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ORIGINAL ARTICLE

New Poroid Hymenochaetaceae (Basidiomycota, Hymenochaetales) from Chile

Mario Rajchenberg[1,2] · María Belén Pildain[1,3] · Daniel Cajas Madriaga[4] · Andrés de Errasti[1] · Cristian Riquelme[4] · José Becerra[4]

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Abstract

Fomitiporia chilensis and *Phylloporia boldo* are described as new poroid species in the Hymenochaetaceae based on morphological, cultural, ecological, and phylogenetic analyses. *Fomitiporia chilensis* pertains to the *Fomitiporia punctata* species complex, being related to its Neotropical taxa. It is distinguished by pulvinate to effuse basidiomes that develop an indurated margin, by contextual tissue between the tube strata and basidiospores larger than 6.0 μm, and by growth on dead tissues of *Peumus boldus* and *Cryptocarya alba*. Its closest phylogenetic relatives are *Fomitiporia neotropica* and *Fomitiporia impercepta*, which differ by flatter basidiomes and by microscopical features. *Phylloporia boldo* grows and sporulates exclusively on living *Peumus boldus*. It is distinguished by a pileate basidiome with sulcate, indurated pileal surface, a dimitic hyphal system and by relatively large basidiospores 5.4–6.0 × 4.4–5.0 μm with dull chestnut walls. It was found to be phylogenetically related to *Phylloporia dependens*, described from China; both species being distantly related to other species in *Phylloporia*.

Keywords *Fomitiporia* · *Phylloporia* · Patagonia · South America · Taxonomy · Phylogeny · New taxa

Introduction

The knowledge of Chilean larger fungi is rather good thanks to the works of Singer (1969), Garrido (1988), and Valenzuela (1993), among others. A baseline for this knowledge has been the works by Mujica and Vergara (1945), Mujica and Oherens (1967), and Mujica et al. (1980), which offered a database of pathogenic and non-pathogenic fungi. In the last decade, Minter and Peredo López (2006) began a web page where taxonomic information on Chilean fungi can be approached, and Gorjón and Hallenberg (2012, 2013) updated and published the most valuable information on corticioid fungi. Polypores have been included in those works but, by far, specialists have given them little attention. The knowledge of polypores from southern South America has been summarized by Rajchenberg (2006), and studies on their phylogeny have been published (Rajchenberg et al. 2011 and Rajchenberg et al. 2015; Miettinen and Rajchenberg 2012; Rajchenberg and Pildain 2012; Pildain and Rajchenberg 2013; Dai et al. 2014), but the focus of these works has been on the eastern slope of the Andes Cordillera forests, and the incorporation of Chilean specimens has been fortuitous. Exceptions are the recent records of taxa by Sandoval and Rajchenberg (2011), Sandoval-Leiva (2014), and Pildain et al. (2017 and 2018).

Poroid Hymenochaetaceae Donk (Hymenochaetales, Agaricomycotina, Basidiomycota) is a well-known group of wood-inhabiting fungi that include many serious forest pathogens that produce white heart and canker rots worldwide (Gilbertson and Ryvarden 1986 and Gilbertson and Ryvarden 1987; Larsen and Cobb-Poulle 1990; Dai et al. 2007; Dai 2010; Rajchenberg and Robledo 2013), and some

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✉ Mario Rajchenberg
mrajchenberg@ciefap.org.ar

[1] Centro de Investigación y Extensión Forestal Andino Patagónico, C.C. 14, 9200 Esquel, Chubut, Argentina

[2] Universidad Nacional de la Patagonia S.J. Bosco, Sede Esquel, Facultad de Ingeniería, Ruta 259 km 14,6, 9200 Esquel, Chubut, Argentina

[3] Universidad Nacional de la Patagonia S.J. Bosco, Sede Esquel, Facultad de Ciencias Naturales, Ruta 259 km 14,6, 9200 Esquel, Chubut, Argentina

[4] Laboratorio de Química de Productos Naturales, Facultad de Ciencias Naturales y Oceanográficas, Universidad de Concepción, Concepcion, Chile

species have potential medicinal value (Dai et al. 2010; Wu et al. 2012). Most taxa, though, live through the decay of wood as saprotrophs on dead, fallen wood, and few have been shown to be mycorrhizal (Larsson et al. 2006). Because of their ecologic and economic importance, this group has been paid constant attention in the last years by mycologists, and results on its taxonomy and phylogeny do not lose the pace.

During the research of poroid Hymenochaetaceae from southern Chile (Antarctic Region, Subantarctic Domain, Subantarctic Province (Cabrera and Willink 1973)), it became evident that this region with numerous biogeographic districts and forest types (Donoso 1993) host several unknown, possibly endemic taxa that deserve an appropriate study.

The aim of this work is to describe two new species of poroid Hymenochaetaceae from southern Chile.

Materials and methods

Areas studied Field trips were performed during autumns 2014–16 and 2018 in the phytogeographic region of subtropical xerophytic and durifoliated forests (Hueck 1978, Cabrera 1971, Cabrera and Willink 1980) around Concepción city, Chile.

Specimens and strains studied Specimens of poroid Hymenochaetaceae were gathered and dried overnight in a 50 °C oven. Fresh portions of basidiomata and/or their associated wood were separated in order to get cultures from either contextual tissue or the associated wood rot, grown in 2% malt extract agar. Specimens and strains were deposited at the first author's institutional culture collection (CIEFAPcc) and phytopathological herbarium (CIEFAP); type materials are deposited at BAFC; some duplicates are kept at CONC. Morphology of basidiomata followed standardized methods (cfr. Rajchenberg et al. 2015, Drechsler-Santos et al. 2016). Cultures were studied and characterized according to Nobles (1965) and Stalpers (1978). Herbarium codes follow Thiers (2018).

Sequencing DNA was isolated from samples with the UltraClean™ Microbia DNA Isolation (MoBio Laboratories Inc., Carlsbad, CA) as per the manufacturer's instructions. The primer pair LR0R-LR5 (Vilgalys and Hester 1990) was used to amplify the partial 28S large sub-unit of nuclear ribosomal RNA gene (LSU), both in *Phylloporia* and *Fomitiporia* samples. Amplification and sequencing of LSU are described in Rajchenberg et al. (2015). On the other hand, the full internal transcribed spacer (ITS) region, the fragment between exons 4 and 8 of the translation elongation factor 1-a (tef1-α) gene, and the RPB2 gene were amplified for the *Fomitiporia* DNA samples. Amplification reactions were performed with GoTaq® Green Master Mix Protocol (Promega Corp) according to the manufacturer's recommendations with the primers ITS5 and ITS4 for ITS (http://biology.duke.edu/fungi/mycolab/primers.htm), 2212R, 1953R, 983F, 2218R for tef1-a (Rehner and Buckley 2005; Matheny et al. 2007), bRPB2-6F, and bRPB2–7.1R for RPB2 (Matheny 2005) under conditions defined by Decock et al. (2007) and Amalfi et al. (2010, 2012). The amplified fragments were sequenced at the DNA Synthesis and Sequencing Facility, Macrogen (Seoul, Korea). Sequences generated in this study were submitted to GenBank, and their codes are presented in Table 1. Sequences were assembled and edited with with BioEdit 7.0.9.0 (Hall 1999).

