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The phylogenetic position of *Postia* s.l. (Polyporales, Basidiomycota) from Patagonia, Argentina

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Abstract: We investigated the phylogenetic relationships of *Postia* species from Patagonia with rDNA ITS and LSU sequences, together with morphological, cultural and biological features. All species in the genus were included in a ''Postia clade'' irrespective of whether their spores were thin- or thick-walled. This clade is characterized by tetrapolar mating, a normal nuclear behavior, metachromatic generative hyphae and absence of fiber hyphae in culture. One subclade merged the austral taxa *P. pelliculosa* and *P. punctata*, but otherwise no distinct relationships were found regarding spore shape, spore wall thickness and geographical distribution of taxa. The austral *P. venata* and the endemic *P. carbophila*, with thin-walled basidiospores, occupied variable positions in both analyses. *Postia caesia* from Patagonia grouped within the *P. caesia* species complex but on a separate branch. In contrast, *P. rennyi* and *P. balsamea* from Patagonia corresponded well with strains from other geographic areas. The two austral species in *Ryvardenia*, *R. cretacea* and *R. campyla*, characterized by non-metachromatic hyphae, bipolar mating and an astatocoenocytic nuclear behavior, formed an independent subclade among the dimitic genera of the ''Antrodia clade'', far from other *Postia* taxa within which they had been placed previously, supporting their inclusion in a genus of their own. *Postia carbophila* grouped with other *Postia* species and not with *Postia* (*Rhodonia*) *placenta* as suggested previously on the basis of morphological comparisons. Instead, the latter species grouped with taxa in the dimitic genus *Amyloporia* with which it shares heterocytic nuclear behavior. A separation between specimens of *Postia pelliculosa* and *Ryvardenia cretacea* from either side of the Pacific (i.e. SE Australia/New Zealand and S Argentina/S Chile) suggests they could be considered different at the species level from a phylogenetic point of view.

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Introduction

Brown-rot polypores that display a monomitic hyphal system with clamped generative hyphae and thin- to thick-walled basidiospores have been included mostly in the genus *Postia* Fr., one of the largest genera in the the Polyporales (Basidiomycota). The genus was characterized on the basis of its morphological and cultural features, mating system and nuclear behavior of the mycelium by David (1980). In brief, she neatly distinguished species in this genus (treated as *Spongiporus* Murrill, a taxonomic synonym) by their (i) monomitic hyphal system with clamped generative hyphae with walls that are irregularly thickened in many species when examined in 5% KOH (or they display this feature in culture), and which give a positive metachromatic reaction when mounted in cresyl blue; (ii) cylindrical, allantoid to ellipsoid basidiospores with thin to slightly thickened walls; (iii) long (i.e. about 3–4 wk or more) delay of spore germination in malt extract agar in vitro, (iv) regularly simple-clamped hyphae throughout the colony and non-formation of fiber hyphae in culture; (v) tetrapolar mating system; and (vi) normal nuclear behavior of the mycelium (cf. Boidin [1971] for the definitions of nuclear behavioral types, and Rajchenberg [2011] for a review of this feature in polypores).

Most species in *Postia* in the past were referred to *Tyromyces* P. Karst. but, as shown by David (1980), that name applies to species producing a white-rot in the substrate, with basidiospores that germinate rapidly in malt extract agar (i.e. within 3–7 d) and that display an astatocoenocytic nuclear behavior. This has been widely confirmed by phylogenetic studies (Yao et al. 1999, Binder et al. 2005). Because of the large number of species that historically were included in *Postia*, the morphological treatment and delimitation of species has been difficult and we refer to the classical work of Lowe (1975), who used a broad genus concept for this group of species (as *Tyromyces*) and to Gilbertson and Ryvarden (1987) for North America, Ryvarden and Gilbertson (1993, 1994) and Bernicchia (2005) for Europe, Núñez and Ryvarden (2001) for eastern Asia and Dai (2011) for species known from China. The name *Postia* has not been widely accepted by taxonomists and, instead, in some

of the above mentioned works, the name *Oligoporus* Bref. has been used for the same species. For a discussion on this nomenclatural issue see Donk (1960), Larsen and Lombard (1986) and Walker (1996), who support the use of *Postia*, and Ryvarden (1991), who supports the use of *Oligoporus*. The exception between these positions is the one adopted by Erkkilä and Niemelä (1986) and Renvall (1992), who proposed the use of both names: *Postia* for taxa with thin-walled basidiospores and *Oligoporus* for taxa having broadly ellipsoid to short cylindrical basidiospores with thickened, cyanophilous walls, and which have a tendency to form chlamydospores in nature and/or culture.

