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Phylloporus and *Phylloboletellus* are no longer alone: *Phylloporopsis* gen. nov. (*Boletaceae*), a new smooth-spored lamellate genus to accommodate the American species *Phylloporus boletinoides*

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Abstract: The monotypic genus *Phylloporopsis* is described as new to science based on *Phylloporus boletinoides.* This species occurs widely in eastern North America and Central America. It is reported for the first time from a neotropical montane pine woodland in the Dominican Republic. The confirmation of this newly recognised monophyletic genus is supported and molecularly confirmed by phylogenetic inference based on multiple loci (ITS, 28S, *TEF1-α,* and *RPB1*). A detailed morphological description of *P. boletinoides* from the Dominican Republic and Florida (USA) is provided along with colour images of fresh basidiomata in habitat, line drawings of the main anatomical features, transmitted light microscopic images of anatomical features and scanning electron microscope images of basidiospores. The taxonomic placement, ecological requirements and distribution patterns of *P. boletinoides* are reviewed and the relationships with phylogenetically related or morphologically similar lamellate and boletoid taxa such as *Phylloporus*, *Phylloboletellus*, *Phyllobolites* and *Bothia* are discussed.

INTRODUCTION

Phylloporus is a genus of lamellate fungi in the family *Boletaceae* that is primarily distributed throughout the tropics (Kirk *et al.* 2008, Neves & Halling 2010, Neves *et al.* 2012, Zeng *et al.* 2013). Singer (1945b) moved *Phylloporus* to the subfamily *Xerocomoideae* due to its *Phylloporus*-type hymenophoral trama and olive-brown spore print. Bresinsky & Besl (2003) synonymised *Phylloporus* with *Xerocomus* based on molecular data, though only a few taxa were sampled in that study. Recent molecular phylogenetic analyses with more extensive sampling support the monophyly of *Phylloporus*, showing that *Xerocomus* is highly polyphyletic (Binder & Hibbett 2006, Šutara 2008, Nuhn *et al.* 2013, Wu *et al.* 2014, 2016) and inferring the placement of *Phylloporus* in an expanded *Xerocomoideae* that now also includes taxa with *Boletus*-type hymenophoral trama and pale yellow to brown spore prints (Šutara 2008, Wu *et al.* 2014, 2016).

Neves & Halling (2010) estimated the genus *Phylloporus* to include about 70 species, but several subsequent studies have described a number of additional taxa (Neves *et al.* 2010, 2012, García-Jiménez 2013, Zeng *et al.* 2013, Ye *et al.* 2014, Hosen & Li 2015, 2017). Species of *Phylloporus* form ectomycorrhizal (ECM) associations with plants in the *Casuarinaceae*, *Dipterocarpaceae*, *Fabaceae*, *Fagaceae*, *Myrtaceae*, and *Pinaceae* (Heinemann 1951, Corner 1970, Heinemann & Rammeloo 1987, Singer 1986, Watling 2008, Neves & Halling 2010, Neves *et al.* 2012, García-Jiménez 2013, Zeng *et al.* 2013, Ye *et al.* 2014, Hosen & Li 2015, 2017). Five species of *Phylloporus* have been described from North America (Neves 2007, Neves *et al.* 2010).

Phylloporus boletinoides is a lamellate to subporoid bolete that was formerly described based on material collected by Harry D. Thiers from Alachua Co., northern Florida (Smith & Thiers 1964) and subsequently reported from all along the Atlantic Coast of eastern and south-eastern USA south to the Gulf Coast (Singer *et al.* 1990, Singer & Williams 1992, Both 1993, Bessette *et al.* 2000, 2016, Justice 2008), down into Central America (Ortiz-Santana *et al.* 2007, Neves & Halling 2010), although not reported by Ortiz-Santana *et al.* (2007) as occurring in the Dominican Republic. It is a putative ECM partner of several American pine trees (*Pinus* spp., *Pinaceae*), but is also found in mixed pine and oak (*Quercus* spp., *Fagaceae*) forests (Smith & Thiers 1964, Singer *et al.* 1990, Both 1993, Bessette *et al.* 2000, 2016, Neves & Halling 2010).

Singer *et al.* (1990) re-described the type collection and placed the species in *Phylloporus* sect. *Fibulati*. The species was originally assumed to represent an intermediate taxon between *Suillus* and *Boletinus* (Smith & Thiers 1964). Singer *et al.* (1990) tentatively placed *P. boletinoides* in *Phylloporus* sect. *Manausenses* but later argued that it belonged to a new section that was never formally established (Singer & Williams 1992). As a matter of fact, this species has always been regarded as retaining an isolated position within the genus because of its peculiar morphological features with special reference to the pallid-coloured hymenophore and unusual macro-chemical reactions.

Several collections of *P. boletinoides* from Belize, the Dominican Republic and Florida were carefully examined using morphological and molecular approaches. Outcomes revealed strong morphological affinities with other members of *Phylloporus*, however, phylogenetic analysis of fungal DNA sequences from four gene regions (ITS, 28S, *TEF1-α,* and *RPB1*) indicate they are distantly related and support the recognition of *Phylloporopsis* as a unique and as yet monotypic generic lineage in the *Boletaceae*, in phylogenetic sister inference to the poroid genus *Bothia* and the sequestrate genus *Solioccasus*.

This study also widens the geographical extension of *P. boletinoides* to the Dominican Republic, where it is reported for the first time and is found in association with *Pinus occidentalis*, although several specimens have been spotted directly growing on decayed wood or even on trunks of living trees, determining some uncertainty relative to its trophic status.

MATERIALS AND METHODS

Collection site and sampling

Specimens from Belize, Dominican Republic, and the USA were examined from several public herbaria (CFMR, F, FLAS, JBSD, MICH, USF), as well as private herbaria [personal herbaria of Claudio Angelini (ANGE) and Matteo Gelardi (MG)]. Acronyms of the public herbaria follow Thiers (2018), Herbarium numbers are cited for all collections from which morphological features were examined. Author citations follow the Index Fungorum, Authors of Fungal Names (www.indexfungorum.org/authorsoffungalnames.htm).

Morphological studies

Macroscopic descriptions, macro-chemical reactions (30 % NH_4OH, 30 % KOH, $FeSO_4{\cdot}7H_2O$) and ecological information, such as habitat, time of fruiting and associated plant communities accompanied the detailed field notes of the fresh basidiomata. Colour terms in capital letters (e.g. Light Buff, Plate XV) are from Ridgway (1912). Microscopic anatomical features were observed and recorded from revived dried material. Sections were rehydrated in water, 5 % potassium hydroxide (KOH), or in anionic solution saturated with Congo Red. All anatomical structures were measured from preparations in anionic Congo Red. Colours and pigments were described after examination in water and 5 % KOH. Measurements were made at 1000 × using an ocular micrometer. Basidiospores were measured directly from the hymenium of mature basidiomata, dimensions are given as (minimum) average ± standard deviation (maximum), Q = length/width ratio with the extreme values in parentheses, Qm = average quotient (length/width ratio) ± standard deviation and average spore volume was approximated as a rotation ellipsoid [$V = (\pi.L.W^2)/6$ ± standard deviation]. The notation [n/m/p] indicates that measurements were made on “n” randomly selected basidiospores from “m” basidiomata of “p” collections. The width of each basidium was measured at the widest part, and the length was measured from the apex (sterigmata excluded) to the basal septum. Metachromatic, cyanophilic and iodine reactions were tested by staining the basidiospores in Brilliant Cresyl blue, Cotton blue and Melzer’s reagent, respectively. Line drawings of microstructures were traced free hand based on digital photomicrographs of rehydrated material. Scanning electron micrographs have been obtained using a JEOL JSM IT300LV (High Vacuum – Low Vacuum 10/650 Pa - 0.3-30kV) Scanning Electron Microscope (SEM) operating at 10 kV. Selected air dried lamellae from the hymenophore were fixed on pin stubs using a Carbon Conductive Cement glue and then sputter-coated with carbon to a thickness of 400 Å.

DNA extraction, PCR amplification and DNA sequencing

DNA extraction and PCR amplification were performed from dried basidiomata (Table 1) or from tissues preserved in CTAB buffer as described by Alvarado *et al.* (2015). Primers ITS1F and ITS4 (White *et al.* 1990, Gardes & Bruns 1993) were used for the ITS region; primers LR0R and LR5 (Vilgalys & Hester 1990, Rehner & Samuels 1994) were used for the 28S rDNA, EF1-983F and EF1-1567R (Rehner & Buckley 2005) for the translation elongation factor 1-α (*TEF1-α*) gene. Amplifications of DNA-directed RNA polymerase II subunit 1 region (*RPB1*) were attempted using the fungal primer pair RPB1-Af/RPB1-Cr and the bolete-specific primer pair RPB1-B-F/RPB1-B-R (Wu *et al.* 2014) but were unsuccessful. To increase amplification success, *RPB1*-primers specific to the *Bothia* clade were developed (Table 2). A touchdown PCR was used to amplify the *RPB1* region with the newly developed primer pairs RPB1-32-F/RPB1-835-R, RPB1-147-F/RPB1-1091-R. The cycle parameters were as follows: (1) 94 ºC for 2 min, (2) 94 °C for 40 s, (3) 66 °C for 40 s, minus 1 °C every cycle, (4) 72 °C 90 s, (5) repeat steps 2–4 for nine additional cycles, (6) 94 °C for 45 s, (7) 56 °C for 90 s, (8) 72 °C for 90 s, (9) repeat steps 6–8 for 35 cycles, (10) 72 °C for 300 s. The PCR products were purified with the Wizard SV Gel and PCR Clean-Up System (Promega) following manufacturer’s instructions and sequenced forward and reverse by MACROGEN Inc. (Seoul, Republic of Korea).

Sequence alignment, data set assembly and phylogenetic analyses

The sequences obtained in this study were checked and assembled using Geneious v. 11.1.4 (Kearse *et al.* 2012) and compared to those available in GenBank database by using the Blastn algorithm (Altschul *et al.* 1990). Chromatograms were examined and manually edited for accuracy. Sequences were submitted to GenBank (http://www.ncbi.nlm.nih.gov/genbank/) and accession numbers are reported in Table 1. Homologous sequences from vouchered specimens and from environmental samples were selected and retrieved from Halling *et al.* (2007), Trappe *et al.* (2013), Zeng *et al.* (2013), Zhu *et al.* (2015) and Orihara & Smith (2017). A general combined Maximum likelihood tree including all the *Boletaceae* sequences deposited in GenBank and UNITE (http://unite.ut.ee/) databases was generated to detect the phylogenetic position of our collections in the major clades of *Boletaceae* as circumscribed by Wu *et al.*

Table 1. Specimens and sequences used for the molecular phylogenetic analyses (i.e., the ITS dataset and the 28S + *TEF1-α* and 28S + *RPB1* combined datasets). Sequences newly generated for this study are highlighted in bold.