Phylogenetic analysis The LSU sequences of *Inonotus hispidus* (GenBank AF311014) was chosen as outgroup for *Phylloporia* analyses based on the results of Zhou (2016). While for the complete data set of ITS analysis of *Fomitiporia*, *Phellinus uncisetus* was designated as outgroup (Decock et al. 2007). On the other hand, for the *Fomitiporia* combined phylogenetic analyses, *F. castilloi* was designated as outgroup (Amalfi and Decock 2013; Amalfi et al. 2014; Morera et al. 2017). Nucleotide sequences were initially edited with BioEdit 7.0.9.0 (Hall 1999), then aligned automatically with MAFFT (Katoh and Standley 2013) and manually adjusted in MEGA version 6 (Tamura et al. 2013).

The final LSU dataset for *Phylloporia* resulted in 98 sequences with 902 characters including gaps, 938 characters for the ITS dataset of *Fomitiporia* (93 taxa), the LSU, ITS, TEF, and RPB2 of *Fomitiporia* datasets comprised 19 sequences representing 7 putative species, 795, 773, 1132, and 788 characters including gaps, respectively, which were manually combined for concatenated analyses. The substitution models that best fitted the sequence alignments were determined using the AIC criterion (Akaike 1974) implemented in jModelTest (Posada 2008; http://darwin.uvigo.es). The following models were used: TrN+I+G for LSU of *Phylloporia*; in *Fomitiporia*, TVM+I+G for the ITS complete dataset, TIM+I for LSU, HKY+G for ITS, TrNef+I for TEF, and TrN+G for RPB2. Maximum likelihood (ML) phylogenetic trees for individual loci (LSU of *Phylloporia*) and *Fomitiporia* combined data were estimated under these models in RAxML 7.2.8 (Stamatakis 2014) and Bayesian inferences (BI) of phylogenies in Mr. Bayes v.2.2 (Ronquist et al. 2012) with four incrementally heated simultaneous Monte Carlo Markov chains (MCMC) run over 10 million generations. Trees were sampled every 1000 generations; convergence after removal of the first 10% (10000) of trees was determined by observing that the standard deviation of split frequencies reached < 0.01; effective sample size (ESS) values for all parameters were > 200 and that parameters had reached a stationary stage after a 10% burn-in. Tracer 1.6 was used to check the effective sample sizes. For the remaining trees, a majority rule consensus tree showing all compatible partitions

Table 1 Detail of voucher specimens, isolates, and new sequences generated during this study

Species	Isolate	Specimen Voucher	Host	GenBank accession numbers			
				ITS	28S	RPB2	TEF 1α
Fomitiporia chilensis	CIEFAPcc 518	MR12556	*Cryptocarya alba*	MK131088	MK193749	MK140499	
	CIEFAPcc 519	BAFC52942 (MR12557)	*Cryptocarya alba*	MK131089	MK193750	MK140500	MK156786
	CIEFAPcc 520	MR12567	*Cryptocarya alba*	MK131093	MK193753	–	–
	CIEFAPcc 522	MR12581	*Lithraea caustica*	MK131092	–	–	–
	CIEFAPcc 523	MR12588	*Peumus boldus*	MK131091	–	–	MK156785
	CIEFAPcc 586	BAFC 52944 (MR12609)	*Peumus boldus*	MK131090	MK193751	MK140501	MK156788
	CIEFAPcc 587	MR12610	*Peumus boldus*	MK131094	MK193752	MK140502	MK156789
	CIEFAPcc 589	MR12612	*Peumus boldus*	MK131095	MK193755	MK140503	MK156790
	CIEFAPcc 593	MR12617	*Cryptocarya alba*	–	MK193754	MK140504	MK156787
Phylloporia boldo	CIEFAPcc 532	MR12573	*Peumus boldus*	–	MK193759	–	–
	CIEFAPcc 534	BAFC 52947 (MR12575)	*Peumus boldus*	–	MK193756	–	–
	CIEFAPcc 584	BAFC 52945 (MR12606)	*Peumus boldus*	–	MK193758	–	–
	CIEFAPcc 585	BAFC 52946 (MR12607)	*Peumus boldus*	–	MK193757	–	–

was computed to obtain estimates for Bayesian posterior probabilities (PP).The final alignments were deposited in TreeBASE (http://www.treebase.org/treebase/index.html), under accession ID23647.

Results

Phylogenetic analyses

The full ITS and the combined LSU+ITS+TEF+RPB2 concatenated dataset for *Fomitiporia* (Fig. 1) phylogenetic analyses showed that Chilean collections are grouped in a monophyletic group with strong support (ITS: BPP = 1, MLB = 100; LSU+ITS+TEF+RPB2 concatenated dataset: BPP = 1, MLB = 100%). This new taxon is closely related to *Fomitiporia neotropica* (ITS: BPP = 1, MLB = 92; LSU+ITS+TEF+RPB2 concatenated dataset: BPP = 1, MLB = 98%) and *Fomitiporia impercepta* (LSU+ITS+TEF+RPB2 concatenated dataset: BPP = 1, MLB = 80%) within the resupinate habit group defined by Amalfi et al. (2014). The phylogenetic analyses of LSU sequences of *Phylloporia*, which includes collections from Patagonia, Chile, is presented in Fig. 2. The trees generated from both datasets using ML and BI analyses were congruent; therefore, only the Bayesian trees with both BPP and MLB values are shown. Phylogenetic analyses indicated that the Chilean collections of *Phylloporia* are closely related with *P. dependens* and form a strong monophyletic group (BPP = 1, MLB = 95%). Within this group, two well-defined species were observed, *P. dependens* (BPP = 86, MLB = 65%) and the new species *P. boldo* (BPP = 1, MLB = 100%).

Taxonomy

Fomitiporia chilensis Rajchenb. and Pildain sp. nov. (Figs. 3, 4, 5)

MB829086

Holotype: CHILE, Región Bío-Bío, Concepción, Collico Norte, Reserva Coyan Mahuida, on fallen branch of *Cryptocarya alba* (Lauraceae), 4 May 2015, leg. M. Rajchenberg 12557 (BAFC 52942); culture CIEFAPcc 519. ITS = MK131089, LSU = MK193750, EF = MK156786, RPB2 = MK140500

Etymology: *chilensis* refers to Chile, the country where the taxon has been found.

