4.9–)5.6–8.4 μm wide, hyaline, inamyloid. *Pileipellis hyphae* hymeniform, in KOH yellow; thin walled, intercalary cells cylindric. *Pileus trama* radial, hyphae hyaline, with elements 5.6–7 μm wide, smooth, thin walled. *Stipitipellis hyphae* vertically oriented, parallel, giving rise to clusters of caulocystidia, 18.9–36.4 μm long, 7–10.5 μm wide, clavate, hyaline. *Stipe trama* hyphae parallel, cylindric, hyaline, 6.3–11.2 μm wide. *Clamp connections* absent.

MYCORRHIZAL HOST: *Castanopsis*.

DISTRIBUTION: From northern Thailand, this species is only known from the type locality.

MATERIAL EXAMINED: THAILAND. Chiang Mai Province: Mae Sae, Highway 1095 at Km 55, 19°14′33.6″N, 98°38′29.4″E, 982 m, 3 June 2006, *Neves 104* (HOLOTYPE: MFLU08 1118, ISOTYPE: NY); 10 June 2006, *Neves 124* (MFLU08 1112, NY). Sangasabhasri lane to Huai Kok Ma village, Doi Suthep National Park, 18°48′24.3″N, 98°54′38″E, 1150 m, 7 June 2006, *Neves 118* (MFLU08 1116, NY).

Of the three collections examined, all basidiomes are of a small diameter and have a distinctly curved stipe. The small size, the curved stipe, and the light pink reaction with NH_4 on the pileus are diagnostic.

Phylloporus cyanescens (Corner) M.A. Neves & Halling, **stat. nov.** (Fig. 3)

MYCOBANK: MB 563627

Phylloporus bellus var. *cyanescens* Corner, Nova Hedwigia. 799. 1970.

Pileus 4–6 cm broad, at first convex, with age plane or eventually concave, dry; dark brown to cocoa brown; surface subtomentose to barely subvelutinous, becoming areolate with age, with NH_4 a blue-green flash then light lavender. *Flesh* white, staining pale blue to dark gray. *Hymenophore* lamellate, decurrent. *Lamellae* subdistant,

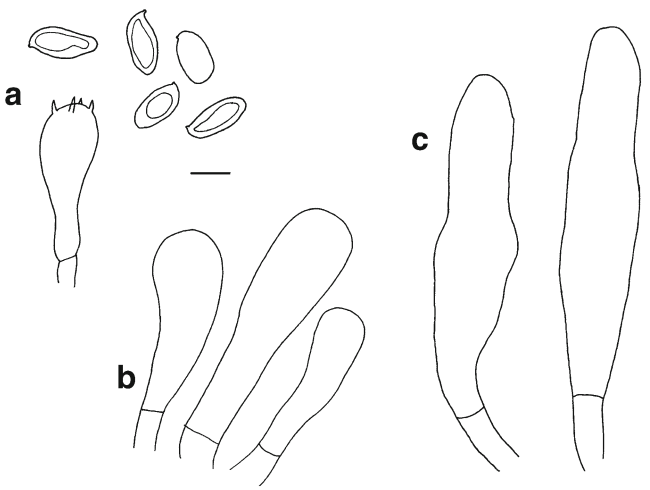

Fig. 2 *P. castanopsidis* **a.** Spores and basidium. **b.** Caulocystidia. **c.** Pleurocystidia. (Scale bar=10 μm.)

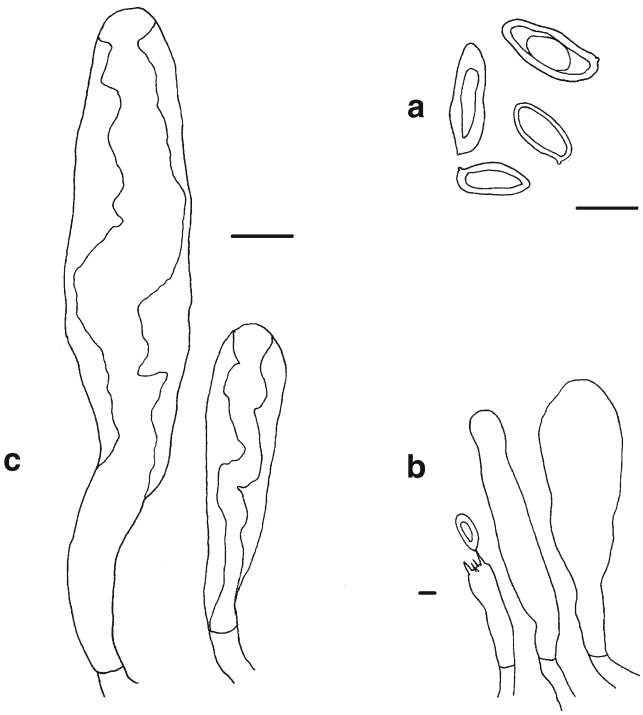

Fig. 3 *P. cyanescens* **a.** Spores. **b.** Basidium with spore and pleurocystidia. **c.** Pleurocystidia. (Scale bar=10 μm.)

anastomosing, bright yellow, staining blue-green. *Stipe* 4-5 cm long, 8–15 mm wide, mostly equal, dry; upper half subpubescent to matted subtomentose, white to light yellow; lower half subpubescent to matted subtomentose, white; base white; interior solid; flesh white, staining pale blue then grayish fuscous. *Basal mycelium* white. *Fleeting-amyloid reaction* positive.