Taxon	Voucher No.	Locality	ITS	28S	*TEF1-α*	*RPB1*
Afroboletus luteolus	00-436	Africa	-	KF030238	KF030397	-
Afrocastellanoa ivoryana	Arora 126	Mukuvisi, Zimbabwe	-	KX685721	KX685715	-
Australopilus palumanus	REH 6791	Queensland, Australia	-	JX889650	JX889691	-
Austroboletus fusisporus	HKAS75207	China	-	JX889720	JX889718	-
Austroboletus gracilis	112/96	MA, USA	-	DQ534624	KF030425	KF030358
Austroboletus mutabilis	BRI AQ0795793	Queensland, Australia	-	KP242263	-	KP242078
Austroboletus subvirens	BRI AQ0794171	Queensland, Australia	-	KP242227	-	KP242045
Baorangia bicolor	MB07-001	NY, USA	-	KF030246	-	KF030370
Baorangia pseudocalopus	HKAS 63607	China	-	KY418895	-	KJ184564
	HKAS 75739	China	-	KJ184558	-	KF030369
	HKAS 75081	Nanhua County, Yunnan Prov., China	-	KF112356	KF112168	-
Boletus aereus	REH 8721	Humboldt County, California, USA	-	KF030339	-	KF030377
Boletus edulis	Be3	Bavaria, Germany	-	KF030282	GU187682	-
	BD380	Colorado, USA	-	HQ161848	-	-
Boletus pulchriceps	DS4514	Chiricahua Mnts, AZ, USA	-	KF030261	KF030409	-
Boletus rufomaculatus	4414	Chestnut Ridge Park, NY, USA	-	KF030248	KF030406	KF030369
Boletus semigastroideus	PBM3076	Auckland, New Zealand	-	KF030352	KF030430	-
Boletus variipes var. *fagicola*	A.H. Smith 4249	Cheboygan Co, Michigan, USA	-	JQ327014	JQ327017	-
Borofutus dhakanus	HKAS 73792	Bangladesh	-	JQ928617	JQ928575	-
Bothia castanella	MB 03-053	MA, USA	DQ867110	DQ867117	KF030421	KF030382
	NY28003	NY, USA	DQ867111	-	-	-
	NY8669	NY, USA	DQ867112	-	-	-
	NY28002	NY, USA	DQ867113	-	-	-
	MB 03-067	MA, USA	DQ867114	-	-	-
Bothia fujianensis	HKAS 82693	Fujian Prov., China	KM269196	-	-	-
	HKAS 82694	Fujian Prov., China	KM269195	KM269193	KM272860	-
Buchwaldoboletus lignicola	HKAS 76674	Yichun, Heilongjiang Prov., China	-	KF112350	KF112277	KF112642
Butyriboletus appendiculatus	Bap1	Bavaria, Germany	-	AF456837	JQ327025	-
Butyriboletus roseoflavus	HKAS 54099	China	-	JX290184	-	KF739741
Caloboletus firmus	MB 06-060	Chestnut Ridge Park, NY, USA	-	KF030278	KF030408	KF030368
Caloboletus inedulis	MB 06-044	Erie Co., NY, USA	-	JQ327013	JQ327020	KF030362
Chalciporus piperatus	MB 04-001	Rutland State Park, MA, USA	-	DQ534648	GU187690	GU187453
Fistulinella prunicolor	REH 9502	Fraser Island, Queensland, Australia	-	JX889648	JX889690	-
Gymnogaster boletoides	REH 9455	Cooloola, Queensland, Australia	-	JX889673	JX889683	-
Gyrodon lividus	Gl1	Bavaria, Germany	-	AF098378	GU187701	-
Harrya chromapes	HKAS 50527	Dêqên, Yunnan Prov., China	-	KF112437	KF112270	-
Heliogaster columellifer	KPM-NC 23012	Odawara, Kanagawa Pref., Japan	-	KX685724	KX685718	-
Hourangia cheoi	HKAS 52269	China	-	KF112385	KF112286	-
Hourangia microcarpa	HKAS 83763 (Wu1324)	China	-	KP136945	KP136923	-
Hourangia nigropunctata	HKAS 76657	China	-	KF112388	KF112287	-
Hourangia sp.	HKAS 68178	China	-	KF112453	KF112301	-
Imleria badia	xb2	Bavaria, Germany	-	KF030357	KF030422	-

Table 1. (Continued).

Taxon	Voucher No.	Locality	ITS	28S	*TEF1-α*	*RPB1*
	S-F119691	Sweden	-	KJ806971	KJ806969	-
Imleria obscurebrunnea	HKAS 52557	Ning'er, Yunnan Prov., China	-	KF112374	KF112190	KC215225
Lanmaoa angustispora	HKAS 74759	China	-	KM605140	-	KM605167
Lanmaoa asiatica	HKAS 63592	Heqing, Yunnan Prov., China	-	KM605142	KM605152	KM605163
Lanmaoa carminipes	MB 06-061	Erie Co., NY, USA	-	JQ327001	JQ327022	-
Leccinellum aff. *griseum*	KPM-NC 17831	Hyogo Pref., Japan	-	JN378508	JN378449	-
Leccinellum cremeum	HKAS 90639	China	-	-	-	KT990936
Leccinellum crocipodium	KPM-NC 18041	Yazu-cho, Tottori Pref., Japan	-	KC552053	KC552094	-
Leccinum monticola	HKAS 76669	China	-	KF112443	-	KF112592
Leccinum scabrum	KPM-NC 17840	Burn O' Vat, Scotland, UK	-	JN378515	JN378455	-
Leccinum versipelle	KPM-NC 17833	Scotland, UK	-	JN378514	JN378454	-
Neoboletus magnificus	HKAS 74939	Baoshan, Yunnan Prov., China	-	KF112320	KF112148	-
Nigroboletus roseonigrescens	GDGM 43238	Guangdong Prov., China	-	KT220588	KT220595	-
Octaviania decimae	KPM-NC 17763	Mt. Hiei, Kyoto Pref., Japan	-	JN378465	JN378409	-
Octaviania kobayasii	KPM-NC 17785	Mt. Kasuga, Nara Pref., Japan	-	JN378478	JN378420	-
Octaviania nonae	KPM-NC 17748	Amami-oshima, Kagoshima Pref., Japan	-	JN378459	JN378403	-
Octaviania tasmanica	MEL2341996	Tasmania, Australia	-	JN378495	JN378436	-
Octaviania yaeyamaensis	KPM-NC 17819	Ishigaki Isl., Okinawa Pref., Japan	-	JN378491	JN378432	-
Paragyrodon sphaerosporus	MB06-066	Iowa, USA	-	GU187593	-	-
Paxillus vernalis	MB062 (CUW)	China	-	AY645059	-	-
Phylloboletellus chloephorus	XAL3388	Veracruz, Municipio Coatepec, El Grande, Mexico	-	DQ534658	-	-
Phylloporopsis boletinoides	**JBSD127411**	**Jarabacoa, Dominican Republic**	**MH571675**	**MH571711**	**MH588312**	**-**
	JBSD127412	**Jarabacoa, Dominican Republic**	**MH571676**	**MH571712**	**MH588313**	**-**
	JBSD127413	**Jarabacoa, Dominican Republic**	**MH571677**	**MH571713**	**MH588314**	**-**
	JBSD127414	**Jarabacoa, Dominican Republic**	**MH571678**	**MH571714**	**MH588315**	**-**
	FLAS-F-60407	**Putnam County, Florida, USA**	**MG845193**	-	-	-
	FLAS-F-60413	**Putnam County, Florida, USA**	**MG845194**	-	-	-
	FLAS-F-61158	**Putnam County, Florida, USA**	**MH211774**	**-**	**-**	**-**
	Farid 617 (USF 296126)	**Tampa, Florida, USA**	**MG817716**	**MG817715**	**-**	**MG820263**
	CORT014483	**Mountain Pine Ridge, Belize**	**MH571679**	**MH571715**	**MH588316**	**-**
	CORT010991	**Kountze, Texas, USA**	**-**	**MH571716**	**MH588317**	**-**
	F1118420	**Sarasota, Florida, USA**	**MH571680**	**MH571717**	**-**	**-**
Phylloporus attenuatus	HKAS 76168 (holotype)	Tangail, Bangladesh	-	NG_059569	KR094791	-
Phylloporus bellus	HKAS 56763	Yunnan, SW China	-	JQ967196	JQ967153	-
Phylloporus brunneiceps	HKAS 56903	Yunnan, SW China	-	JQ967198	JQ967155	-
Phylloporus catenulatus	HKAS 76157	Bangladesh	-	KR094779	KR094789	-
Phylloporus gajari	HKAS 76158	Gazipur, Bangladesh	-	KR231697	KR231695	-
Phylloporus imbricatus	HKAS 54647	Yunnan, SW China	-	JQ967202	JQ967159	-
	HKAS 68642	China	-	KF112398	KF112299	-
Phylloporus leucomycelinus	HKAS 74678	eastern USA	-	JQ967206	JQ967163	-
Phylloporus luxiensis	HKAS 57036	Yunnan, SW China	-	JQ967207	JQ967164	-
	HKAS 75077	China	-	KF112490	KF112298	KF112636

Table 1. (Continued).

