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Remarkable nutritional diversity of basidiomycetes in the Corticiales, including a new foliicolous species of *Marchandiomyces* (anamorphic Basidiomycota, Corticiaceae) from Australia[1]

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Fungi in the basidiomycete order Corticiales are remarkably diverse nutritionally, including a variety of saprotrophs, plant and fungal pathogens, and lichen-forming fungi. Tracing the origin of this diversity depends on a clearer understanding of the phylogenetic relationships of fungi in the order. One of its core members is the genus *Marchandiomyces*, originally established for lichen pathogens that form orange or coral bulbils. We describe here a new species in the genus, *M. marsonii* sp. nov., which is unusual in its appearance, habit, and geographic provenance. It is foliicolous on leaves of *Pandanus* (screw pines, Pandanaceae) and produces flattened, coral bulbils resembling apothecia of the ascomycete genus *Orbilia*. It is also the first member of the genus to be collected from Australia. An isolate of the new fungus and several additional cultures of related plant pathogenic fungi were obtained and investigated phylogenetically using parsimony, likelihood, and Bayesian analyses of nuclear small and large subunit ribosomal sequences. Our phylogeny makes clear that *Marchandiomyces* species and their close relatives contribute significantly to the ecological diversity of the Corticiales and that this diversity is derived mainly from lignicolous ancestors.

Key words: basidiomycetes; bulbilliferous fungi; Corticiales; foliicolous; *Laetisaria*; lichenicolous fungi; *Limonomyces*; *Marchandiomyces*; rDNA sequence analyses.

The largest and most diverse class in the phylum Basidiomycota is the Agaricomycetes, which includes a variety of mushroom-formers and crustlike resupinate forms with modes of nutrition ranging from saprotrophs to pathogens and mutualists. Recent molecular phylogenetic analyses have begun to clarify the composition of the major clades of Agaricomycetes as well as the evolutionary development of its ecological and morphological diversity (Hibbett et al., 2000; Hibbett and Thorn, 2001; Lim, 2001; Langer, 2002; Hibbett and Binder, 2002; Larsson et al., 2004; Binder et al., 2005; Hibbett, 2006; Matheny et al., 2006, 2007; Larsson, 2007).

One of the clades of the Agaricomycetes consistently resolved in molecular phylogenies is now recognized formally as the Corticiales K.-H. Larss. (Hibbett et al., 2007), including a single family Corticiaeae Herter (Larsson, 2007). This order is almost entirely composed of resupinate species and characterized by smooth hymenophores, a monomitic hyphal system with clamps, and smooth basidiospores with pink walls, all characteristics of the sexual, basidiospore-producing forms of the fungi (teleomorphs). Asexual forms (anamorphs), named separately by convention in mycology, have only recently been recognized in the Corticiales and assigned with certainty to the group. Members of the order have a wide range of nutritional ecologies, including mutualistic and pathogenic forms as well as lignicolous saprobes (Diederich et al., 2003; Binder et al., 2005; DePriest et al., 2005; Lawrey et al., 2007).

One of the important core genera in the Corticiales is *Marchandiomyces*, an anamorphic genus originally established by Diederich (1990) for the coral lichen pathogen *Marchandiomyces corallinus* (Roberge) Diederich & D. Hawksw., commonly collected in the eastern United States and Europe. An orange, primarily European lichen pathogen [*M. aurantiacus* (Lasch) Diederich & Etayo] was later added to the genus by Etayo and Diederich (1996). Both species produce bulbils—small, round masses of tightly coiled hyphae that function as resting or dispersal structures (Clémençon, 2004). The relations of these asexual anamorphs to sexual teleomorphic forms were uncertain, although both species were assumed to be basidiomycetous (Hawksworth, 1979). This was confirmed unequivocally by the molecular phylogenetic study of Sikaroodi et al. (2001) and the discovery of the teleomorph of *M. aurantiacus* (named *Marchandiobasidium aurantiacum* Diederich & Schultheis by Diederich et al. [2003]).