The morphology and biology of *Postia* species found in the Patagonian Andes forests of southern Argentina have been summarized by Rajchenberg (2006). Nine taxa are known, of which two are endemic (*P. carbophila* Rajchenb. and *P. minuta* Rajchenb.), four are austral (*P. dissecta* [Lév.] Rajchenb., *P. pelliculosa* [Berk.] Rajchenb., *P. punctata* Rajchenb. & P.K. Buchanan and *P. venata* [Rajchenb. & J.E. Wright] Rajchenb.) and three are widespread in distribution (*P. balsamea* [Peck] Jülich, *P. caesia* [Schard.] P. Karst. and *P. rennyi* [Berk. & Broome] Rajchenb.). Several of these species possess particular morphological features that may conflict with their placement in the genus. *Postia pelliculosa* is one of the major wood-rotting species of several *Nothofagus* species in the Patagonian Andes forests and is widespread in the southern hemisphere, being recorded also from eastern Africa (Ryvarden and Johansen 1980), Cameroon (Douanla-Meli 2007), Australia (Cunningham 1965) and New Zealand (Buchanan and Hood 1992). It differs from other *Postia* species in having relatively thick-walled basidiospores. *Postia punctata* has basidiospores that are slightly thick-walled and, because of their shape and size, could be compared with species of *Ryvardenia* Rajchenb. (see below). *Postia carbophila* was compared to the morphologically similar *Postia placenta* (Fr.) M.J. Larsen & Lombard, proposed as the type species for the genus *Rhodonia* Niemelä (Niemelä et al. 2005) based on phylogenetic research (Schigel et al. unpubl). *Postia balsamea* is widespread in the northern hemisphere where it is circumpolar but is rare in the southern hemisphere. It is known only from the Patagonian Andes forests of Argentina, where it decays standing *Saxegothaea conspicua* (Podocarpaceae) and fallen *Nothofagus* spp. *Postia caesia* is widely distributed in temperate and tropical regions worldwide and is recognized as a species complex (Yao et al. 2005) whose affinities are little known. *Postia rennyi* is known from Europe and Asia but is unknown from the Americas except from Patagonia. For these reasons it was interesting to validate the identity of these species with means other than morphology.

In addition to the above-mentioned species, two other austral taxa have been treated under *Postia*: *P. campyla* (Berk.) Rajchenb. and *P. cretacea* (Lloyd) Rajchenb. but were separated into the genus *Ryvardenia* Rajchenb. (Rajchenberg 1994). This genus, with *Polyporus cretaceus* Lloyd as the type species, is characterized by: (i) basidiomes with a dimitic hyphal system with dissepiments presenting long, terminal, thick-walled hyphae that may be considered skeletal hyphae, but which is monomitic in the context; (ii) tissues that are characterized by the presence of thick-walled, clamped generative hyphae with glossy/refringent walls that are not metachromatic in cresyl blue and that contrast with thin-walled generative hyphae; (iii) basidiospores that are obovate, broad-ellipsoid to drop-shaped and thick-walled, (iv) spores that germinate in 2–4 wk after discharge on malt extract agar (known only for the type species); (v) a bipolar mating system; (vi) an astatocoenocytic nuclear behavior of the mycelium; and (vii) in culture by the generative hyphae of the advancing mycelium being simple-septate but, backwards, by forming homogeneously sclerified, clamped generative hyphae and fiber hyphae that are never irregularly thick-walled (Rajchenberg 1994, 2006).

Postia pertains to the ''Antrodia clade'', which includes most of the known brown rotting polypores (Hibbett and Donoghue 2001, Garcia Sandoval et al. 2011). Perhaps because it is a well circumscribed group with well delimited genera that include many taxa of pathological importance in forestry, this clade has been the focus of numerous phylogenetic studies and several genera or groups of genera have been, or are being treated regularly (Kim et al. 2001, 2003, 2005, 2007; Lindner and Banik 2008; Yu et al. 2010; Lindner et al. 2011; Rajchenberg et al. 2011; Bernicchia et al. 2012). The genus *Postia*, though, has rarely been the focus of this type of research and few works have been published, all dealing with a small group of species (Yao et al. 2005, Hattori et al. 2011). It is clear though from the literature that the genus is well separated from *Antrodia* P. Karst., from other dimitic genera in the clade such as *Fomitopsis* P. Karst., *Daedalea* Pers., *Piptoporus* P. Karst. and *Fibroporia* Parmasto, and from other monomitic genera such as *Parmastomyces* Kotl. & Pouzar and *Auriporia* Ryvarden. Both species in *Ryvardenia*, *R. campyla* and *R. cretacea* are of particular interest because they were treated in several different genera such as *Piptoporus* and *Postia* but also in the white-rotting genera *Spongipellis* Pat., *Tyromyces* and/or *Grifola* S.F. Gray. Because of their morphological and biological peculiarities deviating from *Postia*, pointed

out above, it was deemed interesting to investigate with molecular methodologies the phylogenetic support for this southern hemisphere genus and to check its relationships within the Polyporales.

The aims of the present study were (i) to evaluate the phylogenetic relationships of *Postia* species found in the forests of the Patagonian Andes of Argentina, (ii) to evaluate the position of the related genus *Ryvardenia*, (iii) to validate the determinations of *Postia* taxa displaying widespread distribution vis à vis specimens from the northern hemisphere and (iv) to validate the taxonomic position of several austral or endemic *Postia* taxa that present peculiar morphological features.

MATERIALS AND METHODS

Strains and herbarium specimens.—Most strains studied, with their voucher specimens, are deposited at the author's institutional culture collection (CIEFAPcc) and phytopathological herbarium (CIEFAP); some are duplicates deposited elsewhere, and if so the information is indicated in the text. Herbarium designations follow Holmgren et al. (1990), and culture collection designations follow the World Federation for Culture Collection website (http://www.wfcc.info). Strains included in this study are detailed (TABLE I).

DNA extraction and PCR conditions.—For DNA extraction, *Postia* and *Ryvardenia* species from Patagonia collections were cultured in malt peptone broth with 10% (v/v) of malt extract (Merck) and 0.1% (w/v) Bacto peptone (Difco), 2 mL medium in 15 mL tubes. The cultures were incubated at 25 C for 15 d in darkness. Total DNA was extracted with the UltraClean™ Microbial DNA Isolation Kit (MO BIO laboratories Inc., Solana Beach, California), according to the manufacturer's instructions. Herbarium specimens were examined under a dissecting microscope before extraction of DNA and cleaned with a toothbrush. Samples were ground with a mortar and pestle, extracted at 70 C for 10 min in 300 µL bead solution (UltraClean™ Microbial DNA Isolation Kit, MO BIO laboratories Inc., Solana Beach, California) and continued according to the above protocol indicated by the manufacturer Kit instructions. DNA quantification was performed by ultraviolet spectroscopy.