Basidiospores olive in mass, 9–12.5 μm long, (2.7–) 3.5–5.5 μm wide, mean Q=2–3.3, lacrymoid to subfusoid, smooth, inamyloid, in KOH golden yellow to light brown melleous. *Basidia* 29–38 μm long, 6.5–10 μm wide, clavate, hyaline, 4 -sterigmate. *Hymenial cystidia* 32–81 μm long, 7.5–18 μm wide, numerous on sides and edges of lamellae, thin walled, ampullaceous or clavate-ventricose to ventricose to utriform to obtuse to mucronate, encrusting pigment sometimes present. *Hymenophoral trama* bilateral. *Pileipellis hyphae* a trichodermium or forming a palisade, in KOH pale yellow; elongated or short (eg. 11–16×10 μm, but mostly 20–28×6–7 μm), smooth, thin walled. *Stipitipellis hyphae* vertically oriented, parallel, giving rise to dermatocystidia, 30–55(–87) μm long, 5–20 μm wide, with incrusting pigment present. *Clamp connections* absent.

MYCORRHIZAL HOSTS: *Quercus* and *Castanopsis*.

DISTRIBUTION: First described from Malaya (Corner 1970), the species also have been reported from Australia (Watling and Gregory 1991, as a variety).

MATERIAL EXAMINED: AUSTRALIA. Victoria: Otway Range, Colac Otway Shire, Otway State Forest, 38º41′56″ S, 143º28′42″E, 72 m, 08 May 2005, *Halling 8681*(NY).

The diagnostic features are the white basal mycelium, the cyanescent lamellae and flesh that turn grayish, and thin-walled non-incrusted hymenial cystidia. *Phylloporus cyanescens* has longer spores and stronger, cyanescent flesh when compared to *P. bellus*. The molecular phylogeny does not support a similarity based on the collections included in the analyses, since var. *cyanescens* is positioned in a different clade from the two collections of *P. bellus* var. *bellus*. Singer (1978) conjectured that var. *cyanescens* could be *P. foliiporus*, a species from southern USA and Japan, and not a variety of *P. bellus*. However, the cystidia of var. *cyanescens* are longer and not melleous at the apex as in *P. foliiporus*. The molecular phylogenies based on LSU and ITS genes also separate these three taxa.

Elevation of var. *cyanescens* to species rank is warranted based on the morphological differences among the three taxa and on the phylogeny provided by the LSU and ITS analysis.

Phylloporus dimorphus M.A. Neves & Halling **sp. nov.** (Figs. 1, 4)

MYCOBANK: MB 563622

ETYMOLOGY: dimorphus (two forms), due to the presence of two differently shaped spores

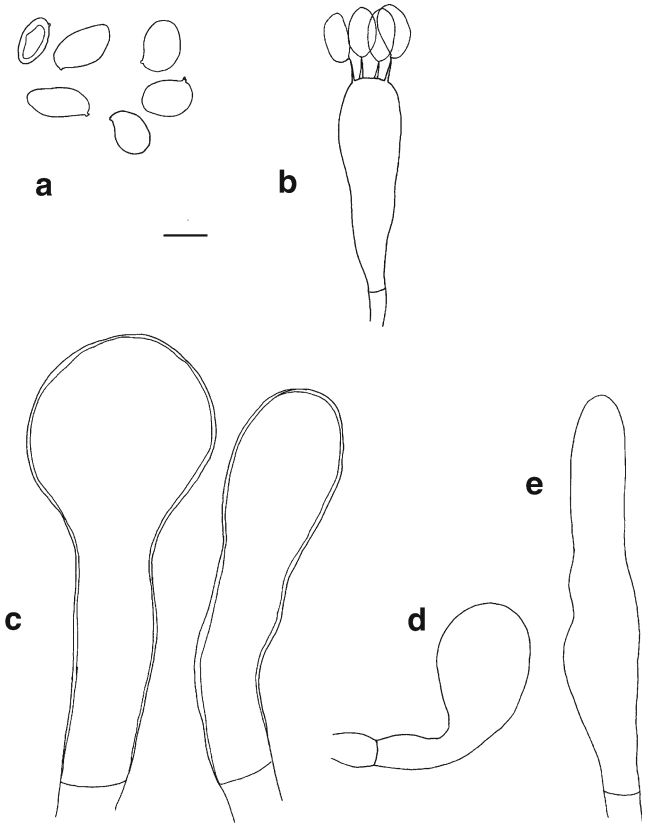

Fig. 4 *P. dimorphus* **a.** Spores. **b.** Basidium. **c.** Caulocystidia at the top of the stipe. **d.** Caulocystidium at the base of the stipe. **e.** Pleurocystidium. (Scale bar=10 μm.)