Later molecular studies (DePriest et al., 2005; Lawrey et al., 2007) made clear that *Marchandiomyces* species and their close relatives are not always associated with lichens. Several species in the genus are lignicolous (*M. lignicola* Lawrey & Diederich and *M. nothofagicola* Diederich & Lawrey), a common and apparently basal habit in the Corticiales (DePriest et al., 2005). Even more surprising was the discovery that the basidiolichen formerly called *Omphalina folicacea* P.M.Jørg., originally assumed to be an omphalinoid agaric but subsequently placed in the Hymenochaetales (Palice et al., 2005), is instead a member of the Corticiales closely related to *Marchandiobasidium aurantiacum*, renamed *Marchandiomphalina foliacea* (P.M.Jørg) Diederich, Lawrey & Binder (Diederich and Lawrey, 2007).

The wide range of nutritional modes observed in *Marchandiomyces*-like species, combined with the close affinities they

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have with numerous plant pathogens [*Laetisaria fuciformis* (McAlpine) Burds., *Waitea circinata* Warcup & P.H.B. Talbot] and saprobes (*Corticium roseum* Pers.), suggests an unusually high level of evolutionary flexibility in these fungi (DePriest et al., 2005; Lawrey et al., 2007). Understanding the origin of this diversity will require clarification of the phylogenetic relationships of the fungi in the order.

We recently had the opportunity to study a foliicolous fungus from Australia with bulbils similar in color to those of *Marchandiomyces corallinus*, but differing in size and shape. We were able to isolate the fungus and obtain from it nuclear small (nuc-SSU) and large (nuc-LSU) subunit rDNA sequences, which we analyzed to determine its phylogenetic position and possible relationship to *Marchandiomyces*. In addition to the unknown culture, we obtained cultures of three plant pathogens representing genera closely related to *Marchandiomyces*, and their DNA was also sequenced. Preliminary analysis made use of a core data set from two recent comprehensive phylogenetic studies of the Agaricomycetes (Binder et al., 2005; Lawrey et al., 2007). A second data set extensively sampled the Corticiales, using 36 terminals. Our objectives were (1) to determine the phylogenetic position of the unknown *Marchandiomyces*-like fungus in relation to existing species (2) and to consider possible nutritional transitions that have taken place in lineages containing these fungi.

MATERIALS AND METHODS

Specimens collected and anatomical methods—Specimens of the *Marchandiomyces*-like unknown were collected by Guy Marson from dead, hanging leaves of *Pandanus oblatus* H.St.John (screw pine, Pandanaceae) near Cairns, Australia.

Dry herbarium specimens were examined and measured using a binocular microscope Leica (Wetzlar, Germany) MZ 7.5 (magnification up to 50×) and photographed using a Nikon (Melville, New York USA) Coolpix 4500. Entire unsectioned and sectioned bulbils were studied in water, KOH, or lactophenol cotton blue either with or without pressure on the coverslip. Microscopic photographs of bulbils were prepared using a Zeiss (Düsseldorf, Germany) Photomikroskop III with a Canon (Lake Success, New York, USA) PowerShot G5.

Isolation of fungal culture—A culture of the *Marchandiomyces*-like fungus was isolated from bulbils from herbarium material as discussed in Lawrey (2002). Bulbils germinated within two days on potato dextrose agar (PDA, Difco, Detroit, Michigan, USA) without antibiotics, and outgrowths were isolated for liquid culture in either potato dextrose or Sabouraud's (Difco) medium with dextrose. Approximately 2 µg dry mycelial mass was harvested after two weeks and extracted for DNA analysis.

In addition to the unknown culture, we obtained cultures of *Limonomyces culmigenus* (R. K. Webster & D. A. Reid) Stalpers & Loer. (ATCC 22523), *L. roseipellis* Stalpers & Loer. (CBS 299.82), and *Laetisaria arvalis* Burds. (type strain CBS 131.82) for DNA extraction and analysis. Previous studies (Andjic et al., 2005; Lawrey et al., 2007) suggested that these species may have phylogenetic affinities with the *Marchandiomyces* clade. Isolates were maintained in liquid culture (Sabouraud's with dextrose) until approximately 2 µg dry mycelial mass had accumulated, and this mycelium was extracted for DNA analysis.