Taxon	Voucher No.	Locality	ITS	28S	*TEF1-α*	*RPB1*
Phylloporus maculatus	HKAS 56683	Yunnan, SW China	-	JQ967210	JQ967167	-
Phylloporus pachycystidiatus	HKAS 54540	Yunnan, SW China	-	JQ967211	JQ967168	-
Phylloporus parvisporus	HKAS 54768	Yunnan SW China	-	JQ967214	JQ967171	-
Phylloporus pelletieri	Pp1	Austria	-	AF456818	JQ327036	KF030390
Phylloporus pelletieri	Q7199c	Slovakia	-	JQ003668	-	-
	K 128205	England, UK	-	JQ967215	-	-
Phylloporus rhodoxanthus	SAR 89.457	eastern USA	-	U11925	-	-
	MAN075	eastern USA	-	JQ003674	-	-
	REH8714	eastern USA	-	JQ003675	-	-
	MAN099	eastern USA	-	JQ003676	-	-
	JLM1808	eastern USA	-	JQ003688	-	-
	BD374		-	HQ161851	-	HQ161820
Phylloporus rubeolus	HKAS 52573	Yunnan, SW China	-	JQ967216	JQ967172	-
Phylloporus rubrosquamosus	HKAS 54542	Yunnan, SW China	-	JQ967217	JQ967173	-
	HKAS 52552	China	-	KF112391	KF112289	-
Phylloporus rufescens	HKAS 59722	Hainan, southern China	-	JQ967220	JQ967176	-
Phylloporus yunnanensis	HKAS 52225	Yunnan, SW China	-	JQ967222	JQ967178	-
Phylloporus sp.	HKAS 74679	Hunan, central China	-	JQ967228	JQ967184	-
	HKAS 74680	Fujian, SE China	-	JQ967229	JQ967185	-
	HKAS 74681	Hainan, southern China	-	JQ967227	JQ967183	-
	HKAS 74682	Yunnan, SW China	-	JQ967230	JQ967186	-
	HKAS 74683	Yunnan, SW China	-	JQ967231	JQ967187	-
	HKAS 74684	Fujian, SE China	-	JQ967232	JQ967188	-
	HKAS 74685	Yunnan, SW China	-	JQ967233	JQ967189	-
	HKAS 74687	Yunnan, SW China	-	JQ967235	JQ967190	-
	HKAS 74688	Yunnan, SW China	-	JQ967236	JQ967191	-
	HKAS 74689	Hainan, southern China	-	JQ967237	JQ967192	-
Porphyrellus brunneus	REH 9527	Fraser Island, QLD, Australia	-	JX889647	JX889689	-
Porphyrellus porphyrosporus	KPM-NC 22667	Mt. Tarumae, Hokkaido, Japan	KX685713	KX685722	KX685716	-
	KPM-NC 25017	Rishiri Island, Hokkaido, Japan	KX685714	KX685723	KX685717	-
	MB 97-023	Walhalla, Bavaria, Germany	DQ534563	DQ534643	GU187734	-
	HKAS 76771	Yanbian, Jilin Prov., China	-	KF112482	KF112243	-
Porphyrellus sp.	HKAS 53366	Sanming, Fujian Prov., China	-	KF112480	KF112241	-
	HKAS 75078	Chuxiong, Yunnan Prov., China	-	KF112481	KF112242	-
Pseudoboletus parasiticus	Xps1	Bavaria, Germany	-	AF050646	KF030443	-
Retiboletus fuscus	HKAS59460	Yunnan Prov., China	-	JQ928626	JQ928580	-
Retiboletus griseus	Both sn	NY, USA	-	KF030308	KF030414	KF030373
Rossbeevera griseovelutina	TNS-F-36989	Hyogo, Japan	-	KC552031	KC552076	-
Rossbeevera vittatispora	MEL2128491	NSW, Australia	-	KX685725	KX685719	-
Royoungia boletoides	Trappe 27456	NSW, Australia	-	JX889655	JX889696	-
Rubroboletus rhodosanguineus	4252	Chestnut Ridge Park, NY, USA	-	KF030252	KF030412	-
Solioccasus polychromus	J. Trappe 15399	Australia	JX888459	-	-	-
	REH 9417	Queensland, Australia	-	JQ287642	JQ287644	-
Spongiforma thailandica	DED 7873	Thailand	-	EU685108	KF030436	-
Strobilomyces strobilaceus (as *S. floccopus*)	Sf1	Bavaria, Germany	-	DQ534626	JQ327037	-
Suillellus amygdalinus	112605ba	Mendocino Co., CA, USA	-	JQ326996	JQ327024	-
Sutorius aff. *eximius*	HKAS 52672	Kunming, Yunnan Prov. CHINA	-	KF112399	KF112207	-
Sutorius eximius	REH 8594	Jardin de Dota, Costa Rica	-	JQ327008	JQ327027	-

Table 1. (Continued).

Taxon	Voucher No.	Locality	ITS	28S	*TEF1-α*	*RPB1*
Turmalinea persicina	KPM-NC 18001	Iwakura Kyoto Pref., Japan	-	KC552038	KC552082	-
Tylopilus alpinus	HKAS 55438	China	-	KF112404	-	KF112538
Tylopilus ballouii	Osmundson 1198	Thailand	-	EU430740	-	EU434340
	REH 9467	Fraser Island, Queensland, Australia	-	JX889676	JX889686	-
Tylopilus felleus	AT2001011	Uppsala, Sweden	-	JQ326993	JQ327015	
	HKAS 90203	China	-	KT990545	-	KT990913
	MCVE98230	Italy	JF908787	-	-	-
Tylopilus ferrugineus	MB 06-053	Erie Co., NY, USA	-	JQ326994	JQ327016	-
Tylopilus microsporus (*T. neofelleus*)	HKAS 59661	Yunnan Prov., China	-	KF112450	KF112225	-
Tylopilus otsuensis	HKAS 53401	Chenzhou, Hunan, China	-	KF112449	KF112224	-
Tylopilus plumbeoviolaceus	MB06-056	NY, USA	-	KF030350	KF030439	-
Veloporphyrellus alpinus	HKAS 57490	Yunnan Prov., China	-	KF112380	KF112209	KF112555
Xerocomellus chrysenteron	Xch1	Bavaria, Germany	-	AF050647	KF030415	-
Xerocomellus zelleri	REH 8724	Humbolt Co., CA, USA	-	KF030271	KF030416	KF030366
Xerocomus magniporus	HKAS 59820	Yunnan, SW China	-	JQ678699	JQ967195	-
Xerocomus perplexus	MB00-005	USA	-	JQ003702	KF030438	-
Xerocomus subtomentosus	KM167686	England, U.K.	-	KC215222	-	-
	Xs1	Bavaria, Germany	-	AF139716	JQ327035	KF030391
Zangia citrina	HKAS 52684	China	-	HQ326941	-	-
Zangia erythrocephala	HKAS 75046	Nujiang, Yunnan, Prov., China	-	KF112414	KF112269	-
Zangia roseola	HKAS 52661	China	-	JQ928623	-	JQ928595
Uncultured *Boletaceae*	clone 47C_G1_H9	Jonathan Dickinson State Park, Hobe Sound, Florida, USA	KX899732	-	-	-
	clone 4C_G2_C3	Big Lagoon Start Park, Pensacola, FL, USA	KX899785	-	-	-

Table 2. Newly designed *RPB1*-primers specific for the *Bothia* clade.

Primer name	Sequence (5' → 3')
RPB1-32-F	AGGCYGATATCGTGAGTCGC
RPB1-147-F	CTCGAGYTATCGAGGCGT
RPB1-835-R	ACCCTCRTCYTCRTCCTTGGG
RPB1-1091-R	CCATCYACYGCTATACTCGG

(2014) (data not shown). Consequently, phylogenetic analyses were restricted to the major clade including *P. boletinoides* sequences and to selected genera in the *Boletaceae*.

Three phylogenetic analyses were performed: two large phylogenetic analysis using a 28S/*TEF1-α* dataset and a 28S/*RPB1* dataset were focused on the generic position of *P. boletinoides* in the *Boletaceae*. According to the results by Orihara & Smith (2017), species of *Paxillaceae* were chosen as outgroup taxa for the combined datasets. The third phylogenetic analysis based only on an ITS dataset was restricted to the taxa closely related to *P. boletinoides*; *Tylopilus felleus* was used as outgroup taxon Alignments were generated for the ITS, 28S, *TEF1-α*, and *RPB1* datasets with MAFFT (Katoh *et al.* 2002) with default conditions for gap openings and gap extension penalties. Alignments were then manually adjusted using Geneious v. 11.1.4 (Kearse *et al.* 2012). We estimated the best fit substitution model for each single alignment using the Bayesian information criterion (BIC) with jModelTest 2 (Darriba *et al.* 2012) and therefore selected the GTR+G model for all alignments. The ITS dataset was not partitioned.

Phylogenetic hypotheses were constructed with Bayesian inference (BI) and Maximum likelihood (ML) criteria. The BI was performed with MrBayes v. 3.2.6 (Ronquist *et al.* 2012) with one cold and three incrementally heated simultaneous Monte Carlo Markov chains (MCMC) run for 10 M generations, under the selected evolutionary model. Two simultaneous runs were performed independently. Trees were sampled every 1 000 generations, resulting in sampling of 10 001 trees per single run with the first 2 500 trees (25 %) discarded as burn-in. For the remaining trees of the two independent runs, a majority rule consensus tree showing all compatible partitions was computed to obtain estimates for Bayesian posterior probabilities (BPP).

ML analysis was performed using RAxML v. 7.3.2 (Stamatakis 2006) with 1 000 bootstrap replicates (Felsenstein 1985) and the GTRGAMMA algorithm. Support values from bootstrapping runs (MLB) were mapped on the best ML tree using the "-f a" option of RAxML and "-x 12345" as a random seed to invoke the novel rapid bootstrapping algorithm. BI and ML analyses were run on the CIPRES Science Gateway (Miller *et al.* 2010). Only BPP values