Pileus plano-convex, brownish yellow, dry, subtomentose, 6.2–7.9 cm broad, with NH_4 vinaceous red. Spores subfusoid or ellipsoid. Context pale yellow, staining pale blue. Lamellae decurrent, bright yellow, intervenose, staining blue (then brown). Stipe tapering downwards, pale yellow to light ochraceous. Context whitish yellow. Basal mycelium yellow. Spores 8.4–9.8×3.5–4.2 μm. Clamp connections absent.

Pileus 6.2–7.9 cm broad, at first convex, with age plano-convex, dry, entire or irregularly pitted, becoming irregularly pitted; disc smooth, at first brownish yellow, then ochraceous brown (5C4); margin slightly inrolled, ochraceous; surface finely felted, becoming subfibrillose, with NH_4 vinaceous red. *Flesh* pale yellow, staining pale blue; odor slightly acrid; with NH_4 no reaction. *Hymenophore* lamellate, decurrent. *Lamellae* subdistant, anastomosing, shallowly intervenose, bright yellow (2A6), staining blue (then brown); edges eroded. *Stipe* 6–7 cm long, 1.1 mm wide, tapering downwards, strict or curved, dry; upper half longitudinally ribbed, pale yellow to light ochraceous (5B4, 4B4); lower half longitudinally ribbed or scurfy, white or buff tan; with age yellow (concolorous with the lamellae); base pale yellow, staining not present; interior solid; with age whitish yellow, with NH_4 no reaction. *Basal mycelium yellow. Fleeting-amyloid reaction positive.*

Basidiospores 8.4–9.8 μm long, 3.5–4.2 μm wide, mean Q=2.36, subfusoid to fusoid; or 7–7.7 μm long, 4.2–4.9, μm wide, mean Q=1.69, ellipsoid to ovoid, smooth, inamyloid, in KOH ochraceous. *Basidia* 28–30.8 μm long, 7–7.7 μm wide, clavate, hyaline or pale yellow (some), 4 -sterigmate. *Hymenial cystidia* 53.2–60.9 μm long, 7.7–8.4 μm wide, more common towards the edge of lamellae, thin walled, hyaline, clavate-ventricose or cylindric, encrusting pigment absent. *Hymenophoral trama* bilateral; hyphae cylindric, 7.7–10.5 μm wide, hyaline, inamyloid. *Pileipellis hyphae* a trichodermium, in KOH hyaline, inamyloid; elements (4.9–)6.3–8.4 (–10.5) μm wide, cylindric, smooth, thin walled, granular content absent, intercalary cells cylindric. *Pileus trama* interwoven, hyphae light yellow, inamyloid, smooth, thin walled. *Stipitipellis hyphae* vertically oriented, parallel, giving rise to clusters of caulocystidia, 51.5–76.3 μm long (31.5–49), 8.4–25.2 μm wide (12.6–16), clavate or subfusoid (on the lower half) or sphaeropedunculate (on the upper half), hyaline, with incrusting pigment present (sometimes). *Stipe trama* hyphae parallel, cylindric, hyaline, inamyloid. *Clamp connections* absent.

MYCORRHIZAL HOST: *Castanopsis*.

DISTRIBUTION: Only known from northern Thailand.

MATERIAL EXAMINED: THAILAND. Chiang Mai Province: Ban Pha Deng village, Pathummikaram temple, 19°06′28.8″N, 98°44′47.3″E, 1050 m, 12 June 2006, *Neves 128* (HOLOTYPE: >MFLU08 1109, ISOTYPE: NY). Doi Inthanon National Park, Highway 1009 at 25 Km marker, 18°32′19.5″N, 98°33′42.5″E, 1050 m, 5 June 2006, *Neves 111* (MFLU08 1108, NY).

The two different spore morphologies are evident in the collections and a very diagnostic character for this taxon. The different shape of the caulocystidia at the base versus the apex of the stipe, and the vinaceous red reaction with NH_4 on the pileus are also diagnostic. *Phylloporus bellus* is a close species, reported from the neotropics by Singer and described by Massee from the Paleotropics, that produces spores in two size classes in some collections, but the caulocystidia are morphologically consistent. The vinaceous red reaction to NH_4 does not occur in *P. bellus*.