Molecular techniques—Genomic DNA was extracted from fungal tissue using the Bio 101 Fast DNA Spin Kit for tissue (Qbiogen, Illkirch, France) according to the manufacturer's protocol with slight modifications. About 10 ng of extracted DNA were subjected to a standard PCR in a 50-µL reaction volume. We amplified approximately 1700 bp of portions of the nuc-LSU and ITS2 rDNA using primers (LR0R, LR3R, LR8R, LR5, LR7, LR9) available from the Vilgalys laboratory web site (http://www.biology.duke.edu/fungi/mycolab/primers.htm). The nuc-SSU with an approximate length of 1750 bp for most species was completely sequenced using NS17UCB, NS19UCB, NS3, NS21UCB, NS23UCB, NS24UCB, NS22UCB, NS20UCB, NS2, and CNS26 (White et al., 1990; Gargas and Taylor, 1992). After confirming the PCR product using ethidium bromide following separation on a 1% agarose gel, the products were purified with magnetic beads (Agencourt Biosciences, Beverly, Massachusetts, USA).

The purified PCR products were used in standard sequencing reactions with BigDye Terminator Ready Reaction Mix (Applied Biosystems, Foster City, California, USA). The sequencing reactions were then purified using Sephadex G-50 (Sigma-Aldrich, St. Louis, Missouri, USA), dried in a speed vacuum, denatured in HiDi Formamide (Applied Biosystems) and run on a SCE-9610 capillary machine (SpectruMedix, Reedsville, Pennsylvania, USA). The data collected were analyzed using the program BaseSpectrum (SpectruMedix), and about 600 bases were collected for each primer used. These sequences were then transferred to the program Sequencher (GeneCodes, Ann Arbor, Michigan, USA) for manual base calling and to make contiguous alignments of overlapping fragments.

Phylogenetic analyses—Initial placement of the unknown was done using a core data set of nuclear small and large subunit (nuc-SSU, nuc-LSU) rDNA sequences from 142 species (see the supplement in Binder et al. [2005] for strain information and GenBank accession numbers), with sequences added from a study of lichen-associated and sclerotial taxa to yield 268 terminals (Lawrey et al., 2007). The core data set was tested for positive conflict by bootstrapping nuc-SSU and nuc-LSU partitions separately in PAUP* version 4.0b10 (Swofford, 2002), using the neighbor-joining (NJ) nonparametric bootstrap (1000 replicates) with a maximum likelihood distance. Likelihood models were selected and parameters estimated using the Akaike information criterion (with the program MODELTEST version 3.7; Posada and Crandall, 1998). We found no evidence of topological conflict between partitions >70% and therefore combined the data. Missing data at the terminal ends of sequences were trimmed, but no other sequence data were excluded. Parsimony analysis of this core alignment indicated the unknown was a member of the Corticiales (Binder et al., 2005), closely related to *Marchandiomyces* spp. and other related fungi. We then assembled a Corticiales alignment containing nuc-SSU and nuc-LSU sequences from the core data set plus additional sequences obtained from GenBank or AFTOL (Assembling the Fungal Tree of Life; http://www.aftol.org). The Corticiales data set was aligned by eye in the program MacClade version 4.08 (Maddison and Maddison, 2005) and submitted to the TreeBASE database (S2053; http://www.treebase.org).