PCR products amplified in this study were nuclear ribosomal LSU (LR0R–LR5) (Vilgalys and Hester 1990) and ITS (ITS5–ITS4) (White et al. 1990) partial sequences. PCR reaction mixtures for amplification of both regions were the same as those described by Rajchenberg et al. (2011); the mixture included dNTPs (0.25 mM of each), 2.5 mM $MgCl_2$; PCR buffer supplied with the polymerase enzyme; 0.1 µM each primer; 100–500 ng DNA; and 1.25 U GoTaq polymerase (Promega, Madison, Wisconsin). The final reaction volume was 50 µL. The PCR reactions were performed in a thermal cycler (My Cycler™, BioRad) and the conditions for ITS were: an initial denaturation at 95 C (2 min), followed by 30 cycles of denaturation at 94 C (1 min), primer annealing at 56 C (1 min) and elongation at 72 C (1 min), and a final elongation step was allowed at 72 C (10 min). For LSU the PCR conditions were: an initial denaturation at 95 C (2 min), followed by 35 cycles of denaturation at 94 C (30 s), primer annealing at 53 C (1 min) and elongation at 72 C (1 min), with a final elongation step at 72 C (5 min). PCR products were separated on a 1% (w/v) ethidium bromide-stained agarose gel, and the bands were visualized under UV illumination. The amplified fragments were purified and sequenced on an ABI 3700 automated sequencer (Perkin-Elmer, Foster City, California) at the DNA Synthesis and Sequencing Facility, Macrogen (Seoul, Korea). Sequences generated in this study were submitted to GenBank (TABLE I).

Sequence and phylogenetic analyses.—Sequence data generated in this study were manually edited with BioEdit 7.0.9.0 (Hall 1999), and additional sequences were retrieved from the GenBank nucleotide database. Two sequence datasets were analyzed for this study, one for ITS and one for LSU, which were treated separately because of lack of overlap (TABLE I).

Alignment of LSU and ITS sequence datasets were performed automatically with MEGA 4.0 (Tamura et al. 2007) and are available from TreeBASE (http://purl.org/phylo/treebase/phylows/study/TB2:S12727). Analyses were conducted via maximum parsimony (MP) in PAUP* 4.0b10 (Swofford 2002) and Bayesian inference (BI) in MrBayes 3.0B4 (Ronquist and Huelsenbeck 2003). MP analysis for both datasets were performed with gaps treated as missing characters, equal weighting of characters and transformations, heuristic searches with random addition of sequences (1000 replicates), TBR (tree bisection reconnection) branch swapping and MAXTREES was set to autoincrease. A bootstrap analysis was performed with 1000 replicates with simple stepwise addition of sequences. The GTR+I+G and TVM+I+G models of evolution were identified with Modeltest 3.7 (Posada and Crandall 1998) under selection AIC for the LSU and ITS sequence datasets respectively. BI posterior probabilities for both datasets were conducted with the suggested nucleotide evolution models for 10^7 generations, by running four chains with 100 000 generations using the program default priors on model parameters. Trees were rooted with *Antrodiella semisupina* (Berk. & M.A. Curtis) Ryvarden and *Bjerkandera adusta* (Willd.) P. Karst.

Species of *Fomitopsis*, *Daedalea*, *Oligoporus*, *Amyloporia* Singer, *Antrodia*, *Neolentiporus* Rajchenb. and *Postia* (*Rhodonia*) *placenta* were included in the analyses because they have been associated with *Postia* taxa in other phylogenetic studies (Hibbett and Donoghue 2001, Kim et al. 2001, Wu et al. 2004, Yu et al. 2010). LSU sequences for *Postia placenta* were not available; therefore results for this species were obtained only for the ITS analysis. Similarly, material of *A. nothofaginea* and *P. rennyi* from Patagonia could not be obtained and were excluded from the LSU analysis.

RESULTS

The phylogenetic relationships of *Postia* and *Ryvardenia* species from Patagonia were estimated with two

TABLE I. Specimens presented in this study with GenBank accession numbers for the ITS and LSU sequences (newly sequenced strains are indicated in boldface)