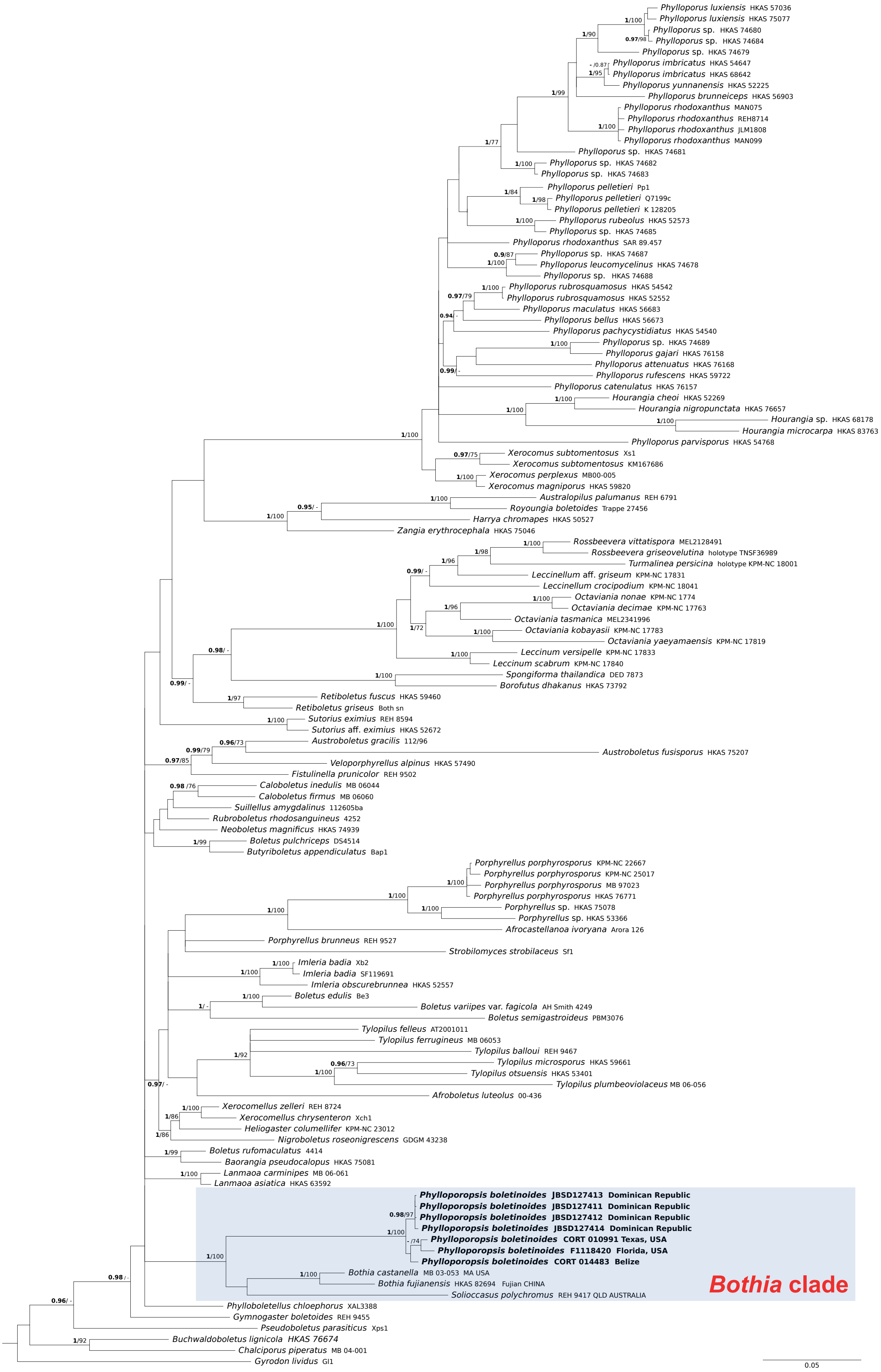

Fig. 1. Phylogeny of the *Boletaceae* based on a Bayesian and Maximum likelihood inference analysis of a combined matrix of two nuclear gene regions (28S and *TEF1-α*). Bayesian posterior probability (BPP) values (in bold) ≥ 0.95 and Maximum likelihood bootstrap (MLB) values ≥ 70 % are shown on the branches. Newly sequenced collections are in bold.

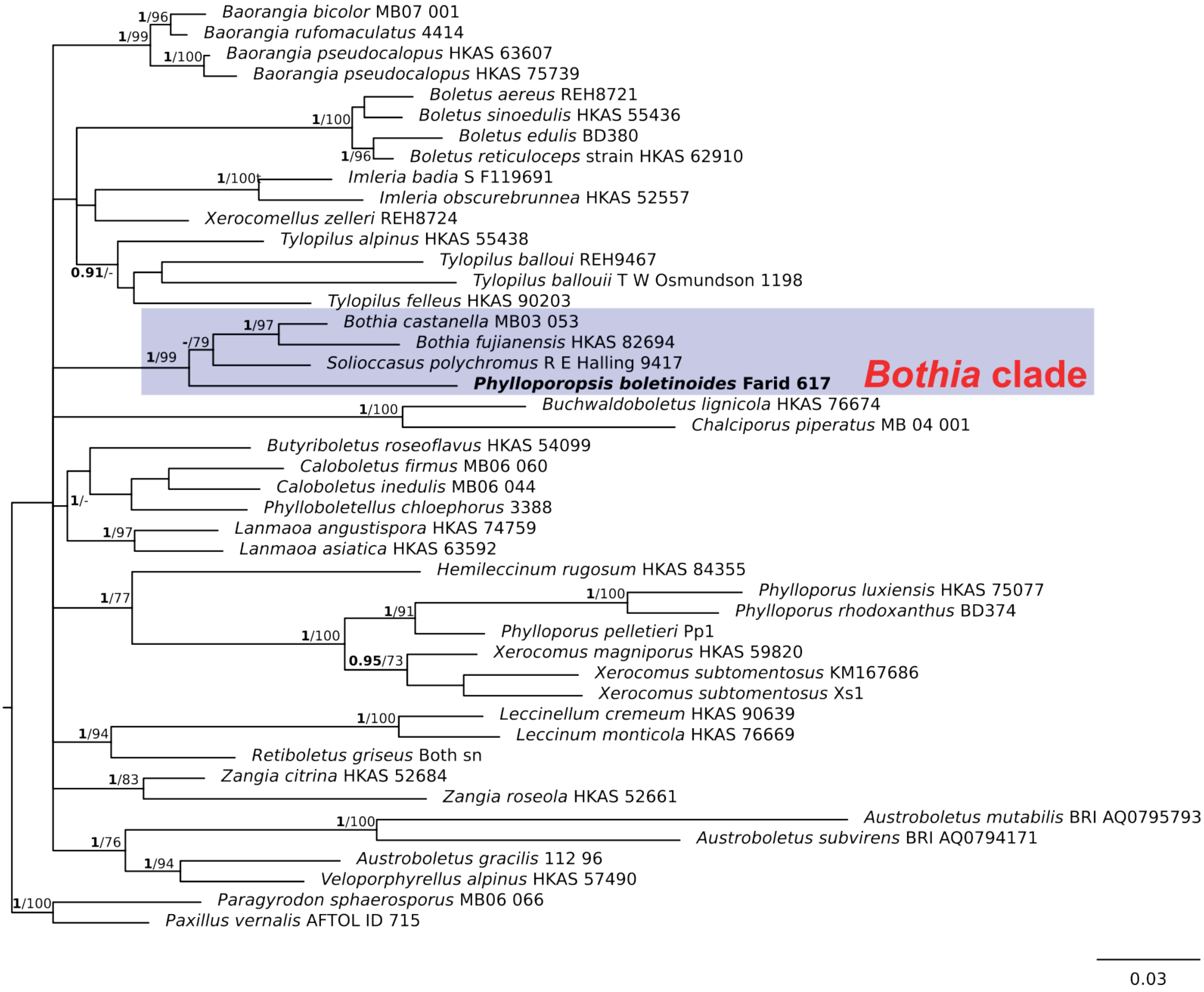

Fig. 2. Phylogeny of the *Boletaceae* based on a Bayesian and Maximum likelihood inference analysis of a combined matrix of two nuclear gene regions (28S and *RPB1*). Bayesian posterior probability (BPP) values (in bold) ≥ 0.95 and Maximum likelihood bootstrap (MLB) values ≥ 70 % are shown on the branches. Newly sequenced collections are in bold.

≥ 0.95 and MLB values ≥ 70 %, are reported in the resulting trees (Figs 1–3). Branch lengths were estimated as mean values over the sampled trees. Pairwise percent identity values (P %IV) of the ITS sequences were calculated using Geneious v. 11.1.4 (Kearse *et al.* 2012). Alignments and phylogenetic trees are available at TreeBASE (www.treebase.org) under ID S22978.

RESULTS

Molecular analysis

Both Bayesian and Maximum Likelihood analyses produced comparable topologies and therefore only Bayesian trees with BPP and MLB values are shown (Figs 1–3). The combined 28S/*TEF1-α* and 28S/*RPB1* dataset comprised 123 and 45 taxa, respectively. The ITS dataset comprised 21 taxa.

In the 28S/*TEF- α* dataset (Fig. 1), all the *P. boletinoides* collections (from Dominican Republic, Belize, Florida and Texas) form a strongly supported clade (BPP = 1.0, MLB = 100 %) which is part of a larger clade that includes *Bothia* and *Solioccasus* (BPP = 1.0, MLB = 100 %). We hereafter refer to this clade that includes *Bothia*, *Solioccasus* and *P. boletinoides* as the *Bothia* clade. *Phylloporus* clusters with *Xerocomus* and *Hourangia* in a strongly supported clade (BPP = 1.0, MLB = 100 %) that is distantly related to the *Bothia* clade. In the combined 28S/*RPB1* dataset (Fig. 2), *P. boletinoides* was strongly supported in the *Bothia* clade (BPP = 1.0, MLB = 99 %). *Phylloporus* clusters with *Xerocomus* in a strongly supported clade (BPP = 1.0, MLB = 100 %). The other boletoid genus characterized by a lamellate hymenophore, *Phylloboletellus*, is resolved in an isolated and uncertain position within the *Boletaceae* (Figs 1, 2). In the ITS analysis (Fig. 3), the newly generated sequences of *P. boletinoides* (BPP = 1, MLB = 100 %) are closely related to two environmental sequences: KX899732 (clone 47C_G1_H9), an environmental sequence from Jonathan Dickinson State Park, Hobe Sound, FL, USA, and KX899785 (clone 4C_G2_C3), an environmental sequence from Big Lagoon State Park, Pensacola, FL, USA. Both sequences were obtained from ectomycorrhizal samples on *Pinus clausa* (Sand Pine). The pairwise % identity values (P %IV) of the ITS sequences of the *Phylloporopsis* clade is 98 %.