Diagnosis: Basidiome pulvinate to effused, with an indurated margin, contextual tissue between strata and pores (4–)6–7.5/mm, hyphal system dimitic, basidiospores globose to subglobose, thick-walled, hyaline and dextrinoid. Grows on dead tissue of *Peumus boldus* and *Cryptocarya alba*

Basidiome perennial, adnate, first resupinate but soon pulvinate, nodular, or globular (hemispheric, then pseudo-pileate) in general shape, when growing on a vertical substrate also forming a long lacrymoid pseudo-pileate body, when resupinate or pulvinate with receding growth, generally forming an indurated margin; first small 2 × 2.5 × 0.5 cm but up to 19 long, 17 wide, and 7 cm thick. Margin first velutinate, up to 4 mm wide, whitish in the growing area but soon yellowish brown, irregular in form, verrucose here and there or not, becoming indurated with age, the indurated margin little to much developed up to 1–10 cm wide, grayish brown to dark gray, smooth, zonated or not or zonated in parts, cracking with age. Pore surface light yellowish brown to dark brown, receding, pores round to angular, (4–)6–7.5/mm. Context up to 3 mm thick, presenting a black crust against the substrate up

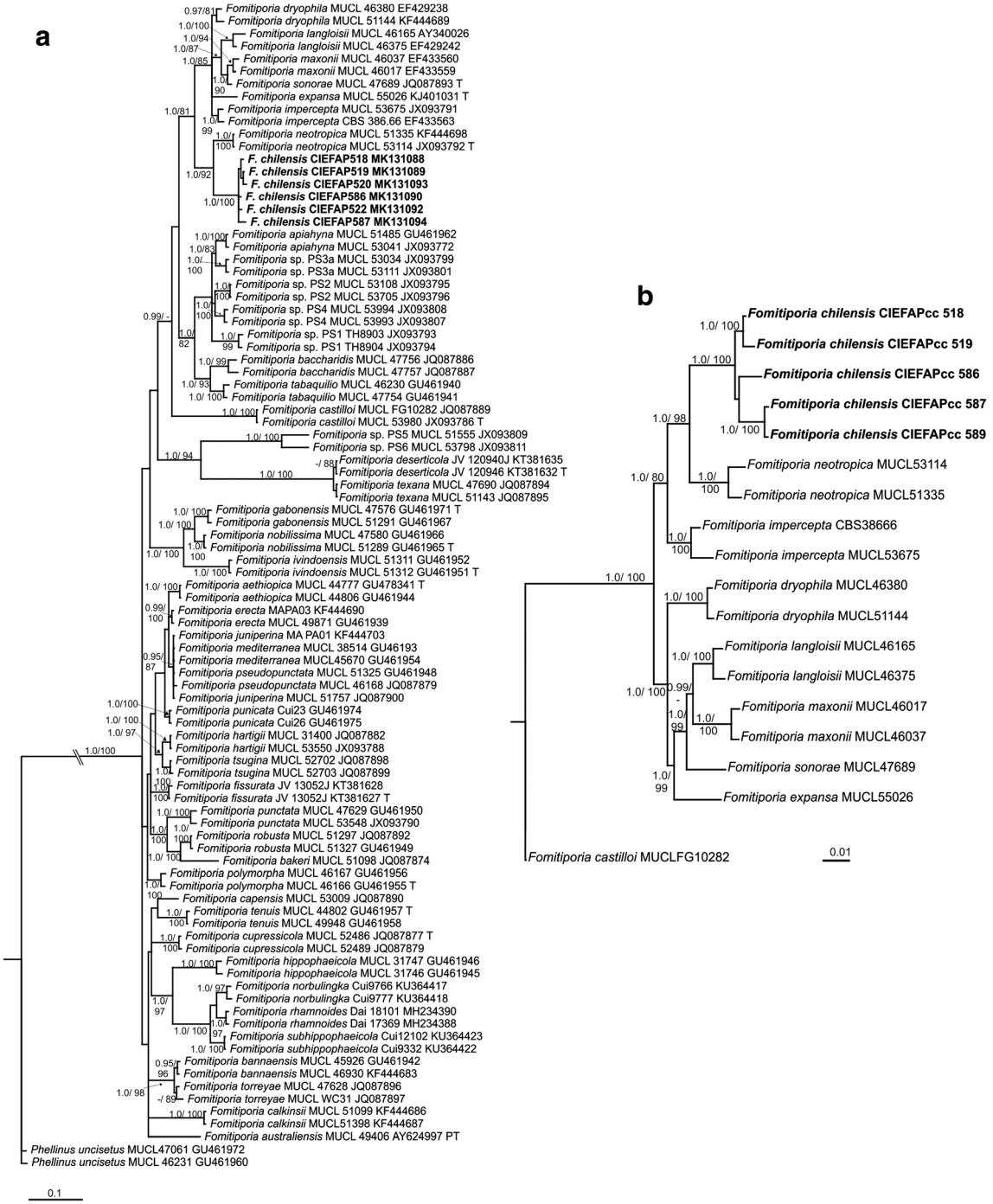

Fig. 1 The 50% majority-rule consensus tree from Bayesian inference of the ITS (**a**) and combined ITS, LSU, tef1, and RPB2 sequences (**b**). Branches are labeled with bootstrap value greater than 75% (ML) and 95% (BPP). Boldface = Patagonian specimens

to 1–2 mm thick that may also develop in the middle of the contextual tissue, where it is thinner. Tubes up to 7 mm long in each stratum, strata distinct, separated by contextual tissue. Consistency dense and woody

Hyphal system dimitic. *Generative hyphae* simple-septate 1.8- to 2.5-μm diam., walls hyaline becoming yellowish, thin- to slightly thick-walled. *Skeletal hyphae* 2- to 3.5-μm diam., but up to 4-μm diam. in context. Hyphae lacking encrustations but polyhedric crystals of variable sizes occasionally present.

Basidia broadly ellipsoid to barrel-shaped, 12–18 × 8–10 μm, with four sterigmata up to 4 μm long.; upon collapsing forming a bee-nest like structure. *Cystidioles* fusiform, lageniform to slightly ventricose, mammiform or with a long apical tube, thin-walled, hyaline, 11–13 × 6–7 μm, few along