Species	Strain/herbarium	Location	Host	GenBank accession nos. ITS	GenBank accession nos. LSU
Postia balsamea	**CIEFAPcc 296/ MR11974**	Argentina, Neuquén, Nahuel Huapi	*Saxegothaea conspicua*	**JX090116**	—
	CIEFAPcc 340/ MR12338	Argentina, Neuquén, Nahuel Huapi	*Nothofagus dombeyi*	**JX090108**	—
	CIEFAPcc 351/ MR12413	Argentina, Chubut, Los Alerces	*Nothofagus dombeyi*	**JX090105**	**JX090131**
(as *Oligoporus*)	JB8609_9	—	*Malus* sp.	JF950570	—
	K(M)31063	—	—	AY599566	—
	KEW35	—	—	—	AF518640
Postia caesia	**CIEFAPcc 174/ MR12276**	Argentina, Chubut, Los Alerces	*Nothofagus antarctica*	**JX090109**	**JX090129**
	CIEFAPcc 350/ MR12421	Argentina, Chubut, Los Alerces	*Nothofagus dombeyi*	**JX090110**	**JX090130**
	K(M)31967	UK, New Forest, Hamps	*Alnus*	AY599567	—
	WD1974	Japan, Kochi	—	—	AB569120
	WD1984	Japan, Kochi	—	—	AB569119
Postia carbophila	**CIEFAPcc 162/ MR10758**	Argentina, Río Negro, El Bolsón	*Nothofagus sp.*	**JX090114**	**JX090132**
	CIEFAPcc 257/ MR12281	Argentina, Chubut, Los Alerces	*Nothofagus dombeyi*	**JX090115**	—
Postia dissecta	**CIEFAPcc 328/ AG s.n.**	Argentina, Chubut, Golondrinas	*Austrocedrus chilensis*	**JX090106**	**JX090134**
	CIEFAPcc 349/ MR12423	Argentina, Chubut, Los Alerces	*Nothofagus antarctica*	**JX090107**	**JX090135**
Postia lactea (as *Oligoporus*)	KEW55	—	—	—	AY293205
Postia leucomallela	K(M)31064	UK, Surrey	—	AJ006663	AF393072
	K(M)31057	UK, Surrey	—	AY599565	—
Postia pelliculosa	**CIEFAPcc 130/ MR10592**	Argentina, Chubut, Los Alerces	*Nothofagus dombeyi*	**JX090102**	**JX090124**
	CIEFAPcc 221/ MR10671	Argentina, Neuquén, Lanín	*Nothofagus alpina*	**JX090101**	**JX090123**
	CIEFAPcc 64/ DFP	Australia, Victoria, Cumberland Falls	*Nothofagus sp.*	**JX090103**	**JX090126**
	CIEFAPcc 75/ PKB85159	New Zealand, Southland, Longwood St . For.	*Nothofagus menziesii*	**JX090104**	**JX090125**
Postia placenta	FPRL 280	—	—	EF524035	—
	JV0909/16	Slovakia	—	JN592503	—
Postia punctata	**CIEFAPcc 40/ MR11100**	Argentina, Neuquén, Lanín	*Nothofagus dombeyi*	**JX090112**	**JX090128**
	CIEFAPcc 344/ MR12398	Chile, Región X, ca. Santa Lucía	*Nothofagus dombeyi*	**JX090111**	**JX090127**
Postia rennyi (as *Oligoporus*)	**CIEFAPcc 91/ MR10497**	Argentina, Chubut, Futaleufú	*Austrocedrus chilensis*	**JX090117**	—
	KEW 57	—	—	AY218416	AF287876
Postia venata	**CIEFAPcc 346/ MR12368**	Chile, Región X, Yelcho	*Nothofagus dombeyi*	**JX090113**	**JX090133**
Amyloporia nothofaginea	**CIEFAPcc 196/ SPG2802**	Argentina, Chubut, Los Alerces	*Nothofagus dombeyi*	**JF713078**	—
	CIEFAPcc 304/ MR12101	Argentina, Chubut, Lago Puelo	*Nothofagus dombeyi*	**JF713079**	—

TABLE I. Continued

Species	Strain/herbarium	Location	Host	GenBank accession nos. ITS	LSU
Amyloporia xantha	CBS200.91	Turkey, Istanbul	*Pinus* sp.	AJ415569	—
(as *Antrodia*)	FCUG 1396	Germany, Hamburg	—	—	AJ583430
Antrodia albida	CBS308.82	USA, Madison	—	DQ491414	—
Antrodia serialis	CBS 306.82	Germany, Reinhausen	*Picea abies*	DQ491417	—
	GEL 4465	Germany	—	—	AJ406519
Daedalea quercina	HHB8735	USA	—	FJ403214	—
	DAOM 142475	—	—		AF518613
Fibroporia gossypium	**CIEFAPcc 131/ MR10569**	Argentina, Chubut, Futaleufú	*Nothofagus pumilio*	JF713075	**JX090142**
Fibroporia vaillantii	CIEFAPcc 261/ MR11674	Argentina, Río Negro, El Bolsón	*Austrocedrus chilensis*	JF713076	—
Fomitopsis rosea	ATCC 76767	Turkey, Trabzon	—	DQ491410	—
	FP 104278-T	—	—	—	AY333809
Fomitopsis pinicola	UBC F16252/	Canada	—	EF530947	—
	DAOM 189134	—	—	—	AF287858
Neolentiporus maculatissimus	**CIEFAPcc 92/ BAFC 31145**	Argentina, Neuquén, Nahuel Huapi	*Nothofagus dombeyi*	**JX090121**	AF518632
	CIEFAPcc 93/ BAFC 22451	Argentina, Neuquén, Nahuel Huapi	*Nothofagus dombeyi*	**JX090122**	—
Ryvardenia campyla	CIEFAPcc 124/ MR10598	Argentina, Chubut, Los Alerces	*Nothofagus dombeyi*	JQ677140	—
	CIEFAPcc 197/ MR10728	Argentina, Chubut, Futaleufú	*Nothofagus pumilio*	**JX090118**	**JX090141**
	CIEFAPcc 200/ MR10956	Chile, Región X, Chiloé	—	**JX090119**	**JX090140**
	NZFS2826	New Zealand	*Nothofagus fusca*	JQ390051	—
	NZFS2828	New Zealand	*Nothofagus fusca*	JQ390052	—
Ryvardenia cretacea	**CIEFAPcc 182/ MR12329**	Argentina, Neuquén, Nahuel Huapi	*Nothofagus dombeyi*	JQ677142	**JX090137**
	CIEFAPcc 348/ MR12429	Argentina, Neuquén, Nahuel Huapi	*Nothofagus dombeyi*	**JX090120**	**JX090136**
	CIEFAPcc 97, ICMP 11789/ PKB87298	Australia, Tasmania, Great Western Tiers	*Nothofagus cunninghamii*	JQ677138	**JX090139**
	CIEFAPcc 151, CFMR 6449	Australia, Victoria	*Eucalyptus regnans*	JQ677141	**JX090138**