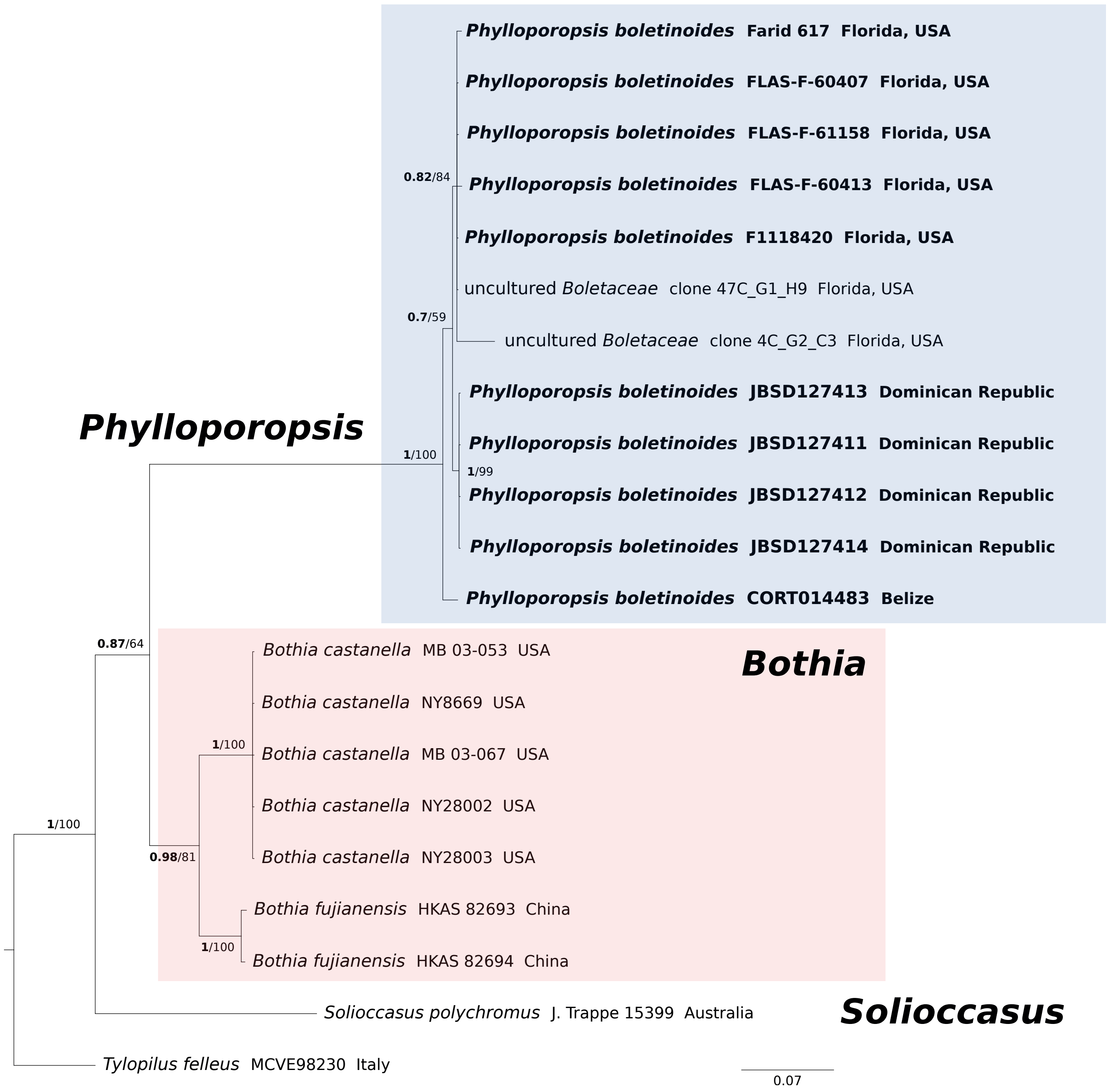

Fig. 3. Bayesian ITS phylogeny restricted to the clade including *Phylloporopsis* (*Bothia* clade). BPP values (in bold) ≥ 0.95 and MLB values ≥ 70 % are shown on the branches. Newly sequenced collections are in bold.

TAXONOMY

Phylloporopsis Angelini, A. Farid, Gelardi, M.E. Smith, Costanzo, & Vizzini, ***gen. nov***. MycoBank MB828149.

Etymology: the generic epithet refers to the morphological affinities shared with *Phylloporus*.

Basidiomata pileate-stipitate with lamellate to subporoid hymenophore, epigeal, evelate, medium-small sized; pileus convex to applanate, velvety-tomentose to fibrillose; hymenophore lamellate to subporoid with anastomosing and interveined gills, strongly decurrent, beige to olive-cream or olive-buff; stipe solid to sometimes hollow at maturity, dry, pruinose to longitudinally fibrillose, reticulation absent; basal mycelium whitish to yellowish, context firm, whitish but cream-yellowish in the stipe; tissues unchangeable or turning light blue especially on hymenophore and pileus context when injured or exposed; taste mild to slightly bitter; olive-brown spore print; purplish-pink or reddish reaction with ammonia on pileus cuticle; basidiospores smooth, ellipsoid-fusiform, spore wall cyanophilic; pleuro-, cheilo- and caulocystidia present; trichodermal pileipellis; hymenophoral trama bilateral-divergent of the “*Phylloporus*-type”; lateral stipe stratum absent; clamp connections absent; ontogenetic development gymnocarpic. According with the phylogenetic analysis of the combined ITS, 28S, *TEF1-α,* and *RPB1* sequences the genus is unrelated to *Phylloporus* and close but separated from *Bothia* and *Solioccasus*.

Typus generis: *Phylloporopsis boletinoides* (A.H. Smith & Thiers) Vizzini, Angelini, A. Farid, Gelardi, Costanzo, & M.E. Smith

Phylloporopsis boletinoides (A.H. Smith & Thiers) Vizzini, Angelini, A. Farid, Gelardi, Costanzo & M.E. Smith ***comb. nov.*** MycoBank MB828150. Figs 4–6.
Basionym: *Phylloporus boletinoides* A.H. Smith & Thiers, Contr. Monogr. North Amer. Species *Suillus*: 105. 1964.

Typus: USA, Florida, Alachua Co., Alachua, west side of Newnan's Lake, east of Gainesville, solitary in deep sandy humus under pines (*Pinus* spp.), low hammock, 31 Jul. 1958, *H.D. Thiers* 4960 [MICH 11740 (**holotype**), SFSU 000741 (**isotype**)].

Basidiomata medium-small. *Ontogenetic development* gymnocarpic. *Pileus* (2.4–)2.7–7.3(–7.5) cm broad, at first hemispherical then persistently convex and finally broadly pulvinate-flattened, never depressed at centre, regularly to hardly unevenly shaped, moderate fleshy, firm at the beginning but progressively softer with age; margin steady to faintly wavy-lobed, initially involute then curved downwards and finally completely plane or even uplifted, not or only a little extending beyond the hymenophore; surface matt, dry, velvety-tomentose to finely fibrillose in all developmental stages, not cracked; cuticle at first deep orange then garnet red, carmin red, dull red, dark red to reddish-brown (Light Coral Red to Hay's Maroon, Plate XIII; Light Corinthian Red to Prussian Red, Plate XXVII), gradually fading with age and becoming beige-ochraceous to pale brownish-pink (Light Buff, Plate XV; Pale Salmon Pink, Plate XIV), especially on the peripheral surface; slowly darkening on handling or when injured; subcuticular layer white (White, Plate LIII). *Hymenophore* lamellate to sub-boletinoid, gills distinctly arcuate-decurrent, somewhat distant, undulate, shorter than pileus context thickness (up to 0.5 cm high), distinctly intervenose with low anastomosing transversal or furcate veins connecting the primary gills, suggesting a subporoid appearance particularly in young specimens, concolourous edges entire (Fig. 4G); at first beige to pale cream-beige (Marguerite Yellow, Primrose Yellow, Plate XXX), in age fading to olive-cream (Light Yellowish Olive, Plate XXX; Deep Olive Buff, Plate XL; Greysh Olive, Plate XLVI) due to mature spores, staining light blue (Pale Green-Blue Grey, Plate XLVIII) on bruising or exposure. *Stipe* (2.5–)2.7–4.5(–5.0) × 0.4–0.7 cm, shorter than or as long as the pileus diameter at maturity, central to slightly off–centre, solid, firm, dry, straight or curved, cylindrical but slightly swollen towards the base, not rooting; surface very finely pruinose to longitudinally fibrillose, devoid of reticulum, evelate; pale orange (Light Salmon Orange, Plate II) in the upper third, concolourous with the pileus to slightly paler elsewhere (Light Coral Red to Hay's Maroon, Plate XIII; Light Corinthian Red to Prussian Red, Plate XXVII), usually with a conspicuous whitish to pale yellowish basal tomentum (White, Plate LIII; Sea-Foam Yellow, Plate XXXI), unchangeable when pressed; rhizomorphs not observed. *Context* firm and tough when young, later soft textured and eventually flabby in the pileus (up to 1.1 cm thick in the central zone), a little more fibrous in the stipe, whitish in the pileus and upper third of the stipe but yellowish (Maize Yellow, Plate IV; Cream Colour, Plate XVI) in the remaining part of the stipe and increasingly deeper towards the base (Buff Yellow, Plate IV); turning light blue (Pale Green-Blue Grey, Plate XLVIII) in the pileus and the connection zone with the stipe after a couple of minutes when exposed to air (Fig. 4H), especially above the tubes and eventually fading to drab whitish, nearly unchangeable elsewhere; subhymenophoral layer whitish; exsiccate brownish. *Odour* indistinct. *Taste* mild but slightly bitter after prolonged mastication. *Spore-print* not obtained. *Macrochemical reactions*: 30 % KOH: brownish-grey to dark brown on context and hymenophore, golden yellow on pileus. 30 % NH_4OH: staining purple-pink on pileus. $FeSO_4$: slowly pale yellowish-green to olive green on context, none elsewhere.

Basidiospores [619/36/19] (8.9–)12.3 ± 1.40(–18.0) × (3.5–)5.1 ± 0.68(–8.2) µm, Q = (1.73) 1.80–3.65(–4.13), Qm = 2.4 ± 0.24, V = 231 ± 68 µm^3, inequilateral, very variable in dimensions and versiform, cylindrical to fusiform or more frequently ellipsoid-fusiform to broadly ellipsoid in side view, ellipsoid to broadly ellipsoid in face view, smooth, apex rounded, with a short apiculus and usually with an indistinct to shallow suprahilar depression although in some spores the depression appears quite pronounced (Figs 5E, F and 6D), thick-walled (0.5–0.7 µm), straw yellow in water, ochraceous-yellow coloured in 5 % KOH, having one or more rarely two to three large oil droplets, occasionally pluri-guttulate when mature, inamyloid to very weakly dextrinoid, cyanophilic (Fig. 5E) and with a very faint metachromatic reaction. *Basidia* (27–)33–54(–58) × 8–14 µm (n = 24), long, subcylindrical, cylindrical-clavate to clavate, moderately thick-walled (0.6–0.9 µm), predominantly 4-spored but also 1-, 2- or 3-spored, usually bearing relatively long sterigmata (2–7 µm), very pale yellowish and containing straw-yellow oil guttules in water and 5 % KOH, bright yellow (inamyloid) in Melzer's, without basal clamps (Figs 5D, 6C); basidioles subcylindrical, cylindrical-clavate to clavate, similar in size to basidia. *Cheilocystidia* (42–)45–108(–118) × (8–)10–22 µm (n = 26), very common, decidedly slender, projecting straight to sometimes flexuous, cylindrical-fusiform, fusiform, ventricose-fusiform to sublageniform, less frequently cylindrical to irregularly cylindrical, subclavate, sausage-like or peanut-like, rarely mucronate or subcapitate, with rounded to subacute tip, smooth, thick-walled (0.8–1.2 µm), very pale yellowish in water and 5 % KOH, bright yellow (inamyloid) in Melzer's, orthochromatic, without epiparietal encrustations (Figs 5D, 6B). *Pleurocystidia* (63–)65–117 × 9–26(–28) µm (n = 29), decidedly frequent, shape, size, colour and chemical reactions similar to cheilocystidia (Fig. 6B). *Pseudocystidia* not recorded. *Pileipellis* a trichoderm consisting of moderately to strongly interwoven, elongated, filamentous and sinuous, frequently branched hyphae tending to be repent in the outermost layer and thus turning into a cutis not or only partially embedded in gelatinous matter at maturity (Figs 5C, 6A); terminal elements 25–88 × 3–14 µm, long and slender, cylindrical to cystidioid, apex rounded-obtuse to sometimes pointed, moderately thick-walled (up to 1 µm), yellowish to pale brownish-yellow in water and 5 % KOH, bright yellow to yellowish-orange (inamyloid to weakly dextrinoid) in Melzer's, smooth; subterminal elements similar in shape, size and colour to terminal elements, all hyphae lacking encrusting pigments. *Stipitipellis* a layer of slender, parallel to subparallel

Fig. 4. *Phylloporopsis boletinoides*. **A–F.** Fresh basidiomata (A: JBSD127411, B: JBSD127412, C: JBSD127413, D: JBSD127414, E: CORT014483, F: CORT010991). **G.** Detail of the lamellate hymenophore (JBSD127411). **H.** Detail of the context turning blue on exposure (JBSD127415). Bars = 1 cm. Pictures: A–D, G–H by C. Angelini; E–F by T.J. Baroni.