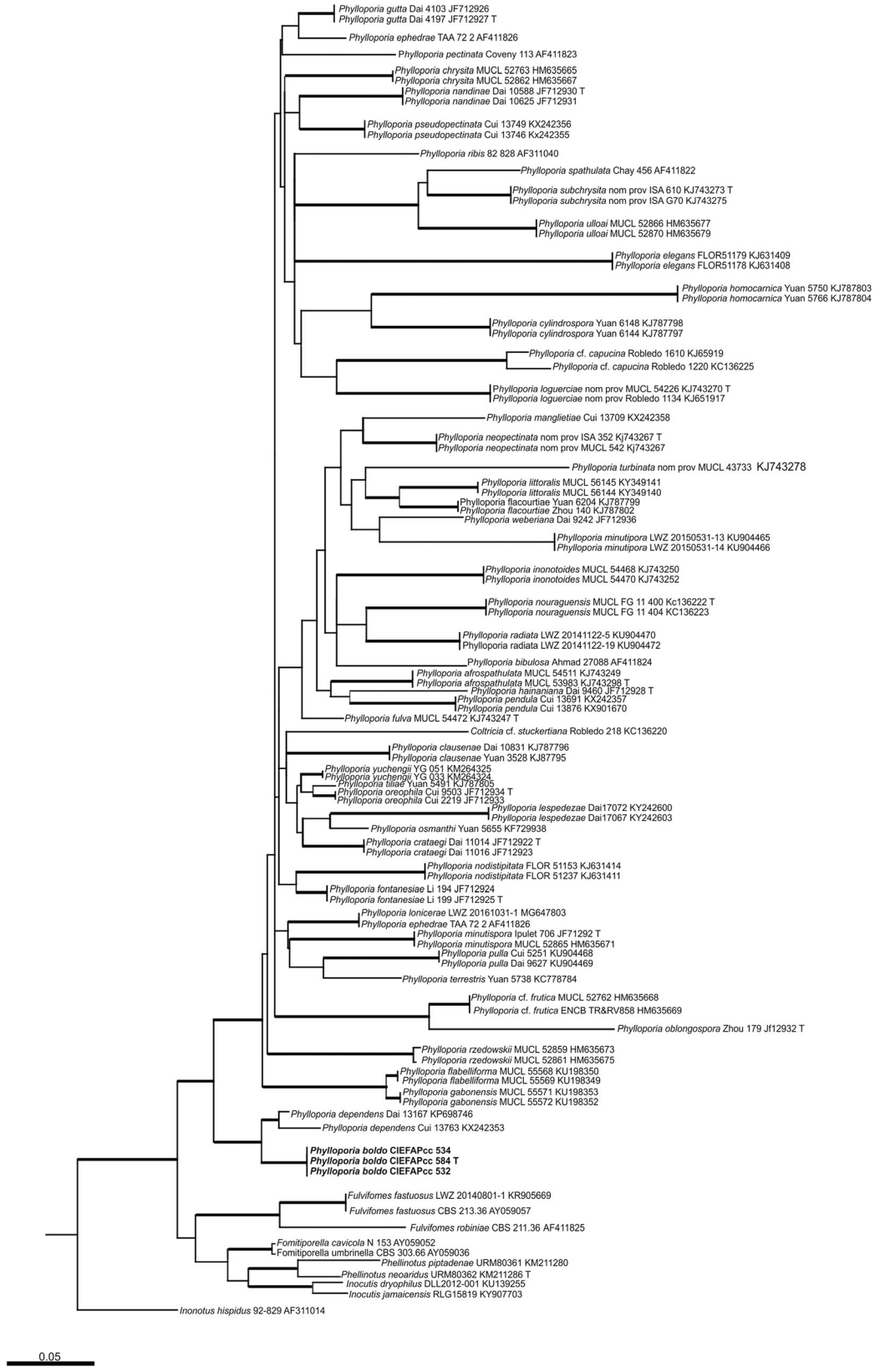

Fig. 2 Phylogram generated from LSU sequence data with Bayesian and RAxML analysis. RAxML bootstraps from 1000 iterations. Bayesian posterior probabilities (BPP) from 1000 iterations (10 million runs sampling every 100th iteration). Thick branches in bold are supported by bootstrap values greater than 75% (ML) and 95% (BPP). Boldface = Patagonian specimens

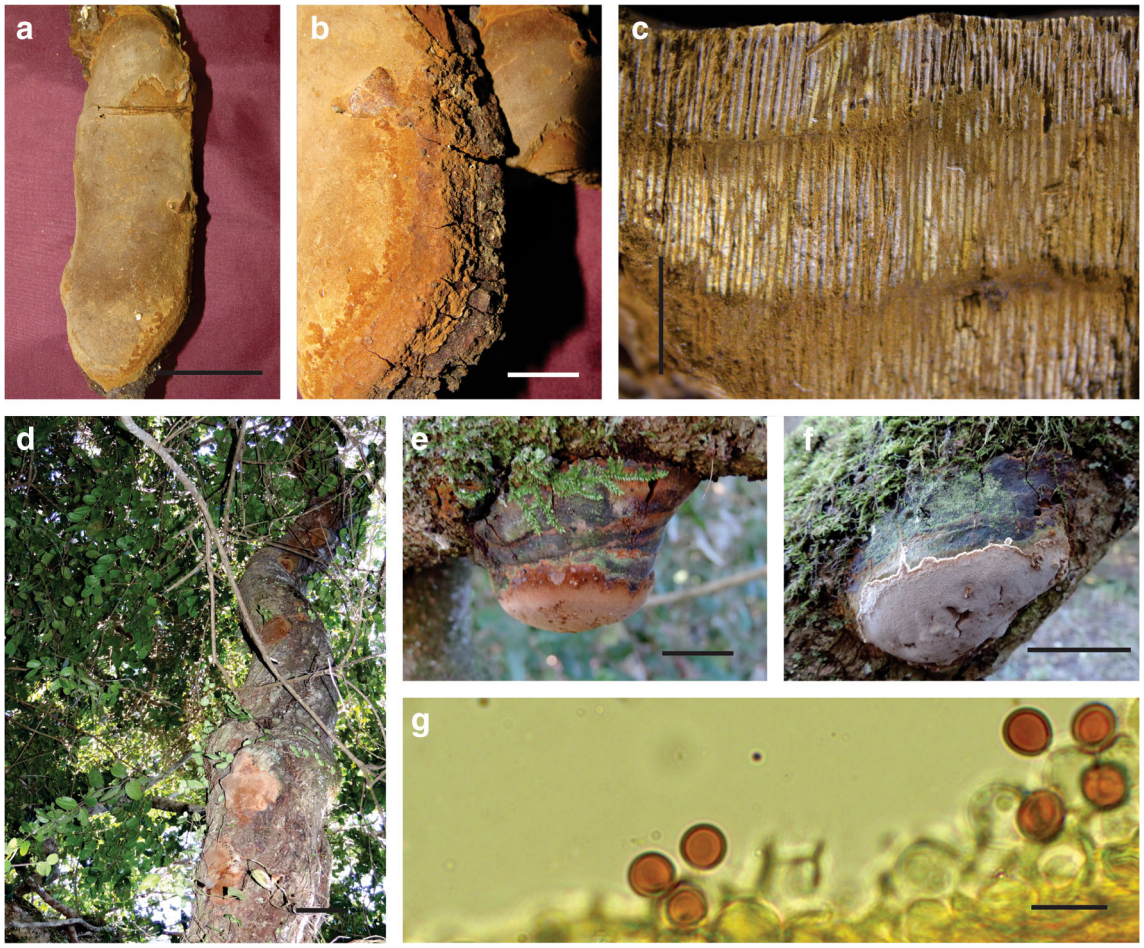

Fig. 3 *Fomitiporia chilensis*, macroscopic and microscopic features of basidiomes. **a**–**b** Holotype (BAFC 52942, MR12557) showing pulvinate habit (**a**) and detail of indurated margin and receding growth (**b**). **c** Specimen MR 2585 showing detail of contextual tissue between strata. **d** Specimen MR12608, columnar habit. **e** Specimen MR12609, effused habit. **f** Specimen MR12610, effused habit with indurated marginal growth. **g** Basidiospores showing dextrinoid reaction of their walls. Bars: **a** = 5 cm; **b**, **c**, **e**, and **f** = 2 cm; **d** = 10 cm; **g** = 10 μm

the tubes but abundant near the pore mouth. *Basidiospores* subglobose to globose, 6.0–6.8 × 5.4–6.2 μm (6.4 ± 0.4 × 5.8 ± 0.4 μm; $Q = 1.7–1.15$, ave$Q = 1.10$), with a small apiculus, walls hyaline, thick-walled, dextrinoid and cyanophilous, becoming slightly yellowish in older parts. *Setae* and/or *setoid elements* absent