datasets: ITS and LSU (FIGS. 1, 2). The ITS sequence dataset included 45 taxa, 668 characters, with 383 of the characters being parsimony informative. Tree length (TL) 1512, consistency index (CI) and retention index (RI) were 0.52 and 0.773 respectively (FIG. 1). The LSU sequence dataset included 36 taxa, 881 characters and 170 parsimony informative. Tree length (TL) 552, CI of 0.53 and RI 0.74 (FIGS. 1, 2).

Overall, the taxa included in this research pertained to the ''Antrodia clade'' (Hibbett and Donoghue 2001) and were grouped into two subclades here named ''Postia clade'' and ''dimitic taxa of Antrodia clade''. Species formerly treated as *Postia* or *Oligoporus* were included in a monophyletic, strongly supported ''Postia clade'', in both types of phylogenetic analysis (MP and BI), and for both DNA datasets. Nevertheless, within the clade relationships between species were not well resolved because only isolates of *P. pelliculosa* formed a strong clade with *P. punctata* as its sister group. South American isolates of *P. pelliculosa* formed a separate branch from the Australasian isolates with a strong bootstrap value for each group (FIGS. 1, 2). Strains of *P. balsamea* formed a group close to the *P. pelliculosa-P. punctata* clade but without bootstrap support. *P. caesia* also grouped with the latter species in ITS analysis but were placed at different phylogenetic positions based on MP and BI analysis of LSU DNA region. Relationships among those clades were not completely resolved. A similar result was obtained in the ITS analysis for *P. venata* and *P. carbophila* that grouped with *P. caesia* and *P. leucomallela* respectively, but those relationships were