Phylloporus infuscatus M.A. Neves & Halling **sp. nov.** (Figs. 1, 5)

MYCOBANK: MB 563623

ETYMOLOGY: infuscatus (darkened), because of the somber color of the context

Pileus convex to plano-convex, dry, subrugulose, 2.7–3.2 cm broad, with NH_4 vinaceous red. Context ochraceous brown. Lamellae decurrent, yellow, staining light blue. Stipe equal, dark gray greenish at the top, yellow at the base. Context ochraceus brown to yellow. Basal mycelium white. Spores 6.3–7.7×3.5–4.2 μm. Clamp connections absent.

Pileus 2.7–3.2 cm broad, at first convex, with age plano-convex, dry, subrugulose; at first dark olive (4E6, 4F5), then olive-ochre to brown (5F5); with NH_4 vinaceous red. *Flesh* ochraceous brown or brown (6E4), staining absent; odor faint; taste mild; with NH_4 vinaceous red. *Hymenophore* lamellate, decurrent. *Lamellae* subdistant, not anastomosing, rarely intervenose, mostly simple, when young bright yellow (3A6), with age yellow, staining light blue; edges even. *Stipe* 3.4 cm long, 4–5 mm wide, equal, strict, dry; upper half when young pruinose, dark gray greenish (4B4) or yellow (pulverulent on the very top), with age finely pruinose, grayish green; lower half when young pruinose, pale yellow (5B3); pruina on upper half when young yellow; base pale yellow; interior solid; flesh above when young ochraceous brown or brown, staining not present, flesh at base when young yellow, staining not present. *Basal mycelium* white. *Fleeting-amyloid reaction* positive.

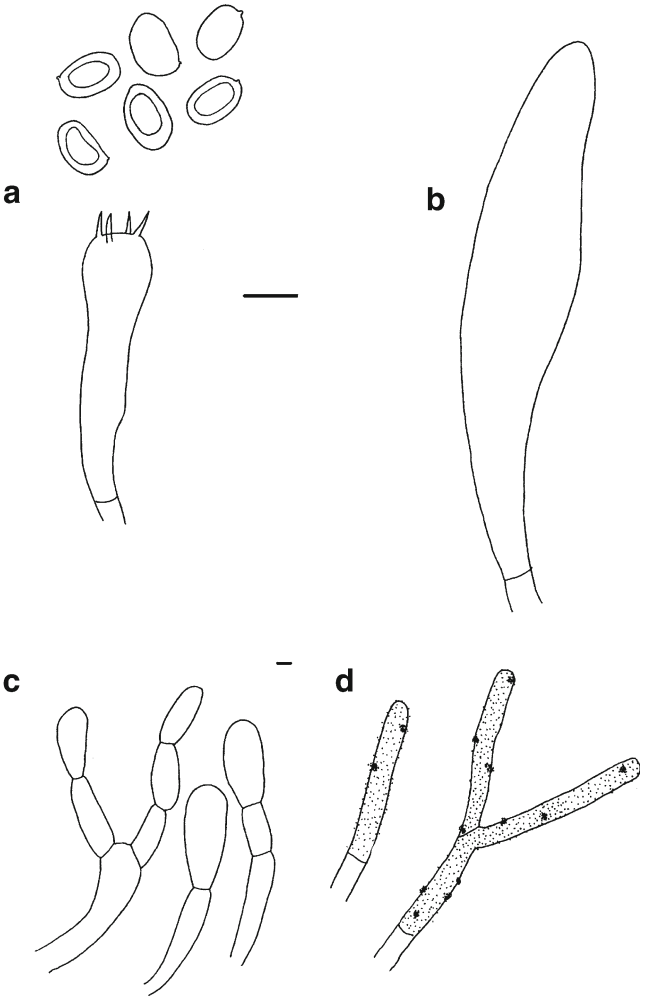

Fig. 5 *P. infuscatus* **a.** Spores and basidium. **b.** Pleurocystidium. **c.** Pileipellis hyphae. **d.** Stipitipellis hyphae. (Scale bar=10 μm.)

Basidiospores 6.3–7.7 μm long, 3.5–4.2 μm wide, mean Q=1.81, oblong or ovoid, smooth, weakly amyloid, in KOH greenish or hyaline. *Basidia* 25.9–30.8 μm long, 6–7 μm

wide clavate, hyaline, 4 -sterigmate. *Hymenial cystidia* 49–63 μm long, 10.5–13.3 μm wide, numerous on sides and edges of lamellae, thin walled, hyaline, fusoid or cylindric or clavate, encrusting pigment absent. *Hymenophoral trama* bilateral; hyphae cylindric. *Pileipellis hyphae* a trichodermium, in KOH tan (some); elements (7–)9.1–15.4 μm wide, in sphaerocyst-like chains, encrusted with pigment (red vinaceous exudate comes out when cuts are mounted in KOH), thin walled. *Stipitipellis hyphae* vertically oriented, parallel, giving rise to clusters of caulocystidia, 5.6–8.4 μm wide, subcylindric or cylindric (with pruina on the surface), yellow (in some hyphae), with incrusting pigment present (fine crystal-like incrustations at the surface). *Stipe trama* hyphae parallel, cylindric, hyaline, inamyloid. *Clamp connections* absent.