Associated wood rot: white and fibrous

Ecology and hosts: on stumps, fallen branches or dried branches still attached to living trees of *Cryptocarya alba* (Lauraceae) and *Peumus boldus* (Monimiaceae)

Distribution: apparently widely distributed on the above-mentioned hosts

Studied specimens—CHILE, Región Bío-Bío, Concepción, Collico Norte, Reserva Coyan Mahuida, on fallen branch of *Cryptocarya alba*, 4 May 2015, leg. M. Rajchenberg 12556, 12564, and 12569. Ibid., on dry branch of standing *C. alba*, leg. ipse 12567. Ibid., on standing *C. alba*, leg. D.A. Cajas Madriaga 1608, 2 May 2016. Ibid., Reserva Nacional Nonguén, Los Olivillos path, on living branch of *C. alba*, leg. M. Rajchenberg 12617, 26 May 2016. River mouth of Bío-Bío river, Santuario de la naturaleza Península de Hualpén, Estación de Biología Terrestre, Universidad de Concepción, 5 May 2015, leg. M. Rajchenberg and R. Reinoso Cendoya MR 12585 on living trunk of *Peumus boldus*. Ibid., 5 May 2015. leg ipse MR 12576 and 12588, on fallen branch. Ibid., higher part of the reserve, on dead, rotten branches attached to living *P. boldus*, 22 May 2016, leg. M. Rajchenberg 12608, 12609 (BAFC 52944), 12610, 12611, and 12612. Arauco, Llico, in remnants of a coastal sclerophyll forest, on dead, standing trunk of *P. boldus*, − 37.213194 long − 73.558500 lat, Mar 2016, leg. D. Alarcón. Concepción, Reserva Forestal Universidad de Concepción, camino Einstein, in mixed forest of *P. boldus*, *Cryptocarya alba*, *Aextoxicon punctatum* and *Nothofagus obliqua*, on stump of angiospermous tree, leg. M.

Rajchenberg 12631, 12632 (BAFC 52943), and 12633 and C. Riquelme, 12 Sep 2018.

Cultures studied: CIEFAPcc 518 (=MR12556), 519 (=MR12557, Type), 520 (=MR12567), 523 (=MR12588), 586 (=MR12609), 587 (=MR12610), 588 (=MR12611), 589 (=MR12612), 590 (=Cajas Madriaga 1608), 593 (=MR12617), 633 (=MR12633), and 635 (=MR12632).

Culture description: growth moderate, 7–9 cm at wk. 6; margin regular to irregular and fan-shaped, subfelty, whitish. Mat homogeneously felty, in some strains becoming progressively abundant up to locally wooly or with wooly flakes, margin white cream, soon becoming yellowish to chestnut yellowish. Around the inoculum formation of crusty areas. Reverse unchanged or forming chestnut areas. Odor none. Margin with generative hyphae, simple septate, 1- to 1.5- to 4-μm diam., thin-walled, hyaline, backwards becoming slightly yellowish to yellowish and slightly thick-walled. Felty mycelium formed by generative hyphae, thin- to thick-walled, with hyaline to yellowish walls, up to 5-μm diam.; generative hyphae forming intercalary, lateral or terminal vesicles, when terminal resembling allocysts, thin to slightly thick-walled; digitiform branches formed from some of the vesicles, thin- to slightly thick-walled; fiber hyphae more or less abundant and dominating in the aerial tissue, 1- to 2-μm diam., unbranched or poorly so; a plectenchyma formed in older parts on the agar surface, formed by thick-walled, chestnut, much branched hyphae that intermingle with hyphae with digitiform structures forming a very cohesive tissue. A yellowish to chestnut substance present in the agar between the hyphae; generative hyphae with yellowish contents also abundantly present in mature parts.

Code: 2.6.8.11.26.32.37.38–39.46-47.54

Note: strains of this taxon display variation in macromorphology of the mat and may form or not chestnut areas in the reverse. They are characteristic by a moderate growth and the formation of vesicles, abundant fiber hyphae and a plectenchyma.

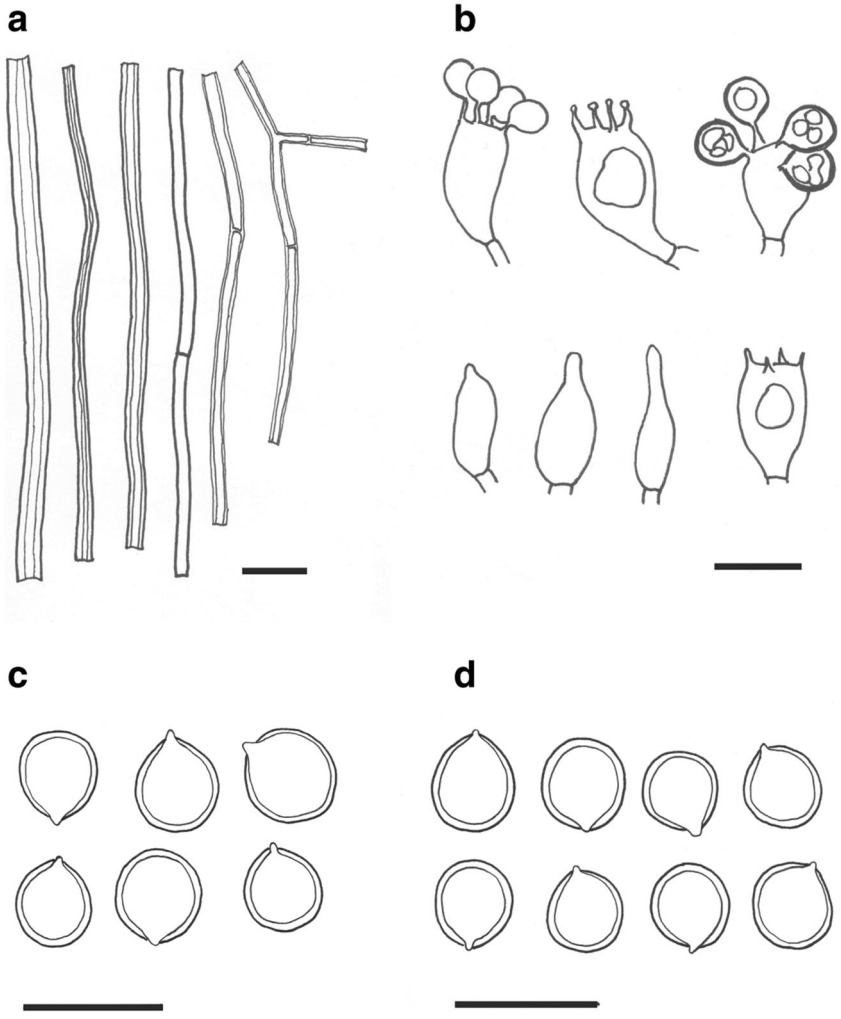

Fig. 4 *Fomitiporia chilensis*, microscopic features of basidiomes. **a** Skeletal and generative hyphae from the dissepiments. **b** Basidia and cystidioles (from holotype). **c** Basidiospores (from holotype BAFC 52942, MR12557). **d** Basidiospores from specimen MR12611. Bars = 10 μm