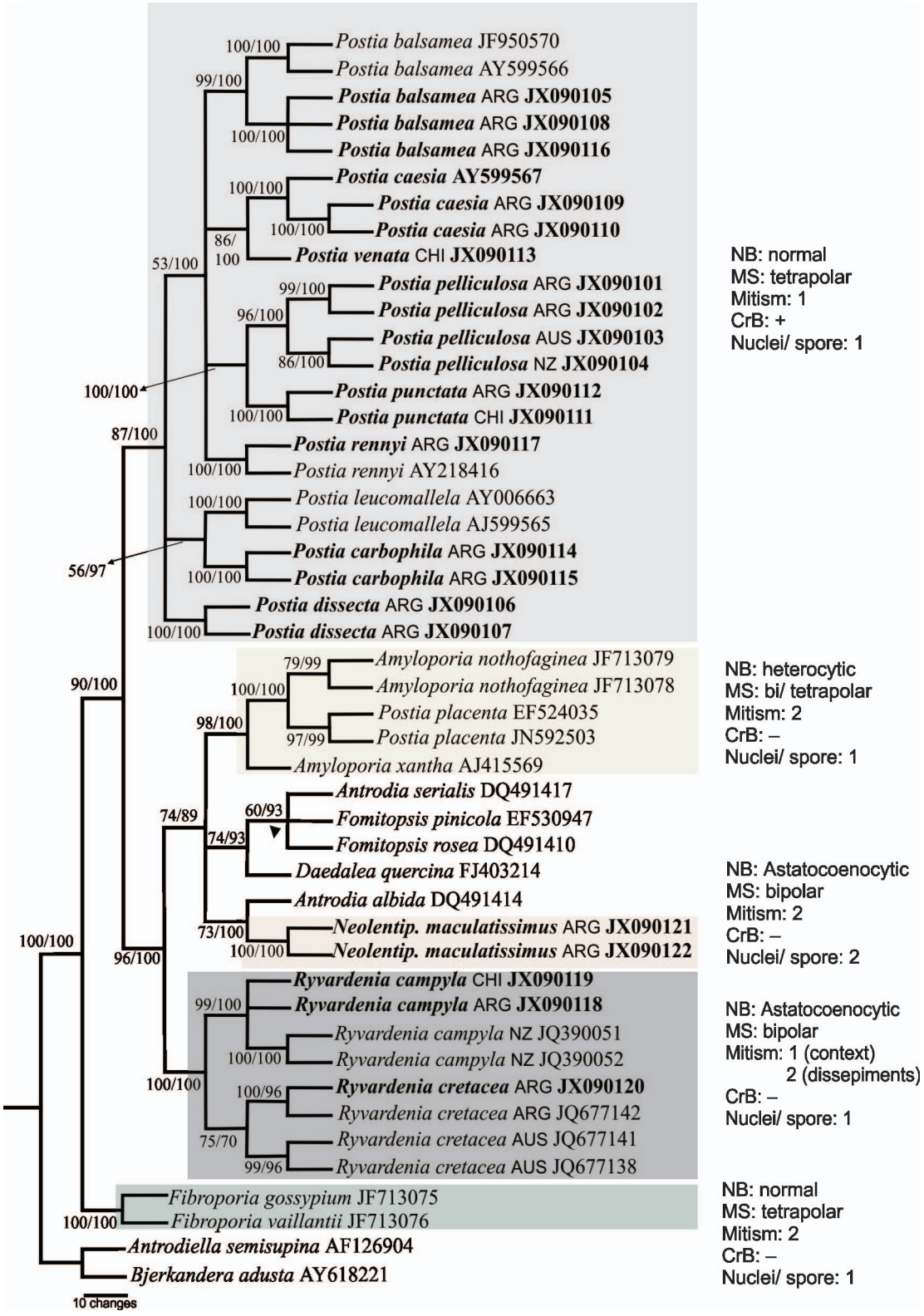

FIG. 1. Phylogenetic relationships of *Postia* and *Ryvardenia* inferred with ITS parsimony (MP) and Bayesian (BI) analysis. Bootstrap values for internal nodes are given on the branches (MP/BI)). Support values < 50% are not indicated. Pointers indicate branches that collapse in the strict consensus tree. GenBank numbers in boldface indicate specimens for which sequencing data were generated. ARG: Argentina; AUS: Australia; CHI: Chile; NZ: New Zealand. NB: nuclear behavior; MS: mating system; Mitism: hyphal system (1) monomitic, (2) dimitic; CrB: metachromatism of generative hyphae (+) positive or (−) negative in cresyl blue.

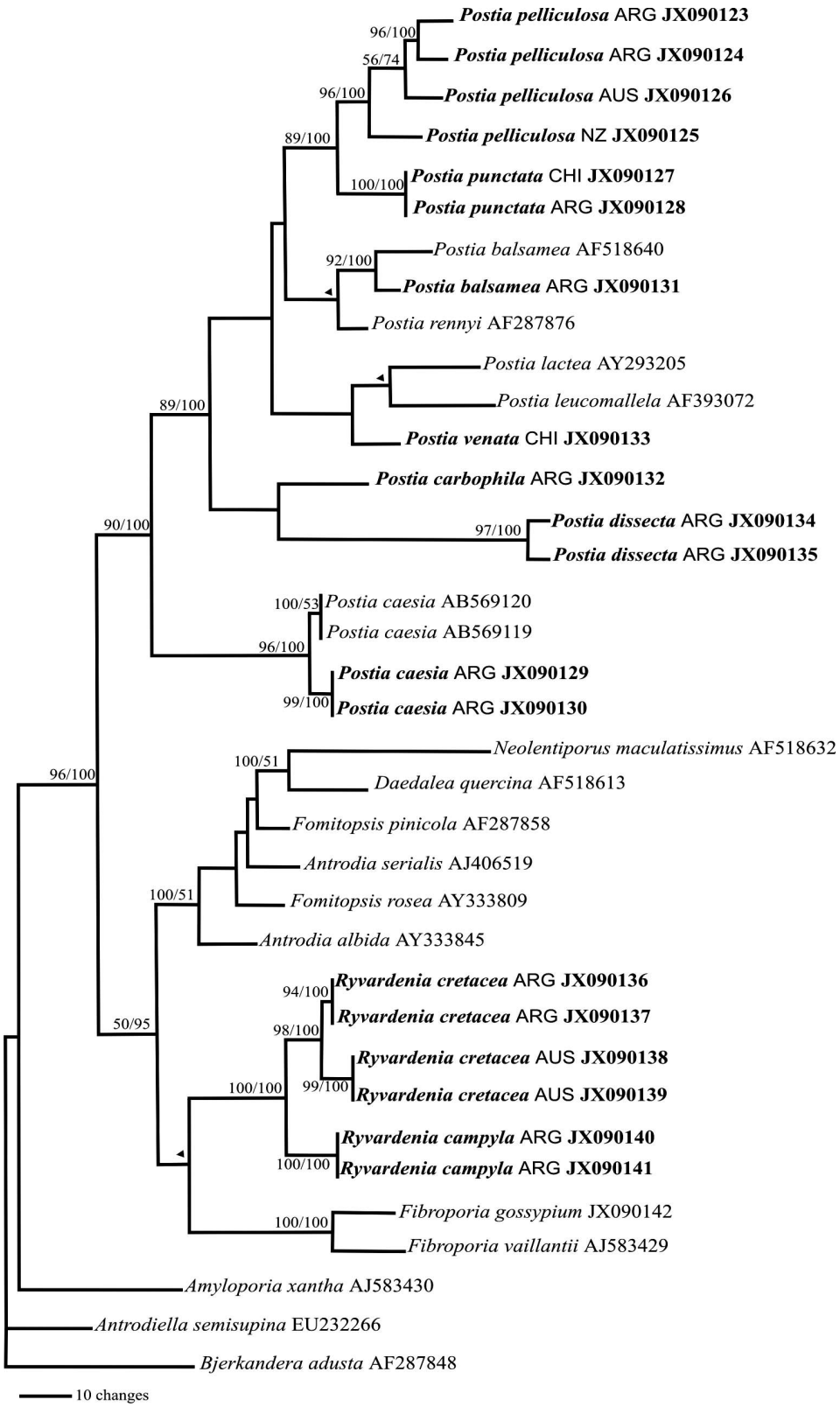

FIG. 2. Phylogenetic relationships of *Postia* and *Ryvardenia* inferred with LSU parsimony (MP) and Bayesian (BI) analysis. Bootstrap values for internal nodes are given on the branches (MP/BI). Support values < 50% are not indicated. Pointers indicate branches that collapse in the strict consensus tree. GenBank numbers in boldface indicate specimens for which sequencing data were generated in this study. ARG: Argentina; AUS: Australia; CHI: Chile; NZ: New Zealand.

unresolved in the LSU analysis (FIGS. 1, 2). Unfortunately, no LSU sequences were obtained for *P. rennyi* from Patagonia.