A
1 cm
27C8
B
1 cm
27C8
C
D
E
F
G
H

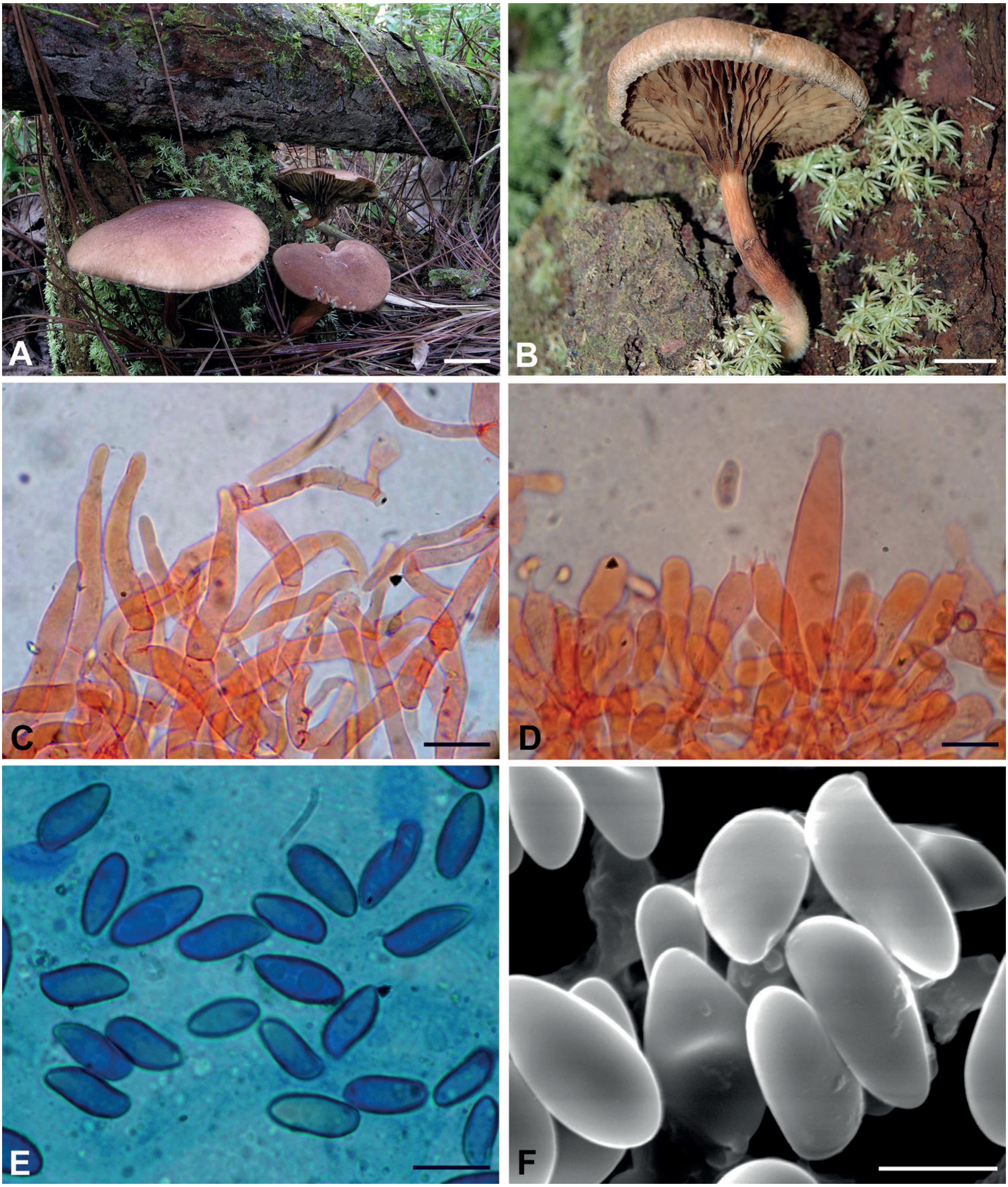

Fig. 5. *Phylloporopsis boletinoides*. **A–B.** Lignicolous basidiomata (A: JBSD127414, B: JBSD127414). **C.** Elements of the pileipellis (JBSD127412). **D.** Basidia and cheilocystidium (JBSD127411). **E.** Cyanophilic basidiospores under optical microscopy (JBSD127412). **F.** Basidiospores under SEM (JBSD127411). C–D in anionic Congo red; E in Cotton blue. Bars C–D = 20 µm; E–F = 10 µm. Pictures: A–B by C. Angelini; C–E by M. Gelardi; F by A. Vizzini.

and longitudinally running, smooth walled, adpressed hyphae, 2–5 µm wide, hyaline to pale yellowish in water and 5 % KOH; the stipe apex covered by a well-developed caulohymenial layer consisting of sterile caulobasidioles, extremely rare, 2-spored, fertile caulobasidia 54 × 10 µm, sterigmata 5 µm long (only one observed in several mounts!) and abundant projecting,

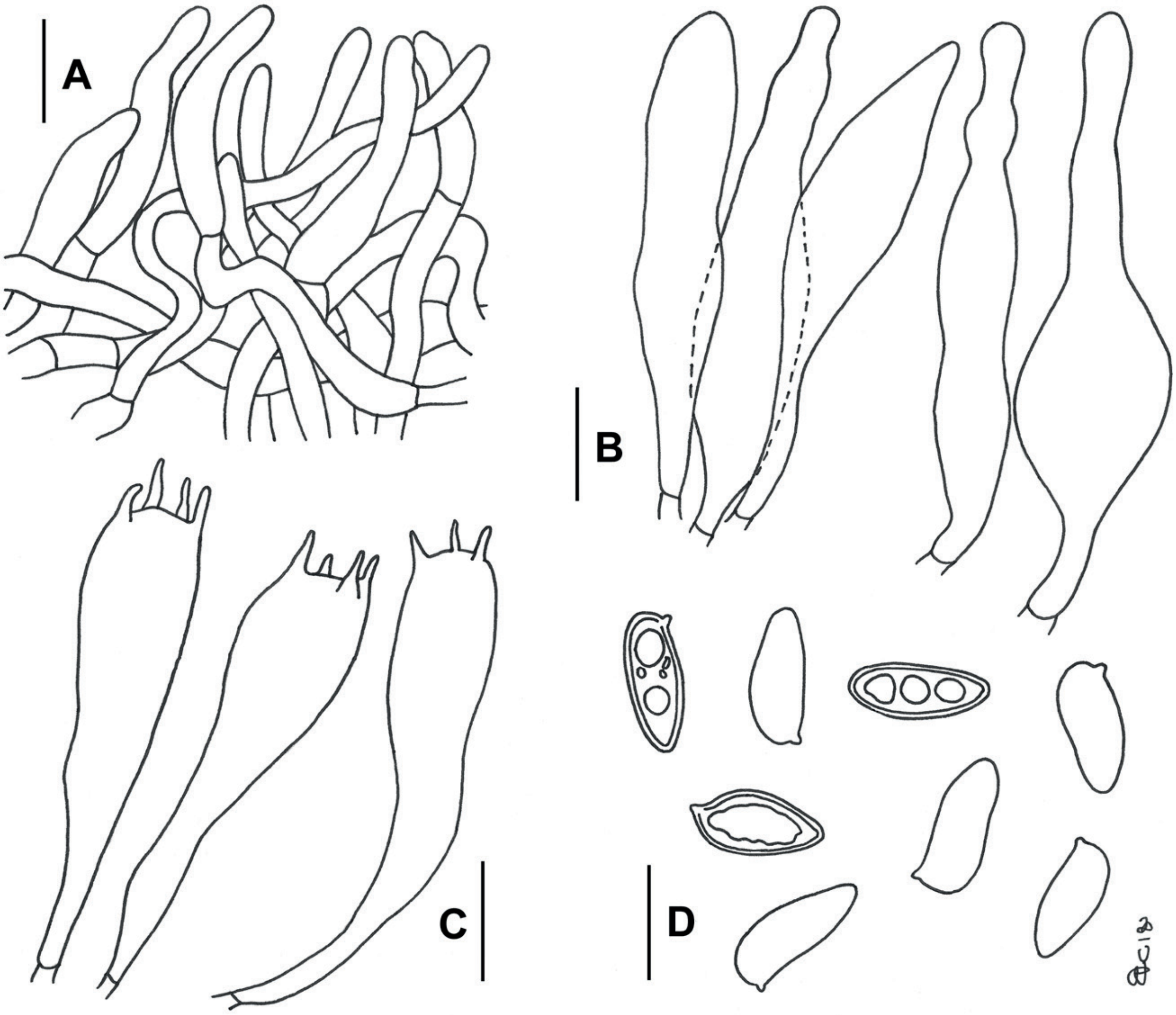

Fig. 6. *Phylloporopsis boletinoides*. Drawings of the anatomical features (JBSD127412). **A.** Elements of the pileipellis. **B.** Cheilo- and pleurocystidia. **C.** Basidia. **D.** Basidiospores. Bars A–B = 20 µm; C–D = 10 µm (F. Costanzo *del*.).

fusiform, ventricose-fusiform, irregular cylindrical to subclavate or occasionally diverticulate caulocystidia similar in colour to hymenial cystidia but decidedly shorter and narrower, 30–60(–63) × 6–15 µm (n = 13), having a wall up to 0.8 µm thick. *Lateral stipe stratum* under the caulohymenium absent. *Stipe trama* composed of confusedly and densely arranged, subparallel to moderately interwoven, filamentous, smooth, hyaline to yellowish in water, inamyloid hyphae, 5–15(–20) µm broad. *Hymenophoral trama* bilateral divergent of the "*Phylloporus*-type", with very slightly divergent to nearly subparallel and tightly arranged, non-gelatinous hyphae (lateral strata hyphae in transversal section touching or almost touching each other, 0–3 µm apart, 3–12 µm broad), hyaline to very pale yellowish in water and 5 % KOH, inamyloid in Melzer's; lateral strata (20–)30–40(–50) µm thick, mediostratum 10–20(–30) µm thick, axially arranged, consisting of a tightly adpressed, non-gelatinous bundle of hyphae, 3–9 µm broad; in Congo Red the mediostratum is concolorous with the lateral strata. *Oleiferous hyphae* very common. *Clamp connections* absent in all tissues. *Hyphal system* monomitic.