MYCORRHIZAL HOST: *Castanopsis*.

DISTRIBUTION: Collected only once in northern Thailand.

MATERIAL EXAMINED: THAILAND. Chiang Mai Province: Mae Sae, Highway 1095 at Km 55, 19°14′33.6″N, 98°38′ 29.4″E, 982 m, 10 June 2006, *Neves 123* (HOLOTYPE: MFLU08 1128, ISOTYPE: NY).

This taxon has unusual characteristics for a *Phylloporus*. The hymenophore features and the microscopic characteristics together with molecular data confirm placement in *Phylloporus*. The green color of the pileus is diagnostic and so is the dark color of the flesh, rather than the usual red-brown pilei and the light yellowish or whitish color of the flesh in the rest of the genus. It is important to note that the color of the flesh is not due to oxidation.

Phylloporus pumilus M.A. Neves & Halling **sp. nov.** (Figs. 1, 6)

MYCOBANK: MB 563624

ETYMOLOGY: pumilus, smaller than other species in the genus

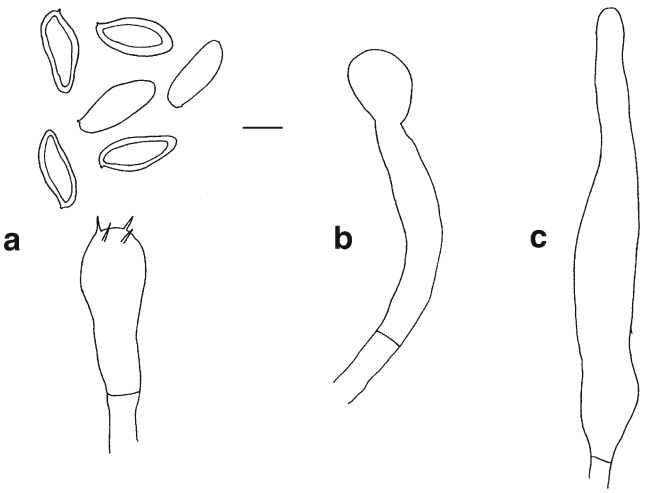

Fig. 6 *P. pumilus* **a.** Spores and basidium. **b.** Caulocystidium. **c.** Pleurocystidium. (Scale bar=10 μm.)

Pileus plano-convex to convex, brown, dry, areolate, 0.5–0.9 cm broad, with NH_4 dark or blue. Context white. Hymenophore alveolate decurrent, yellow. Stipe equal, pinkish brown. Basal mycelium white. Spores 10.5–11.9×3.5–4.9 μm. Clamp connections absent.

Pileus 0.5–0.9 cm broad, at first convex or plano-convex, with age plano-convex, dry, even; disc subtomentose, at first cocoa brown or dark brown (areolate tufts, with white between); margin smooth; becoming matted tomentose, with NH_4 dark or blue (around the drop). *Flesh* white, staining absent; odor mild. *Hymenophore* alveolate, decurrent. Tubes dull yellow, becoming wax yellow; then concolor with tubes, unchanging when injured. *Stipe* 0.7–1 cm long, less than 1 mm wide, equal, strict or curved, dry; upper half when young subpruinose, pinkish brown. *Basal mycelium* white. *Fleeting-amyloid reaction* positive (weakly).

Basidiospores 10.5–11.9 μm long, 3.5–4.9 μm wide, mean Q=2.67, subfusoid, smooth, with SEM smooth, in KOH greenish or melleous. *Basidia* 17.5–23.1 μm long, 6.3–7 μm wide, clavate, hyaline, 4 -sterigmate. *Hymenial cystidia* 52.5–63 μm long, 5.6–11.2 μm wide (4.2 on the top when lanceolate), numerous on sides and edges of lamellae (abundant), thin walled, hyaline, fusoid or lanceolate, encrusting pigment absent. *Hymenophoral trama* bilateral; 3.5–4.9(–5.6) μm wide, hyaline. *Pileipellis hyphae* a trichodermium, dextrinoid; cylindric, smooth, thin walled. *Stipitipellis hyphae* vertically oriented, parallel, giving rise to clusters of caulocystidia (some capitate), 3.5–4.9 μm wide, cylindric. *Stipe trama* hyphae parallel. *Clamp connections* absent.

MYCORRHIZAL HOST: *Dipterocarpus*.

DISTRIBUTION: Two collections of this taxon were gathered in Indonesia (Java) and are only known from the type locality.