Isolates of *Ryvardenia* formed a strong clade in both DNA dataset analyses, which was included in the group of ''dimitic taxa of Antrodia clade''. The latter as a whole was either strongly resolved in the ITS analyses and BI LSU analyses or weakly so in the MP LSU analyses. *Ryvardenia*, *Neolentiporus*, *Fomitopsis*, *Daedalea*, *Antrodia* and *Amyloporia* were included in the ''dimitic taxa of Antrodia clade'' in all analyses. *Fibroporia* presented an erratic disposition, being outside the clade in the ITS analysis and within it in the LSU analysis (FIGS. 1, 2).

The genus *Ryvardenia* formed a strongly monophyletic group supported in both datasets, which confirmed its description as an independent taxon. Its species, *R. cretacea* and *R. campyla*, were distinctly defined (FIGS. 1, 2). MP and BI analysis for both datasets grouped the isolates of *R. cretacea* from Chile and Argentina sister to those from Australia and New Zealand (FIGS. 1, 2). Isolates of *P. placenta* grouped in the same clade as *Ryvardenia*, *Amyloporia*, *Neolentiporus*, *Daedalea*, *Fomitopsis* and *Antrodia*, being closely related to *Amyloporia nothofaginea* as a sister species.

DISCUSSION

Sequences of two nuclear rDNA regions were used to infer the phylogenetic relationships of *Postia* and *Ryvardenia* species from Patagonia. Not all the isolates were analyzed in both datasets; *Postia rennyi* from Patagonia and *Postia placenta* only were included in the ITS analysis. The analyses identified two clades. ''Postia'' comprised species formerly treated as *Postia* or *Oligoporus* and included all *Postia* species from Patagonia as well as other taxa of the genus. Its morphological distinguishing feature is a monomitic hyphal system with metachromatic generative hyphae. Species with both thin- to thick-walled basidiospores grouped in this clade, suggesting that the thickness of the spore wall might not be essential in delimiting the genus, especially because no consistent substructure regarding spore thickness as a feature could be found. Only the two austral species, *P. pelliculosa* (spores very thick-walled) and *P. punctata* (spores slightly thick-walled) with ellipsoid to broadly ellipsoid basidiospores longer than 5 µm, formed a consistent phylogenetic group. They also formed a weakly supported group with other taxa with slightly thickened (i.e. *P. balsamea*) to thickened walls (i.e. *O. rennyi*).

Postia rennyi, the type species of *Oligoporus*, received variable bootstrap values according to the analyses. It occupied an undefined position in the ITS and LSU PAUP analyses. Therefore, and because no distinct features could be assigned to it, either molecular, morphological, sexual, cultural, or of nuclear behavior of the mycelium, and because no phylogenetic characterization was found, *Oligoporus* is here considered a synonym of *Postia*. Hattori et al. (2011) also found that *P. rennyi* clustered with species with thin-walled basidiospores in their analyses.

Other species from Patagonia did not follow any consistent relationship or pattern with other taxa in the genus. Species with thin-walled basidiospores formed two subgroups, one including species of the *P. caesia* species complex and the other subgroup including *Postia* type species *P. lactea* (Fr.) P. Karst. (= *P. tephroleuca* [Fr.] Jülich) and *P. leucomallella* (Murrill) Jülich. The austral *P. venata*, with thin-walled basidiospores, merged with either *P. caesia* species complex or *P. tephroleuca* according to ITS or LSU analyses respectively. The austral *P. carbophila*, with thin-walled basidiospores, was positioned unresolved either with *P. leucomallela* or with *P. dissecta* (ITS and LSU phylogenies respectively).

Species well known and distributed in the northern hemisphere but whose presence was unknown until recently from the southern continents (Rajchenberg 2006) grouped well with specimens from Patagonia. This was the case for *P. balsamea*, *P. rennyi* and *P. caesia*. For the latter case a more detailed study is needed to verify their identity because specimens from Patagonia formed a distinct subclade separated from taxa/specimens from the northern hemisphere, where they form a species complex (Yao et al. 2005). The endemic *P. carbophila* was related to *Postia placenta* when originally described on the basis of basidiome coloration, its resupinate habit, spore morphology and the presence of a resinous matter between the hyphae (Rajchenberg 1995). Our study showed that it is distantly related to that species, being perfectly nested within *Postia*. For the placement of *P. placenta* see below.

The other clade identified by the analyses comprised the ''dimitic species of the Antrodia clade'', which included the two *Ryvardenia* species, this genus being well supported as monophyletic in all analyses. This is the first time the genus *Ryvardenia* was included in a phylogenetic study. The results showed it to be within the ''dimitic taxa of Antrodia clade'' and distant from *Postia*, in which species of *Ryvardenia* had been included. Phylogenetic analyses consistently dismissed a close relationship between *Ryvardenia* and *Postia*, thereby supporting the morphological and biological features that led to its creation (Rajchenberg 1994, FIGS. 1, 2). Both species in the genus were very well supported as a clade and well distinguished at the species level.