The Corticiales data set (Table 1) consisted of 36 terminals including five outgroup sequences representing the Gloeophyllales [*Gloeophyllum sepiarum* (Wulfen) P. Karst. and *Heliocybe sulcata* (Berk.) Redhead & Ginns] and the Thelephorales [*Sarcodon imbricatus* (L.) P. Karst., *Thelephora* sp. and *Bankera fuligineoalba* (J. C. Schmidt) Coker & Beers]. The data were tested for conflict using NJ bootstrap values as before and analyzed in PAUP* using maximum likelihood under the GTR+Γ+I model (estimated with MODELTEST 3.7, Posada and Crandall, 1998) with nucleotide frequencies estimated (A = 0.25, C = 0.21, G = 0.28, T = 0.26), a rate matrix of substitutions (A-C = 1.05, A-G = 3.85, A-T = 1.54, C-G = 0.75, C-T = 7.87, G-T = 1.0), proportion of invariable sites = 0.67, and $\alpha = 0.55$. A maximum likelihood bootstrap analysis was performed under the same settings using 1000 replicates with MAXTREES set to 1000. In addition, Bayesian phylogenetic analyses were carried out using the Metropolis-coupled Markov chain Monte Carlo method (MCMCMC) in MrBayes version 3.0b4 (Ronquist and Huelsenbeck, 2003). Analyses were run using a model with six categories of base substitution, a gamma-distributed rate parameter, and a proportion of invariant sites (GTR+Γ+I). Two parallel MCMCMC runs were performed each using four chains and 2 000 000 generations, sampling trees every 100th generation. The proportion of burn-in trees sampled before reaching equilibrium was estimated by plotting likelihood scores as a function of the number of generations. Posterior probabilities (PP) were determined by calculating a 50% majority-rule consensus tree in PAUP* with the proportion of trees gathered after convergence of likelihood scores was reached, and clades with PP ≥0.95 were considered to be significantly supported.

RESULTS

Phylogenetic placement of the new species in the corticioid clade—Maximum likelihood (ML) analysis of an alignment of nuclear small (nuc-SSU) and large (nuc-LSU) subunit rDNA sequences from the Corticiales and closely related Gloeophyllales

TABLE 1. Specimens, cultures, and GenBank accession numbers of fungi used in this study.

Species name	Strain	GenBank accession number	
		Nuc-SSU	Nuc-LSU
Bankera fuligineoalba	DAOM 184178	AF287831	AF393046
Corticium roseum	CBS 205.91	—	EF537893
Cytidia salicina	CBS 727.85	—	DQ915478
Dendrocorticium polygonioides	FO36469g	—	AJ406531
Dendrocorticium roseocarneum	FPL1800	AF334910	AF393053
Erythricium laetum	NH14530	—	AY586655
Erythricium salmonicolor	—	—	AF506709
Galzinia incrustans	HHB-12952-Sp.	AF518578	AF518617
Gloeophyllum sepiarium	P198	AJ540308	AJ583432
Heliocybe sulcata	D.797	AF334915	AF518619
Laetisaria fuciformis	NJ-2 Jackson	AY293139	AY293192
Laetisaria arvalis	CBS 131.82 (type strain)	EU622843	EU622842
Limonomyes culmigenus	ATCC 22523	EU622847	EU622848
Limonomyces roseipellis	CBS 299.82	EU622845	EU622844
Marchandiobasidium aurantiacum	CBS 718.97	AF289661	—
Marchandiobasidium aurantiacum	ATCC MYA 2502	DQ915460	—
Marchandiomphalina foliacea	Palice 2509	AY542864	AY542864
Marchandiomphalina foliacea	Palice 4369	AY542865	AY542865
Marchandiomyces buckii	ATCC MYA 2992 (type strain)	DQ915462	DQ915472
Marchandiomyces corallinus	JL128-98	—	AY583331
Marchandiomyces corallinus	ATCC MYA 2500	DQ915459	—
Marchandiomyces corallinus	ATCC MYA 2346	DQ915457	—
Marchandiomyces corallinus	ATCC MYA 3182	DQ915464	—
Marchandiomyces lignicola	ATCC MYA 835 (type strain)	AY583333	—
Marchandiomyces lignicola	ATCC MYA 3674	DQ915465	—
Marchandiomyces lignicola	ATCC MYA 2347	DQ915458	—
Marchandiomyces lignicola	ATCC MYA 2831	DQ915461	—
Marchandiomyces marsonii	ATCC MYA 4210 (type strain)	EU622838	EU622839
Marchandiomyces nothofagicola	JL261-04	DQ915466	DQ915474
Punctularia strigoso-zonata	HHB-11897-Sp.	AF518586	AF518642
Sarcodon imbricatus	REG Sim1	AY293157	AF518646
Thelephora sp.	DSH 96-010	AF026627	AF287890
Tretopileus sphaerophorus	JCM10092	AB006005	—
Vuilleminia comedens	T-583	AF518594	AF518666
Vuilleminia macrospora	EL21-99	—	AY586726
Waitea circinata	CBS 315.84	—	AY885164