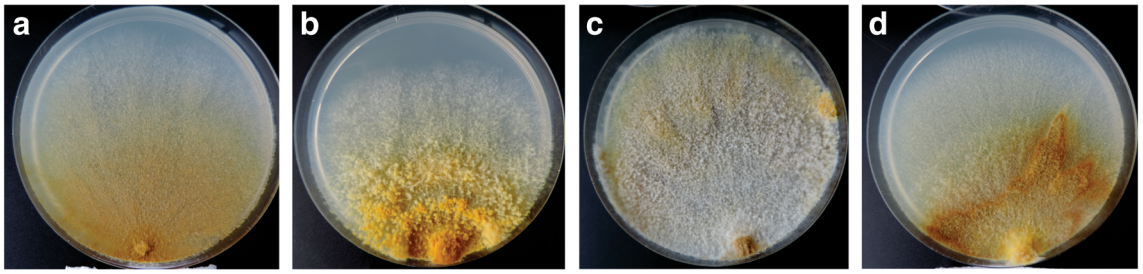

Fig. 5 *Fomitiporia chilensis*, macroscopic features of cultures. **a** Strain CIEFAPcc 519. **b** Strain CIEFAPcc 586. **c** Strain CIEFAPcc 520. **d** Strain CIEFAPcc 594. **e** Strain CIEFAPcc 518. Petri dishes measure 9-cm diam

Remarks *Fomitiporia chilensis* pertains to the *Fomitiporia punctata* (P. Karst.) Murrill species complex in South America (Decock et al. 2007), within the resupinate habit (Amalfi et al. 2014) of the Neotropical lineage. It is macromorphologically similar to *Fomitiporia dryophila* Murrill, with which it shares a cushion-shaped to pseudo-pileate basidiome that develops an indurated margin that cracks with age. *Fomitiporia dryophila* differs by lacking a black line against the substrate which may also develop as a thin line in the contextual tissue and by presenting indistinct tube layers. Microscopically, both species are similar, with subtle differences in basidiospores' size (cfr. Decock et al. (2007) and Raymundo et al. (2012) for recent descriptions and photographs of the species). Biogeographically, *F. dryophila* differs in growing in subtropical areas of SE USA and in Mexico, fruiting on *Quercus* spp. and *Celtis occidentalis*, *Byrsonima crassifolia*, and *Psidium* sp., on dead or living stems/branches. *Fomitiporia neotropica* Camp.-Sant., Amalfi, R.M. Silveira, Robledo Decock (Campos Santana et al. 2014) and *F. impercepta* Morera, Robledo and Urcelay (Morera et al. 2017) are the closest taxa, phylogenetically. The former differs by annual to biannual, flat, and resupinate basidiomata with pores 6–9/mm and the presence of hymenial setae. *Fomitiporia impercepta* is rather similar morphologically but differs by flatter basidiomes less than 1 cm thick and smaller basidiospores, (4.0)5.0–6.0(7.0) × 4.0–6.0(7.0) μm.

Phylloporia boldo Rajchenb. and Pildain sp. nov. (Figs. 6, 7, 8)

MB829087

Holotype: CHILE, VIII Región, Bío-Bío, Concepción, river mouth of Bío-Bío river, Santuario de la naturaleza Península de Hualpén, Estación de Biología Terrestre, Universidad de Concepción, 29 m asl, on living stem of *P. boldus* (Monimiaceae), −73.157603 Long −36.797483 Lat, leg. M. Rajchenberg MR 12606, 22 May 2016 (BAFC 52945). LSU = MK193758

Etymology: "Boldo" refers to the vulgar name of the host species *Peumus boldus* on which the fungus grows.

Diagnosis Basidiome perennial, pileate, flabellate or dimidiate, upper surface sulcate with narrow to wide bands, velutinate in the margin, backwards irregularly tuberculate, context relatively thin, pores 4–5.5/mm, hyphal system dimitic; generative hyphae simple septate, skeletal hyphae mostly unbranched, setae lacking, basidiospores broadly ellipsoid to subglobose, 5.4–6.0 × 4.4–5.0 μm, thick-walled, walls golden in water, chestnut to dull chestnut in KOH solution; associated wood-rot white.

Basidiomes perennial, sessile, pileate, flabellate to dimidiate, triquetrous, sometimes almost ungulate, attached by a single central portion/area with the rest of the basidiome in contact with the host but not attached to it, sometimes with an effused portion and forming effused reflexed basidiomes, solitary or few imbricated, 5–20 cm wide × 3–10 cm radius × 1.5–3.5–5 cm thick; margin thinning but few ones round; pileal surface sulcate with narrow to wide, marked bands 2–7 mm wide, dark brown to almost black, the upper surface strongly indurated, margin first velutinate and yellowish brown but soon afterwards the grooves indurating, the surface creviced, in older parts irregularly tuberculate, breaking irregularly and in some portions becoming slightly to strongly rimose. Context 2–3 mm thick, presenting a black line that separates an upper narrow part that becomes the indurated pilear surface from a lower homogenous part; sometimes, the black line present as the lower indurated limit of the pilear surface; in some basidiomes, the black line is discontinuous. Tubular layer up to 4.5 cm thick. Pores surface light tobacco brown, pores round, 4–5.5/mm.

Hyphal system dimitic. Context monomitic in the upper portion but soon becoming dimitic. Generative hyphae simple-septate, branched, 3–6(−7) μm in diam.; first narrow, with slightly thickened, golden to light chestnut walls, some becoming wider and 1.5–2 μm thick-walled, leaving a distinct lumen, chestnut to dark chestnut. Skeletal hyphae generally straight, few branched, 4–7 μm in diam., with thickened walls, light to dark chestnut. Some skeletal hyphae present secondary, roundish septa that may be confused with true septa and erroneously give the idea that the hyphal system is monomitc. Dissepiments dimitic, with narrower hyphae than in the context. Generative hyphae 2–3.5 μm in diam., much branched and some tortuous, with slightly thickened, golden walls.