The biological features presented by *Ryvardenia* also are present in *Neolentiporus maculatissimus* (Lloyd) Rajchenb. (Rajchenberg 1995, 2006). This austral species is distinguished by cylindrical, binucleated, thin-walled basidiospores, bipolar mating, astatocoenocity and by a hyphal system with irregularly thickened hyphae, which is lacking in *Ryvardenia*. The species also was included in this study; it was not closely related to *Ryvardenia* but instead to the bulk of genera *Antrodia, Daedalea and Fomitopsis* (as already shown by Hibbett and Donoghue [2001]), which display cylindrical to ellipsoid basidiospores that are generally uninucleate (but binucleate in *A. albida*, *A. heteromorpha* [Fr.] Donk and *A. salicina* [Bres.] H. Jahn), a normal nuclear behavior and homothallism or bipolar mating.

Postia placenta, as explained above, was included in this study because the Patagonian *P. carbophila* was considered a related species when described (Rajchenberg 1995). It was long considered a species of *Postia* but was segregated into a genus of its own as *Rhodonia placenta* Niemelä, K.H. Larss. & Schigel based on phylogenetic studies (Niemelä et al. 2005, Schigel et al. unpubl). Boidin et al. (1998), Kim et al. (2001) and Binder et al. (2005) had already shown that this taxon is distinct from the bulk of species in *Postia*. More notable is the fact that this species had been distinguished long before by displaying a heterocytic nuclear behavior (David 1988), a feature unknown in *Postia* (David 1980). Its mating system is tetrapolar according to David (1988) but Nobles (1943) stated it to be bipolar; confirmation of this feature is needed. Our study, based on ITS and LSU analyses, found a potential association of *P. placenta* with taxa in *Amyloporia*, a genus with dimitic species that also displays a heterocytic nuclear behavior and either bipolar or tetrapolar mating (Rajchenberg et al. 2011, Rajchenberg 2011). A strong association was found between *P. placenta* and the recently described taxon *Amyloporia nothofaginea* Rajchenb. & Gorjón which nevertheless is easily distinguished from *P. placenta* by its hymenial coloration (white-cream) and a dimitic hyphal system (but cf. Niemelä et al. [2005] who describe hyphae that become thickened upon maturation, giving the structure a dimitic appearance). The relationship of *P. placenta* to *Amyloporia* needs further research. Kim et al. (2001) and Binder et al. (2005) also reported an association between *P. placenta* and *Amyloporia xantha* (Fr.) Bondartsev & Singer, and Garcia-Sandoval et al. (2011) with *Amyloporia carbonica* (Overh.) Vampola & Pouzar. When we tested this relationship after incorporating more *Amyloporia* taxa (i.e. *A. carbonica* [Overh.] Vampola & Pouzar, *A. sitchensis* [D.V. Baxter] Vampola & Pouzar, *A. stratosa* [J.E. Wright & J.R. Deschamps] Rajchenb., Gorjón & Pildain and *A. sordida* [Ryvarden & Gilb.] Vampola & Pouzar), it remained unchanged (unpubl).

Fibroporia taxa relationships remained rather unclear in this study; they were either associated with the ''dimitic taxa of Antrodia clade'' or occurred as a separate clade. This supports results in which the genus formed a separate clade within *Antrodia* s.l. (Rajchenberg et al. 2011) or occupied a distant position (Kim et al. 2001).

Our study also revealed a strong separation between specimens of *P. pelliculosa* and *R. cretacea* from either side of the Pacific (i.e. SE Australia/New Zealand and S Argentina/S Chile), suggesting they could be considered different species on phylogenetic grounds. Nevertheless, Rajchenberg (1994) and Rajchenberg and Greslebin (1995) reported biological compatibility between strains from both areas through dikaryotic × monosporous or through monosporous × monosporous confrontation tests. For this reason, as well as the lack of morphological differences, we have deferred describing new taxa from the *R. cretacea* species complex (Rajchenberg and Pildain 2012). However future work may reveal more substantial molecular differences not reflected by morphology and compatibility, as has been found for many other cases (Cai et al. 2011). For *R. campyla*, the Argentinean and Chilean strains formed a polytomy vis à vis the two New Zealand strains (ITS analyses, no LSU data was available). In this case it is possible that the unresolved relationship is due to the fact that both New Zealand strains came from a similar place, although this is not completely evident from the available data. More strains thus are needed to verify whether a biogeographical isolation between populations of this species exists, as shown for *P. pelliculosa* and *R. cretacea*.

A better understanding of the phylogenetic relationships within the ''Postia clade'' requires the inclusion of a larger number of strains of certain taxa as well as the incorporation of additional genes. The number of parsimony informative characters within the partial LSU rDNA sequence was fewer than for ITS rDNA sequences; thus it is not surprising that phylogenetic inferences based on analyses of the LSU represent the relationships of higher nodes, and most of relationships below this level could be analyzed based on ITS. However, our phylogenetic analyses of ITS and LSU rDNA sequences for ''Postia clade'' shows that clades representing species were strongly supported and that the majority of relationships above this level are unresolved (FIGS. 1, 2). More characters from unlinked loci and protein-coding genes might help resolve these relationships. Our interest is the need to get a clearer understanding of

the relative importance of spore-wall thickness in the phylogenetic definition of the genus. In this study we were able to couple morphological and biological features of certain genera with molecular-based phylogenies highlighting their importance, offering a more comprehensive view of evolution within each group of organisms and offering a coherent picture of each clade within the Polyporales. Nevertheless, further in-depth studies are needed within the genera and among other genera in the ''dimitic taxa of Antrodia clade'' to understand infrageneric relationships.

The inclusion of taxa from the southern hemisphere, especially from southern South America because of the particular origin of its biota (Sanmartín and Ronquist 2004), helps put into perspective phylogenetic studies in which the southern hemisphere mycota is either not included or generally outnumbered by northern hemisphere taxa.

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