MATERIAL EXAMINED: INDONESIA. Java: Haurbentes Park, 6°32.65′S, 106°26.26′E, 300 m, 14 January 2001, *Halling 8062*; (HOLOTYPE: NY), *Halling 8063* (NY).

This diminutive species is very distinct due to the clearly alveolate hymenophore and the small size of the basidiomes (seen fruiting abundantly on the soil substrate).

Phylloporus rubiginosus M.A. Neves & Halling **sp. nov.** (Figs. 1, 7)

MYCOBANK: MB 563625

ETYMOLOGY: rubiginosus (reddish with metallic tinge), with regard to the color of the pileus and the stipe

Pileus convex to plane, dar reddish brown to deep red brown, dry, 3.3–6.1 cm broad, with NH_4 blue. Context pale yellow, staining blue. Lamellae decurrent, yellow to orangish yellow, intervenose. Stipe equal, red brown. Context pale yellow, staining blue. Basal mycelium yellow. Spores 9.8–11.2×3.5–4.9 μm. Clamp connections absent.

Pileus 3.3–6.1 cm broad, at first convex or plano-convex, with age applanate, dry, even, becoming cracked; when

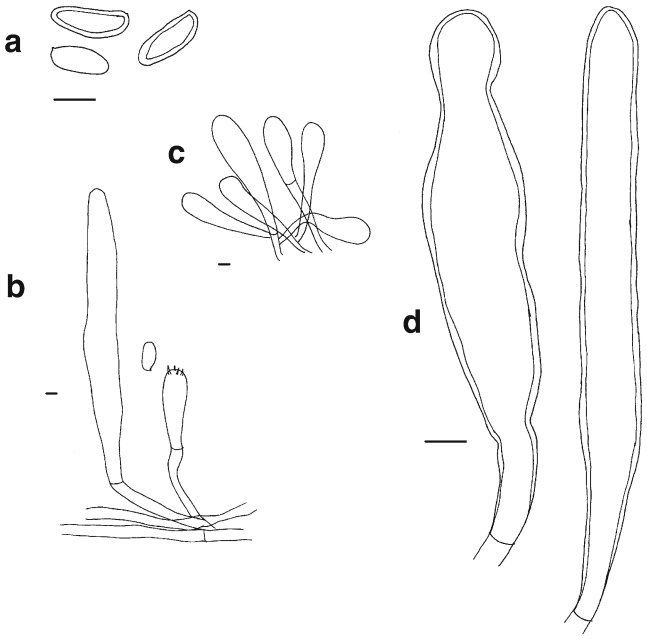

Fig. 7 *P. rubiginosus* **a.** Spores. **b.** Basidium with pleurocystidium and spore. **c.** Stipitipellis hyphae with caulocystidia. **d.** Pleurocystidia. (Scale bar=10 μm.)

young dark reddish brown (6D6, 6E6) or deep red brown, then yellow orange (7 F6, 8E7); surface squamulose, becoming subsquamulose (fracturing in some parts where the yellow flesh can be seen), with NH_4 blue. *Flesh* pale yellow, staining blue (sometimes slowly, after 30 seconds); odor mild; taste inconspicuous. *Hymenophore* lamellate, decurrent (separating from the stipe when older). *Lamellae* close or subdistant, not anastomosing, shallowly intervenose, when young yellow (4A6, 4B5), with age orangish yellow, staining blue; edges even. *Stipe* 3.5–5.5 cm long, 2–3 mm wide, equal, curved, dry; upper half when young finely pruinose, red brown, with age longitudinally ribbed; lower half when young finely pruinose (towards the middle, the pruina become smaller), pale brownish red (8E7); interior solid; flesh above when young pale yellow, staining blue, flesh at base when young yellow. *Basal mycelium* yellow. *Fleeting-amyloid reaction* positive.

Basidiospores 9.8–11.2 μm long, 3.5–4.9 μm wide, mean Q=2.5, subfusoid, smooth, weakly dextrinoid, in KOH straw yellow. *Basidia* 23.8–24.5 μm long, 7–8.4 μm wide, clavate, hyaline, 4 -sterigmate. *Hymenial cystidia* 88.9–100.8 μm long, 9.8–11.2 μm wide, numerous on sides and edges of lamellae, thick walled (1–2 μm), hyaline, fusoid or cylindric, encrusting pigment absent. *Hymenophoral trama* divergent; hyphae cylindric, 5.6–8.4 μm wide, hyaline. *Pileipellis hyphae* a trichodermium, in KOH brownish, inamyloid; elements (4.2–)5.6–7.7 μm wide, cylindric, encrusted with pigment (brown exudate when mounted in KOH), thin walled. *Pileus trama* interwoven, hyphae light yellow. *Stipitipellis hyphae* vertically oriented, parallel, giving rise to clusters of caulocystidia, 32.9–41.3 μm long, 9.8–15.4 μm wide, clavate or sinuous clavate, yellow brown contents, with incrusting pigment present (brown exudate is released when cuts are immersed in KOH). *Stipe trama* hyphae parallel, cylindric, pale yellow. *Clamp connections* absent.