Ecology: usually under *Pinus* spp., occasionally in mixed *Pinus* and *Quercus* forests, solitary to gregarious or less frequently subcaespitose, growing on soil among debris or on rotting wood but also on logs or living trunks of pine trees.
Edibility: unknown.

Materials examined: **Dominican Republic**, La Vega Province, Jarabacoa, 19°11'12.5''N 70°35'25.5''E, 660 m, half dozen young to middle aged specimens growing on soil under *Pinus occidentalis*, 21 Dec. 2013, *C. Angelini* (JBSD127411, ANGE120 and MG709); *ibid*., five mature specimens growing on soil under *P. occidentalis*, 22 Dec. 2013, *C. Angelini* (JBSD127412, ANGE121 and MG710); *ibid*., a single mature specimen growing on soil under *P. occidentalis*, 10 Jan. 2016, *C. Angelini* (JBSD127413, ANGE551); *ibid*., several basidiomata in all developmental stages growing on rotting wood and on a living trunk of *P. occidentalis*, 28 Nov. 2017, *C. Angelini* (JBSD127414, ANGE1007 and MG711); *ibid*., several basidiomata in all developmental stages growing on soil and on rotting wood of *P. occidentalis*, 23 Nov. 2017, *C. Angelini* (JBSD127415, ANGE1013 and MG712). **Belize**, Cayo District, Mountain Pine Ridge, Hidden Valley Inn property, near Lake Lolly Folly, 17°03'13.3''N 88°54'14.0''W, 565 m, seven mostly mature specimens, 7 Jan. 2002, *T.J. Baroni* (9195 TJB = BZ745) (CORT014483). **USA**, Alabama, Baldwin Co., Orange Beach, solitary in a sandy area with oaks nearby, 21 Jul. 1982, *D.P. Lewis* 3196 (F C0235181F); Florida, Alachua Co., W side of Newnan's Lake, solitary in deep sandy humus under pines, low hammock, 31 Jul. 1958, *H.D. Thiers* 4960 (SFSU-F-000741 **holotype**); Austin Cary Memorial Forest, NE of Gainesville, widely scattered under *Pinus* sp., 11 Aug. 1985, *N.S. Weber* 6007 (MICH 65224); Glades Co., Fish eating Creek Wildlife Management Area, 1.2 km S of Highlands Co. line, 1.3 km E of C-731, 9 Sep. 2012, A.R. Franck 3125 (USF 273159); Hillsborough Co., Violet Cury Nature Preserve, under *Quercus* sp. and *Pinus* sp., 15 Jun. 2017, *A. Farid* 617 (USF 296126); Putnam Co., Ordway-Swisher Biological Station, *Pinus* sp. dominated habitat, 23 Oct. 2016, *N. Kraisitudomsook* NAT-033 (FLAS 60407); *ibid*., under *Quercus* sp. and *Pinus* sp., 10 Oct. 2016, *M.E. Smith* s.n. (FLAS 60413); ibid., 19 Oct. 2017, *M.E. Smith* s.n. (USF 298023); Sarasota Co., Myakka Valley Ranches, on soil near *Pinus* sp. and *Quercus* sp., 29 Dec. 1986, *R.S. Williams* 326 (F C0224863); Myakka Valley Ranches-residence, under *Pinus* sp., 12 Jan. 1991, *R. Singer* (F3912) (F 1118420); Mississippi, Harrison Co., National Cemetery, Biloxi, scattered under *Pinus* sp., 10 Sep. 1981, *D. Guravich* 1390 (MICH 65225); Texas, Hardin Co., N of Silsbee, Gore Cemetery, solitary in *Pinus* sp. woodland, 16 Aug. 1982, *D.P. Lewis* 3324 (F 1087286); Orange Co., Vidor, near residence, scattered on *Pinus* sp. stump, 5 Jul. 1976, *D.P. Lewis* 351 (F 1086387); *ibid*., solitary on well-decayed *Pinus taeda* stump, 13 Sep. 1979, *D.P. Lewis* 1982 (F 1089019); Tyler Co., near Kountze, Big Thicket National Preserve, Turkey Creek Unit, along Turkey Creek Trail, 30°31’19.0’’N 94°20’44.7’’W, 60 m, 6 Sep. 1996, *T.J. Baroni* (TJB 8172) (CORT010991).

Known distribution: Eastern and south-eastern USA (Maine, Delaware, New Jersey, Georgia, Florida, Mississippi, Alabama, Texas) (Mycology Collections Portal, MyCoPortal: http://mycoportal.org/portal/index.php. Accessed on June 29, 2018), in Central America reported from Belize and in the Greater Antilles Islands of the Caribbean from the Dominican Republic, more frequently collected in subtropical and at higher altitudes in tropical areas, western and southern limits yet to be established.

DISCUSSION

Taxonomic circumscription of *P. boletinoides*

There are several reliable and essential macro-morphological features that characterize *P. boletinoides.* The pileus is initially reddish-orange then deep red or cinnamon-brown to cocoa brown and becomes pale ochraceous-brown with age. The strongly decurrent lamellate to sub-boletinoid hymenophore showing distinctly anastomosing and interveined lamellae that are beige to olive-cream or olive-buff coloured (becoming dull ochraceous-brown when dried) and sometimes faintly bluing upon handling. The context is whitish but cream-yellowish in the lower quarter of the stipe, unchanging or turning light blue only in the pileus and the connection zone with the stipe. The pileus surface turns purplish-pink or reddish with ammonia. Anatomical traits include the ellipsoid-fusiform, cyanophilic, smooth basidiospores, trichodermal pileipellis, hymenophoral trama of the “*Phylloporus*-type” and absence of lateral stipe stratum (Smith & Thiers 1964, Singer *et al.* 1990, Bessette *et al.* 2000, 2016, Ortiz-Santana *et al.* 2007, Neves & Halling 2010, this study). The Dominican material exhibits predominant reddish hues on the pileus and stipe, larger hymenial cystidia (up to 118 × 28 µm) and slightly larger basidiospores, (11.1–)13.4 ± 1.27(–18.0) × (4.7–)5.8 ± 0.59(–8.2) µm, Q = 2.3 [136/9/5] than those reported in literature (11–13 × 5–6 µm in Smith & Thiers 1964; 10.5–14.5 × 3.5–6.5 µm, Qm = 2.7 in Singer *et al.* 1990; 12–14.4 × 4.8–6.8 µm, Qm = 2.4 in Ortiz-Santana *et al.* 2007; 10.5–11.9 × 4.2–4.9 µm, Qm = 2.4 in Neves & Halling 2010). This morphological variability likely represents a phenotypic continuum along a geographic gradient of the same species (Fig. 3).

The original species description states that the context does not change on exposure (Smith & Thiers 1964). The description was updated by Singer *et al.* (1990) to point out the inconsistent bluing that is present in some collections. Our observations from samples found in Alachua County, Florida, near the type locality and from Dominican collections often show evidence of this bluing. A recent treatment on the boletes from eastern North America (Bessette *et al.* 2016) also notes the inconsistency of the bluing context. Consequently, the occurrence of a blue oxidation in *P. boletinoides* is variable and consistent with Singer’s re-description of the species (Singer *et al.* 1990).

Data obtained from top BLASTn results on GenBank and ITS phylogenetic analysis (Fig. 3) provide molecular confirmation of the ectomycorrhizal association of *P. boletinoides* with *Pinus*. GenBank sequences KX899732 (clone 47C_G1_H9) and KX899785 (clone 4C_G2_C3) were obtained from ectomycorrhizal root samples from *Pinus clausa*. Florida material is typically found under stands of *Pinus* spp., though occasionally in mixed *Pinus* and *Quercus* forests. In the Dominican Republic *P. boletinoides* was found with Hispaniolan pine (*Pinus occidentalis*) at high elevations with no other ectomycorrhizal hosts (no endemic *Fagaceae* occur in the Dominican Republic), despite several specimens were collected on stumps or debris of rotting wood, sometimes on the outer bark (periderm) at the base of living trees (Figs 4D, 5A, B). The occurrence of the Belizean material of *P. boletinoides* on a dead tree (Ortiz-Santana *et al.* 2007) further confirms a lignicolous growth preference, at least at some neotropical sites. Previous studies have shown that some confirmed ECM fungi produce basidiomata in elevated positions on woody substrata (Rayner *et al.* 1985, Henkel *et al.* 2000). Examples of lignicolous growth of ECM *Boletaceae* have been reported from the Americas, Europe, southeast Asia and Australasia and include taxa such as those of the *Boletellus ananas* complex and several *Tylopilus* species (Singer 1945a, Corner 1972, Alessio 1985, Rayner *et al.* 1985, Henkel 1999, Watling 2008). This fruiting behaviour could be explained as a strategy for enhancing spore dispersal, for selective foraging of N in rotting wood, or otherwise as a strategy to preserve basidiomata from becoming water-soaked under wet conditions (Weber & Sundman 1986, Jurgensen *et al.* 1987, Henkel *et al.* 2000, 2012, Lindahl & Tunlid 2015).

Outside of the USA, *P. boletinoides* has a known distribution restricted to Belize and the Dominican Republic but given its ability to adapt to different climatic conditions and to form symbiosis with different pine trees, it is most likely to be expected throughout the neotropical mainland and the Caribbean where pines occur.

Taxonomic and phylogenetic relationships of *Phylloporopsis* to *Bothia, Phylloporus, Phylloboletellus* and *Phyllobolites*

Our phylogenetic analyses provide evidence that *Phylloporopsis* is a monophyletic genus sister to the poroid *Bothia* (typified with *Boletinus castanellus*) and the sequestrate *Solioccasus* (typified with *S. polychromus*) and confirm that it is not related to *Phylloporus sensu stricto* (Figs 1–2). Based on the available data there are no obvious shared morphological or ecological features among these three genera, with exception of the cyanophily of the spore wall (Trappe *et al.* 2013 and personal observations of TJB on *B. castanella* collections), which appears to be the sole synapomorphy known to date, though this feature has been only randomly tested and with contrasting results in the *Boletaceae* (Singer 1986, Watling 2008). Trappe *et al.* (2013) established the genus *Solioccasus* as a sister clade with *Bothia*, although they did not address the shared morphological character between the two genera. More extensive sampling is needed to elucidate the taxonomic boundaries of the *Bothia* clade.