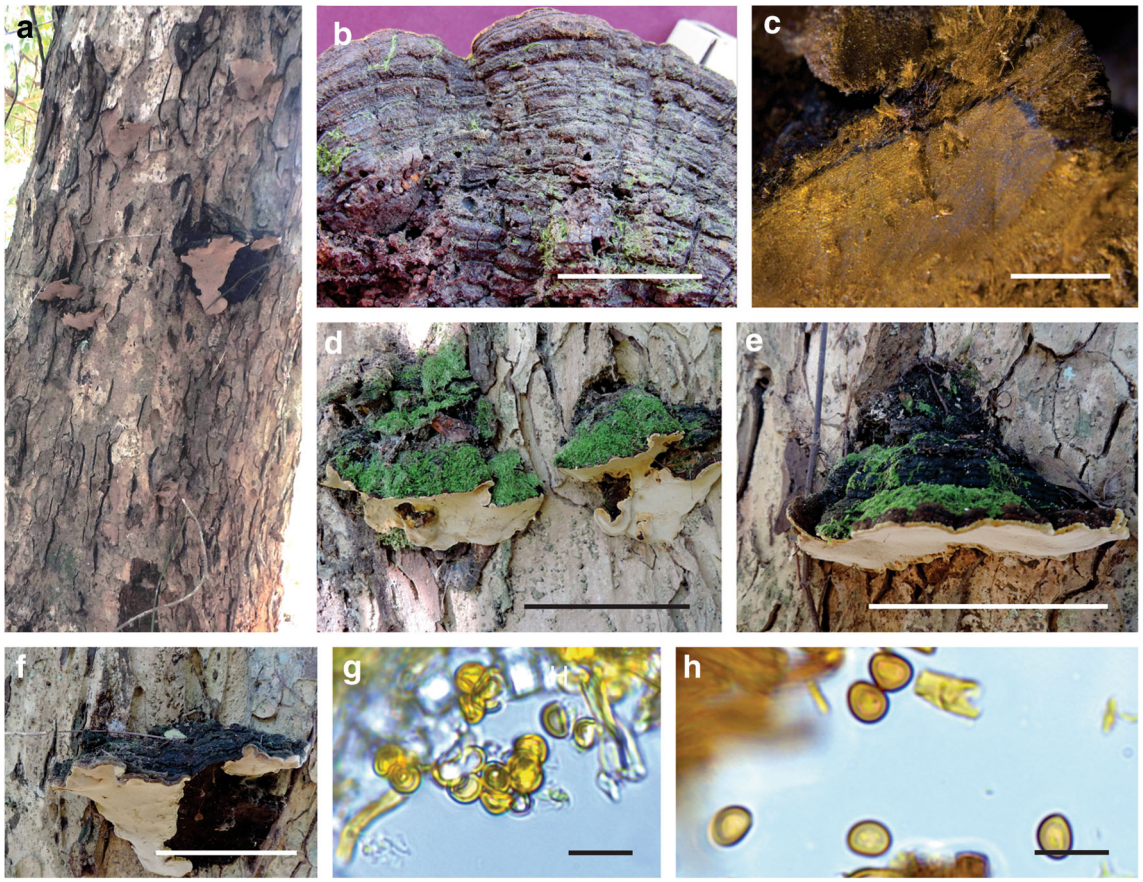

Fig. 6 *Phylloporia boldo*, macroscopic and microscopic features of basidiomes. **a** Tree attacked by *P. boldo*, from where the holotype and isotype were selected. **b**–**c** Specimens from the holotype (BAFC 52945, MR12606). **b** Macroscopic habit. **c** Detail of the pilear surface. **d** Specimen MR12575. **e** Specimen from the isotype (BAFC 52946, MR12607). **d**–**e** Macroscopic habit. **f** Black line below the pilear surface. **g**–**h** Basidiospores from the holotype. **g** Basidiospores in water. **h** Basidiospores in KOH sol. Bars: **b** and **d** = 2 cm; **d**–**f** = 10 cm; **g**–**h** = 10 μm

Skeletal hyphae 2.5- to 3.5- to 4-μm in diam., thick-walled, chestnut, unbranched

Hymenium collapsed and basidia lacking. *Setae* lacking. *Basidiospores* broadly ellipsoid to subglobose, with a lateral flattened or straight side when just formed, but this side becoming roundish when mature, walls thickened 0.3–0.5 μm, golden in water, chestnut to dull chestnut in KOH, with a central oily-like guttula when recently formed that soon disappears, 5.4–6.0 × 4.4–5.0 μm (5.7 ± 0.3 × 4.7 ± 0.3; Q = 1.20–1.22, ave*Q* = 1.21).

Associated wood rot: white

Ecology and hosts: on living stems of the endemic *Peumus boldus* (Monimiaceae, Laurales), so far restricted to this host.

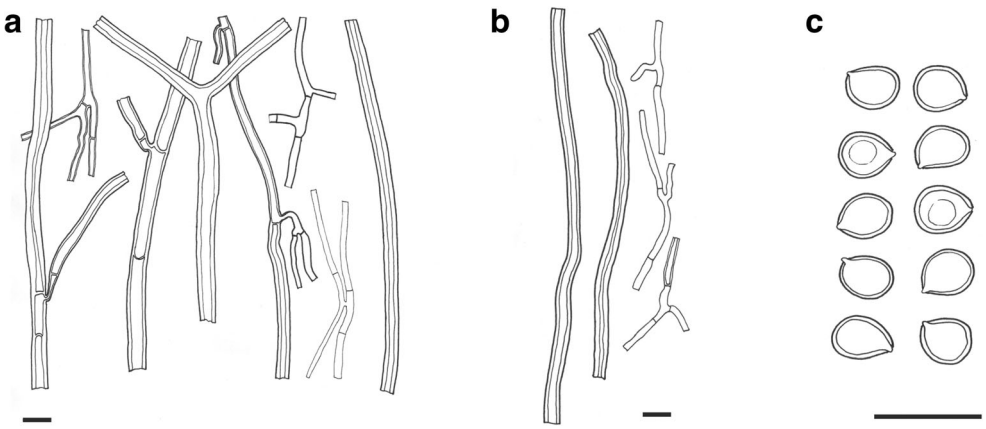

Fig. 7 *Phylloporia boldo*, microscopic features of basidiomes (holotype BAFC 52945, MR12606). **a** Generative and skeletal hyphae from the context. **b** Generative and skeletal hyphae from the dissepiments. **c** Basidiospores. Bars = 10 μm

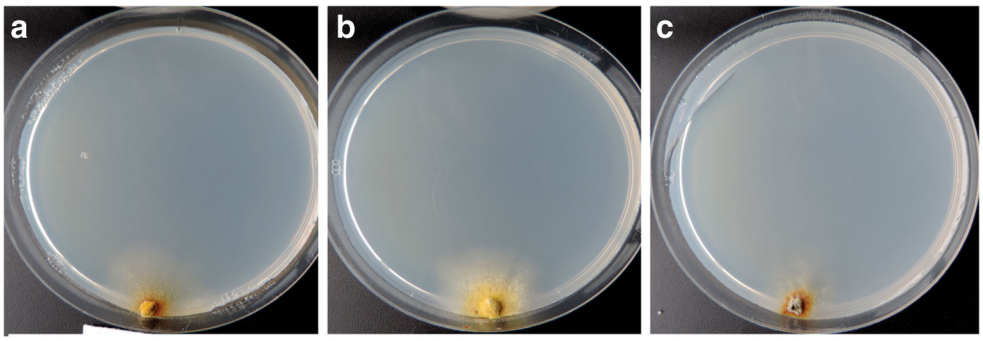

Fig. 8 *Phylloporia boldo*, macroscopic features of cultures. **a** Strain CIEFAPcc 533. **b** Strain CIEFAPcc 584. **c** Strain CIEFAPcc 585. Petri dishes measure 9-cm diam

Distribution Though most of the specimens have been gathered in the province of Concepción, it seems to follow the host, as it has been also found in the province of Melipilla near the capital city Santiago. *Peumus boldus* is distributed from southern Coquimbo in the North to southern Valdivia in the South.