MYCORRHIZAL HOSTS: *Castanopsis, Dipterocarpus*.

DISTRIBUTION: This taxon is only known from two collections in northern Thailand.

MATERIAL EXAMINED: THAILAND. Chiang Mai Province: Sangasabhasri lane to Huai Kok Ma village, Doi Suthep National Park, 18°48′24.3″N, 98°54′38″E, 1150 m, 7 June 2006, *Neves 117* (HOLOTYPE: MFLU08 1110, ISOTYPE: NY), *Neves 119* (MFLU08 1113, NY).

The dark red color of the pileus and stipe is notable. Other diagnostic characters include the blue reaction with the application of NH_4, the slowly cyanescent flesh, and the thick walled hymenial cystidia. The caulocystidia at the apex of the stipe tend to be more strictly clavate, while the ones at the middle and base are sinuous clavate.

Discussion

The methodologies used in this phylogenetic study showed similar results concerning the relationships among the species here included.

This study suggests that *Phylloporus* is a monophyletic genus, closely related to *Xerocomus*. The sister group to *Phylloporus* contains *X. perplexus* and *X. spadiceus* var. *gracilis*, while *X. subtomentosus* comes out in a basal grade to *Phylloporus*. The paraphyly of *Xerocomus* has been seen in other phylogenetic studies (Binder 1999; Binder and Hibbett 2006) and that genus is under examination by different groups of researchers (Peintner et al. 2003; Taylor et al. 2006).

The monophyly of the two isolates of *Phylloporus pelletieri*, the type species of the genus, in the phylogeny is supported by the LSU dataset (results not shown) and by the combined dataset (BS=97% and 98% respectively), however the position of the species is not resolved with confidence. *Phylloporus pelletieri* came out in the tree in a clade distinct from *P. rhodoxanthus* and morphological data also support these as two distinct species. The American species, *P. rhodoxanthus*, has a lamellate hymenophore and encrusted cystidia, compared to the more alveolate hymenophore and smooth cystidia of *P. pelletieri*, a species from Europe.

Xerocomus subtomentosus, the type species of *Xerocomus*, was also included in the studies and it was resolved along with other *Xerocomus* species at the base of the tree (Fig. 8).

Fig. 8 Phylogenetic relationships of *Phylloporus* inferred from the combined rDNA-LSU and rDNA-ITS dataset using maximum likelihood. Bootstrap values (>85%) are shown above the branches. Bayesian posterior probability values (probabilities >0.98) are shown below the branches. Branches present in both ML and MP trees are in bold. The groups within the dashed lines represent taxa with yellow basal mycelium. The letters A – E on the right correspond to the groups mentioned in the text. *Aureoboletus auriporus* was used as outgroup. The arrow marks the node that separates *Phylloporus* from *Xerocomus*

Within *Phylloporus*, five species form distinct groups with good node support that can be recognized in each phylogeny (LSU, ITS, combined). Species that form these well-supported groups (BS>85%, PP>0.98) are *P. leucomycelinus*, *P. centroamericanus*, *P. castanopsidis*, *P. dimorphus*, and *P. pumilus* (labeled from A to E on the tree).

Phylloporus dimorphus (Group B) and *P. castanopsidis* (Group A) are two new species that formed well supported groups and are closely related based on molecular data. They are both associated with *Castanopsis* forests in northern Thailand but can be morphologically differentiated by the two differently shaped spores of *P. dimorphus* and the light pink reaction of the flesh of *P. castanopsidis* when exposed to NH_4.

Phylloporus centroamericanus was the most frequently sampled species in this study, with sequences from seven collections included. This species is easily recognizable by its thick-walled cystidia, and it formed a consistent phylogenetic group (Group D) in the analyses. This is one of the most common *Phylloporus* species collected in oak forests in Costa Rica, followed by species in the *Phylloporus phaeoxanthus* complex.

The *P. phaeoxanthus* group (BS=99, PP=1) contains subspecies *phaeoxanthus* and subspecies *simplex*. *Phylloporus phaeoxanthus* ssp. *simplex* is a taxon included in this group that did not exhibit a significant difference at the molecular level; however, ssp. *simplex* can be morphologically distinguished by having incrusted cystidia, compared to the smooth cystidia seen in *P. phaeoxanthus*.