Though *Phylloporopsis* is phylogenetically related to *Bothia*, the two genera are morphologically quite distinct based on the differently shaped and arranged hymenophore. In *Bothia* the hymenophore is truly boletinoid with compound angular and radially stretched pores that are only slightly decurrent or depressed around stipe apex and always brownish (Halling *et al*. 2007). Conversely, in *Phylloporopsis* the basic structure of the hymenophore is lamellate though anastomosing, always deeply decurrent along the stipe in all developmental stages and olive-cream to olive-buff at maturity. Moreover, the tissues stain brown in *Bothia* and blue in *Phylloporopsis* and the spore print is pale brownish-yellow in *Bothia* while in *Phylloporopsis* it has a distinct olive tinge (Singer *et al*. 1990, Bessette *et al*. 2000, 2016). The two genera are also ecologically different; *Bothia* is associated with *Fagaceae* (mostly *Quercus* spp.) whereas *Phylloporopsis* forms ECM with pine trees (see below). Finally, the geographic range of *Bothia* is only partially overlapping with that of *Phylloporopsis*, since *B. castanella* (= *Boletinus squarrososides* according to Halling *et al*. 2007) is only found in eastern and south-eastern North America [Peck 1900, Murrill 1909, 1914, Snell 1936 (as "*B. squarrososides*"), Singer 1938 (also as "*Phylloporus squarrosoides*"), 1945b (as "*Xerocomus squarrosoides*"), Coker & Beers 1943 (also as "*B. squarrososides*"), Snell & Dick 1958 (also as "*B. squarrososides*"), 1970, Smith & Thiers 1964, Both 1993, Bessette *et al.* 2000, 2016; Roody 2003, Watling 2008, Halling *et al.* 2007]. The only other known representative of the genus, viz. *B. fujanensis* is restricted to south-eastern China (Fujian and probably Taiwan) (Chen *et al.* 1997, Zeng *et al.* 2015). These characters are a sound basis for a clear-cut distinction between the two genera. *Bothia castanella* also differs from *P. boletinoides* in the sometimes coarsely reticulate stipe surface, smaller, ovate to broadly ellipsoid spores [(7–)8–11(–12) × (3.5–)4.5–5.5(–6) µm in *B. castanella*] and the presence of dark brown encrustations on pileus surface hyphae (Halling *et al.* 2007, Watling 2008, Bessette *et al.* 2000, 2016).

Our phylogenetic placement of *Phylloporopsis* suggests that the lamellate hymenophore has evolved at least three times in the *Boletaceae* from poroid ancestors, *viz*. in *Phylloporopsis*, *Phylloporus* and *Phylloboletellus*.

Phylloporopsis is morphologically similar to species of *Phylloporus* despite their distant phylogenetic relationship. However, *Phylloporus* is easily distinguished by the yellow to golden-yellow lamellate hymenophore with the bright yellow colours remaining so even after drying, by the bacillate surface ornamentation of the basidiospores under scanning electron microscope (SEM) (although a number of tropical species have smooth or finely rugulose spores) and the occurrence of clamp connections in some tropical and subtropical species (Singer 1945b, 1946 1978, 1986, Corner 1970, Heinemann & Rammeloo 1987, Šutara 2008, Watling 2008, Neves & Halling 2010, Neves *et al.* 2010, 2012, Zeng *et al.* 2013). Another noticeable distinguishing trait that was considered unifying in *Phylloporus* is the pileus surface that stains vivid blue to bluish-green with ammonia (Singer 1945b, 1986, Watling 2008) as opposed to the reddish to purple-pink reaction observed in *Phylloporopsis*. However, it should be noted that this macrochemical reaction has not been tested on species described from tropical Africa (Heinemann 1951), Malaysia (Corner 1970, 1974), China (Zeng *et al.* 2013, Ye *at al*. 2014) or Bangladesh (Hosen & Li 2015, 2017). In addition, a few taxa from tropical Central and South America, India and Thailand (e.g. *P. fibulatus*, *P. manausensis*, *P. septocystidiatus*, *P. castanopsidis*, etc.) are reported to have negative or non-bluing reaction with both NH_3 and NH_4OH (Singer 1986, Neves & Halling 2010, Neves *et al.* 2012, Pradeep *et al.* 2015). Accordingly, the bluing of the pileus surface with ammonia cannot be considered a synapomorphy within *Phylloporus*. *Phylloporus* is a large assemblage encompassing nearly one hundred species so far as known worldwide (comprising phylogenetic species that have not yet been formally described), it appears to be widespread being best represented throughout temperate and pantropical regions of both hemispheres and is apparently most diverse in Australasia (Corner 1970, 1974, Singer 1978, 1986, Singer & Gómez 1984, Montoya & Bandala 1991, 2011, Halling *et al.* 1999, Watling & Li 1999, Ortiz-Santana *et al.* 2007, Neves & Halling 2010, Neves *et al.* 2012, García-Jiménez 2013, Ye *et al.* 2014, Hosen & Li 2015, 2017, Pradeep *et al.* 2015). The neotropical *P. centroamericanus* is somewhat chromatically similar to *P. boletinoides* but diverges, apart from the generic discrepancies cited above, in the smaller size (pileus 2–3 cm broad), pileus surface tending to become areolate with age, context whitish but pinkish-brown in middle part of the stipe, white basal mycelium, encrusted hymenial cystidia and hymeniform pileipellis with 5–24 µm wide end cells (Singer & Gómez 1984, Montoya & Bandala 1991, Halling & Mueller 2005, Neves & Halling 2010). Affinities can also be found with some eastern Asian species such as *P. rubiginosus* and *P. pachycystidiatus*; the former is separated by the bright yellow to orangish-yellow lamellae, context evenly yellowish and slowly discolouring blue throughout, blue staining reaction on pileus surface with ammonia, smaller spores [9.8–11.2(–13) × 3.5–4.9 µm, Qm = 2.5], thick-walled cystidia (walls up to 2 µm wide) and the occurrence under *Castanopsis* and *Dipterocarpus* in Thailand and south-western China (Yunnan Province) (Neves *et al.* 2012, Ye *et al.* 2014), whereas the latter differs in the slightly smaller size (pileus 3–5 cm in diameter), yellow hymenophore, cream-yellowish context, whitish basal mycelium, bacillate basidiospores under SEM, thick-walled hymenial cystidia (walls 2–4 µm wide), association with *Lithocarpus* spp. and distribution restricted to southern and south-western China (Hainan and Yunnan Provinces) (Zeng *et al.* 2013, Ye *et al.* 2014). There appears to exist a minor affinity between *P. boletinoides* and the type species of the genus *Phylloporus*, *P. pelletieri*, which is separated from the former by the bright yellow to golden yellow hymenophore, bright yellow basal mycelium, unchangeable tissues, blue-green reaction with ammonia on pileus surface, bacillate basidiospores under SEM and the occurrence in temperate Europe (Pilát & Dermek 1974, Breitenbach & Kränzlin 1991, Lannoy & Estadès 2001, Ladurner & Simonini 2003, Watling & Hills 2005, Klofac 2007, Muñoz *et al.* 2008, Šutara 2008, Knudsen & Taylor 2012).

Another known lamellate representative in the *Boletaceae* is *Phylloboletellus*, a monotypic genus based on *P. chloephorus.* This taxon was described from subtropical to tropical montane forests in Argentina (Singer & Digilio 1952) but has an apparent disjunct distribution having later repeatedly been reported from Mexico (García-Jiménez *et al.* 1986, Singer 1988, Singer *et al.*

1992, Bandala *et al.* 2004, García-Jiménez 2013). This species, however, differs from *P. boletinoides* by the subumbonate pileus, yellowish basal mycelium, evenly yellow context discolouring blue on exposure, blue-green reaction with ammonia on pileus surface, hymenophoral trama of the "*Boletus*-type", presence of false or incomplete clamp connections and most of all by the short, broadly ellipsoid, longitudinally winged basidiospores [(9–)9.5–11.7(–12.5) × (7.2–)8–9.5(–10.5) µm, Q = 1.2)] (Singer & Digilio 1952, Singer 1964, 1970, 1981, 1986, 1988, Horak 1968, Petersen 1974, Pegler & Young 1981, Singer *et al.* 1992, Bandala *et al.* 2004, Watling 2008). Moreover, the original material of *P. chloephorus* was collected under *Lauraceae*, *Myrtaceae*, *Sapindaceae*, *Ulmaceae*, etc., but the authors were unable to ascertain its nutritional mode (Singer & Digilio 1952). Later on, the species was thought to be presumably saprotrophic (Singer 1970, 1986, Watling 2008), even though Singer (1981) and subsequently Bandala *et al.* (2004), Binder & Hibbett (2006) and Tedersoo *et al.* (2010) considered it as potentially ectomycorrhizal. *Phylloboletellus chloephorus* var. *mexicanus ad interim* was informally proposed by Singer *et al.* (1992) to circumscribe the Mexican population but the authors themselves were not fully convinced about the separation of the two taxa and claimed that the differences of var. *mexicanus* with respect to the type species were nearly inconsistent, thus it has never been validated thereafter. Previous studies (Binder & Hibbett 2006, Li *et al.* 2011, 2014, Zeng *et al.* 2012) and our own observations (Figs 1–2) have yet to resolve the phylogenetic position of *P. chloephorus*.

It is also worth nothing here the existence of the poorly known genus *Phyllobolites*, which some authors have argued may belong to the *Boletaceae* (Kirk *et al.* 2008, Magnago 2014). *Phyllobolites* was erected based on *Phyllobolites miniatus* from tropical northern South America (Guyana, Suriname, Brazil) (Singer 1964, 1986, Singer *et al.* 1983, Henkel *et al.* 2012, Sulzbacher *et al.* 2013). This taxon has been originally assigned to the broadly conceived family *Paxillaceae*, along with unrelated agaricoid genera such as *Omphalotus*, *Lampteromyces*, *Hygrophoropsis*, *Neopaxillus* and *Ripartites* (Singer 1946, 1986). It is easily delimited by the presence of a membranous ring deriving from a partial veil, ochraceous spore print, fusiform, verrucose basidiospores, abundant pseudocystidia, ixotrichoderm to ixocutis pileipellis, hymenophoral trama subparallel to slightly divergent with gelatinized hyphae and the occurrence under cesalpinoid legumes (Rick 1906, 1961, Singer 1942, 1964, 1986, Singer *et al.* 1983, Watling 2008). Since it has not been possible to locate and re-examine the type specimen of *P. miniatus* from the Brazilian Amazon and since additional material authenticated by Rick was found by Singer to be referred to either *Lentinus* (Singer 1946), *Tapinella* or *Pleurotus*, the assignment of *Phyllobolites* to the *Boletaceae* (Kirk *et al.* 2008, Magnago 2014) or *Paxillaceae* (Singer 1942, 1946, 1953, 1964, 1986, Singer *et al.* 1983, Neves & Capelari 2007, Henkel *et al.* 2012) or even to the order *Boletales* is currently doubtful and it has been suggested that *Phyllobolites* might belong in the order *Gomphales* near the genus *Linderomyces* (a later synonym of *Gloeocantharellus*) (Watling, 2008). A modern re-description and inclusive molecular investigation is needed to resolve its phylogenetic placement.

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