Studied specimens—CHILE, VIII Región, Bío-Bío, Concepción, river mouth of Bío-Bío river, Santuario de la naturaleza Península de Hualpén, Estación de Biología Terrestre, Universidad de Concepción, 29 m asl, on living stem of *P. boldus* (Monimiaceae), −73.157603 long −36.797483 lat, leg. M. Rajchenberg 12573, 12574, and 12575 and R. Reinoso, 5 May 2014 (BAFC 52947). Ibid, leg. ipse MR 12576 and R. Reinoso, on fallen branch of *P. boldus*. Ibid., leg. M. Rajchenberg 12606 (holotype, BAFC 52945) and 12607 (Isotype, BAFC 52946), 22 May 2016. Ibid., Parque Metropolitano Cerro Caracol, Mirador Alemán, 254 m asl, −73.038270 long −36.843645 lat, on *P. boldus*, leg. C. Riquelme 1801, 12 Apr 2018 (CONC). Ibid., Campos Deportivos de Bellavista, 12 m asl, −73.0310340 long −36.784825 lat, on *P. boldus*, leg. C. Riquelme 1807, 22 May 2018 (CONC). Ibid., Reserva Forestal Universidad de Concepción, camino Einstein, in mixed forest of *P. boldus*, *Cryptocarya alba*, *Aextoxicon punctatum*, and *Nothofagus obliqua*, on the base of a standing *P. boldus*, leg. M. Rajchenberg 12630 and C. Riquelme, 12 Sep 2018. Ibid., Arauco, Arauco, in a patch of native forest dominated by *P. boldus* and *C. alba*, −37.260980 long −73.378222 lat, 23 Oct 2014, leg. G. Torres (duplic CONC). Ibid., Coronel, Parque Educativo Jorge Alessandri, 23 m asl, −73.147668 long −36.940811 lat, on *P. boldus*, leg. C. Riquelme 1802, 19 Apr 2018 (CONC). Ibid., Reserva Nacional Nonguén, sendero Los Rojas, 288 m asl, −73.001262 long −36.880745 lat, on *P. boldus*, leg. C. Riquelme 1803, 28 Apr 2018 (CONC). Ibid., San Pedro de la Paz, Laguna Grande de San Pedro, 22 m asl, −73.105659 long −36.847078 lat, on *P. boldus*, leg. C. Riquelme 1805, 5 May 2018 (CONC). Arauco, Arauco, in a patch of native forest dominated by *P. boldus* and *Cryptocarya alba*, on standing, decayed *P. boldus*, −73.378222 long −37.260980 lat, leg. G. Torres, 23 Oct 2014 (CONC). Región Metropolitana, Provincia de Melipilla, Curacaví, La Aurora, −71.006200 long −33.410097 lat, on *P. boldus*, comm. C. Riquelme 1804, 1 May 2018 (CONC)

Cultures studied: CIEFAPcc 532 (=MR12573), 533 (=MR12574), 534 (=MR12575), 584 (=MR12606), and 585 (=MR12607)

Culture description: growth very slow to slow, 2.1–2.5 cm (but in strain CIEFAPcc 532 up to 5.5 cm by the end of the study). Mat generally felty and yellowish or becoming chestnut, margin irregular, subfelty, and whitish (in strain CIEFAPcc 532 mat openly wooly, not dense, whitish in the growing area, becoming yellowish in the middle of the mat and towards the inoculum tightly felty and chestnut). Agar unchanged or becoming chestnut. Margin subfelty. Odor none. Margin with simple septate generative hyphae, 2- to 3-μm diam., walls thin but becoming thickened and chestnut, some may be confused with fiber hyphae but they always present septa. Aerial hyphae formed by generative hyphae 2.5- to 3-μm or wider, 4- to 6-μm diam., narrowing at the septum, some with yellowish contents, thin- to slightly thick-walled; some of the wider hyphae forming digitiform ramifications near the septum in a more or less verticillate disposition. One culture formed fiber hyphae 2.5 to 3.5-μm diam., unbranched, with thickened, golden to chestnut walls.

Code: 2.6.(8).32.37.38.39.47.(48).54.

Note: strains of the new species are characterized by a very slow growth, with exceptional formation of fiber hyphae. Otherwise, they form the typical yellowish mat of many Hymenochaetaceae.

Remarks

The new species resembles *Phylloporia pectinata* (Klotzsch) Ryvarden for its perennial and dimitic basidiome, but this species presents a duplex context and typical golden yellowish basidiospores. Other perennial taxa in the genus (Zhou and

Dai 2012, Zhou 2015 and 2016) differ by presenting small pores in a degree in between 6 and 12/mm, and/or by basidiospores smaller than 5 μm. Other taxa in the genus are annual and monomitic (cfr. Zhou and Dai 2012, and Dai 2010 for descriptions and photographs). *Phylloporia boldo* differs from all species in the genus by its comparatively large basidiospores with chestnut to dull chestnut (not golden) walls in KOH that better resemble those of *Fulvifomes* Murrill. *Phylloporia dependens* Y.C. Dai groups phylogenetically with the new taxon but differs by a monomitic hyphal system and smaller, golden basidiospores (Liu et al. 2015). The association of both species and their low relatedness with all other taxa of *Phylloporia* that have been incorporated in phylogenetic studies suggest the existence of a clade different from *Phylloporia*. Nevertheless, for the time being, it is difficult to assert how this two species might be related, for which reason we prefer to maintain them under the name *Phylloporia*. Because of the morphological similarity of *P. boldo* with *Fulvifomes* species, efforts "to force" their association in the phylogenetic analysis were performed, but all failed.

From a biogeographic point of view, there is no apparent relationship of the new taxon with other species. It is clear that *P. boldo* is an example of a taxon whose relationships remain unknown, due probably to the lack of information on the phylogenetic disposition of taxa from the Southern hemisphere.

Discussion

The two new described species have strong phylogenetic and morphological support. Most important issues have been addressed under the "Remarks" of each of them. Cultural features were typical of the Hymenochaetaceae but did not present any feature characteristic at genus level, as has already been shown for that family (Fiasson and David 1983); nevertheless, they provide useful information for phytopathological studies. We underline the importance of inventories in order to properly establish the mycodiversity of different areas, also for larger fungi. In the case of *F. chilensis*, a relationship with the South American taxa within the *Fomitiporia punctata* species complex was found. For *Phylloporia boldo*, no apparent biogeographic relationship was found with the single species that is phylogenetically related to it, *P. dependens*. This shows that there is a gap of knowledge regarding this group of wood-rotting fungi and that future studies incorporating phylogenetic analyses may provide clues to understand its diversity. Our study shows that the mycobiota of Chile is still fragmentarily known and that we might expect new taxa to be discovered.

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