Phylloporus rhodoxanthus presented an interesting pattern, with the taxon placed in two different parts of the tree (Fig. 8). Three collections (MAN 98, MAN 99, JLM1808) were within the Thailand species group, with *P. castanopsidis* and *P. dimorphus*, while two collections (MAN75, MAN8714) were near *P. arenicola* and *P. phaeoxanthus*. However, it is interesting to note that when the gene sequences were analyzed in separate datasets, the position of these collections changed and they formed well-supported groups (BS >85%). In the LSU dataset, the collection JLM1808 from Alabama still came out related to the Thailand group, while the other four collections formed a group with 90% BS support. When the ITS database was analyzed three *P. rhodoxanthus* collections formed a strongly supported group (BS=100, PP=1).

Phylloporus rhodoxanthus is the most common species in the United States and is found mainly on the east coast, where its distribution overlaps with the distribution of *P. leucomycelinus*. These two species have been confused in the field because *P. leucomycelinus* is not as well known, but they can be separated because *P. leucomycelinus* has a white basal mycelium.

Phylloporus pumilus is a species that forms a distinct group in the analyses (Group C). This is a species from Indonesia with an alveolate hymenophore, and is distinct from the other alveolate species by its diminutive size.

The *P. leucomycelinus* group (Group E) includes the Panamanian collection of *P. caballeroi*; unfortunately no other collections of *P. caballeroi* were successfully amplified for either LSU or ITS. The collection was observed and is correctly identified and distinct from *P. leucomycelinus*. One single collection of *P. leucomycelinus* (4582) fell outside the main group, suggesting possible contamination of the isolated DNA, since the specimen has been observed and it is correctly identified. Closely related to the *P. leucomycelinus* group is a species from Costa Rica, *P. alborufus*, which also has white basal mycelium and a red colored pileus. This species was found in oak forests in Costa Rica and possibly migrated from the Northern Hemisphere to the montane neotropic oak forests as observed in other species (Halling 2001).

The sections proposed for *Phylloporus* by Heinemann and Rammeloo (1987a) and by Singer (1945) and Singer (1978) do not seem to follow a natural arrangement. The cited authors used morphological characters such as the oxidation of the flesh when exposed, spore ornamentation under a scanning electron microscope, and the presence or absence of clamp connections to create these sections. Sections *Phylloporus*, *Sulphurei*, and *Manausensis* were erected by Singer for species from the Americas, while sections *Phylloporus* sensu Heinemann and Rammeloo, *Oxydabiles*, *Immutabiles*, *Tubipedes*, and *Fibulati* were erected based on African species. When these characters are mapped in the molecular phylogenies it is observed that they are distributed in different clades and do not form the basis of natural arrangements.

A larger sample that would include more species that have been classified in these sections would give a better idea of the relationships within *Phylloporus* and the formation of natural sections. Unfortunately, none of the clamp connection-bearing species (section *Fibulati*) were successfully amplified and therefore these could not be included in the molecular analysis. A geographic pattern was not observed, although the sequences of *Phylloporus* included in this work are from various regions of the world. Unfortunately the African species were not available to study, which constitutes a gap in the phylogenetic analyses.

Yellow pigments have been shown to be present in members of the Boletaceae in different parts of the basidiome (Besl and Bresinsky 1997). *Phylloporus* species present these pigments in the basal mycelium, which constitute a valuable taxonomic character in the genus. In *Phylloporus,* the color of the basal mycelium has been traditionally used to separate species, and it was interesting to note that the species with yellow basal mycelium formed two groups within the genus, and was also present in *P. pelletieri* (Fig. 8). The white basal mycelium is present in the outgroup species used in this work, and appears to be plesiomorphic in *Phylloporus*.

The results suggest that yellow pigmentation in the basal mycelium is a character that has evolved three times (Fig. 8), or, alternatively, was gained once and lost four times, though the former explanation is more parsimonious.

This study might suggest that the lamellate hymenophore evolved once in *Phylloporus*, and it represents a reduction of the tubular hymenophore in the Boletaceae.

Large subunit sequence data have been shown to be useful for the delimitation of groups in Boletales (Binder 1999; Bresinsky et al. 1999) and its utility in delimiting *Phylloporus* species within the genus and from *Xerocomus* is demonstrated in the present study. The previous species concept based on the lamellate hymenophore is confirmed by the results of the molecular phylogenetic analyses based on LSU, ITS, and the combined analyses of these two genes. Basal relationships are not resolved, which is similar to the results of other studies that used only LSU (Moncalvo et al. 2000; Moncalvo et al. 2002; Peintner et al. 2003). The lack of success in amplifying ITS sequences from many of the specimens is probably one of the reasons why better resolution was not acquired for the basal relationships of the combined LSU and ITS analysis presented herein. It is expected that the inclusion of other genes in the dataset will improve the results.

The phylogenetic analyses suggest that *Phylloporus* is a monophyletic group distinct from *Xerocomus*; however, further analyses are needed to determine if the genus is monophyletic because the resolution of the backbone of the tree is not completely resolved. Also, broader analyses including diverse genera in the family and more *Xerocomus* species from different groups are needed to answer this question.

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