and Thelephorales resulted in one optimal tree (Fig. 1) with a score of –lnL = 9607.902. Bayesian runs converged after 200 000 generations and 24 402 trees were used to compute posterior probability (PP) values. The Corticiales as a whole and the clade containing described *Marchandiomyces* spp. are both strongly supported by PP values and ML bootstrap support, a result that has been observed before (DePriest et al., 2005; Lawrey et al., 2007). The new species *Marchandiomyces marsonii* forms a weakly supported clade with the turfgrass pathogen *Laetisaria fuciformis* (the generitype), which is sister to another strongly supported clade containing two recently described *Marchandiomyces* species (*M. nothofagicola* from southern Chile and *M. buckii* from the United States) and the grass pathogen *Limonomyces culmigenus*. The plant pathogen *L. roseipellis* (generitype) is basal to the entire clade, which is strongly supported (100%) by PP and moderately supported (79%) by ML bootstrap. This fungus is sister to another well-supported clade containing two additional *Marchandiomyces* species, the *M. corallinus* (the generitype) and *M. lignicola.* The entire *Marchandiomyces* clade appears to be sister to the type species of the Corticiales, *Corticium roseum*, and the *Marchandiomyces* + *Corticium* clade is sister to a weakly supported and diverse clade containing the basidiolichen *Marchandiomphalina foliacea*; the lichen pathogen *Marchandiobasidium aurantiacum*; *Erythricium laetum*, a salmon lignicolous fungus that grows on decayed wood, moist leaves, and possibly living mosses (Binder et al., 2005); *Laetisaria arvalis*, a facultative fungal parasite that has been studied for possible use as a biocontrol agent against *Rhizoctonia* and *Pythium* spp. (Burdsall et al., 1980; Conway et al., 2000; Bobba and Conway, 2003); and two plant pathogens, *Waitea circinata*, a soilborne saprobe and pathogen of cereals, turf grasses and legumes; and *Erythricium salmonicolor* (Berk. & Broome) Burds., a fungus causing pink crust of citrus, coffee, and rubber trees (Burdsall, 1985).

Taxonomic implications of the phylogeny—We interpret the uppermost clade illustrated in the tree (Fig. 1), which includes the generitype *Marchandiomyces corallinus*, to represent *Marchandiomyces* s.s., with two anamorphic species. The clade below this is comprised of two teleomorphic genera, *Laetisaria* and *Limonomyces*, and three anamorphic species, provisionally included in *Marchandiomyces* s.l. Because the generitypes for both *Limonomyces* and *Laetisaria* are included in this clade, and *Limonomyces culmigenus* is apparently more closely related to *Laetisaria fuciformis* than it is to *Limonomyces roseipellis*, there would appear to be a need for taxonomic rearrangements as more species are added. This group is ecologically quite diverse and merits far more attention in the future. The genus *Erythricium* s.s. (represented by the generitype *E. laetum*) is sister to the genus *Marchandiobasidium* (represented by the type *M. aurantiacum*). The clade containing the generitype *Waitea circinata* also includes *Laetisaria arvalis* and *Erythricium salmonicolor*, so taxonomic reevaluations are needed here as additional species are added. We should point

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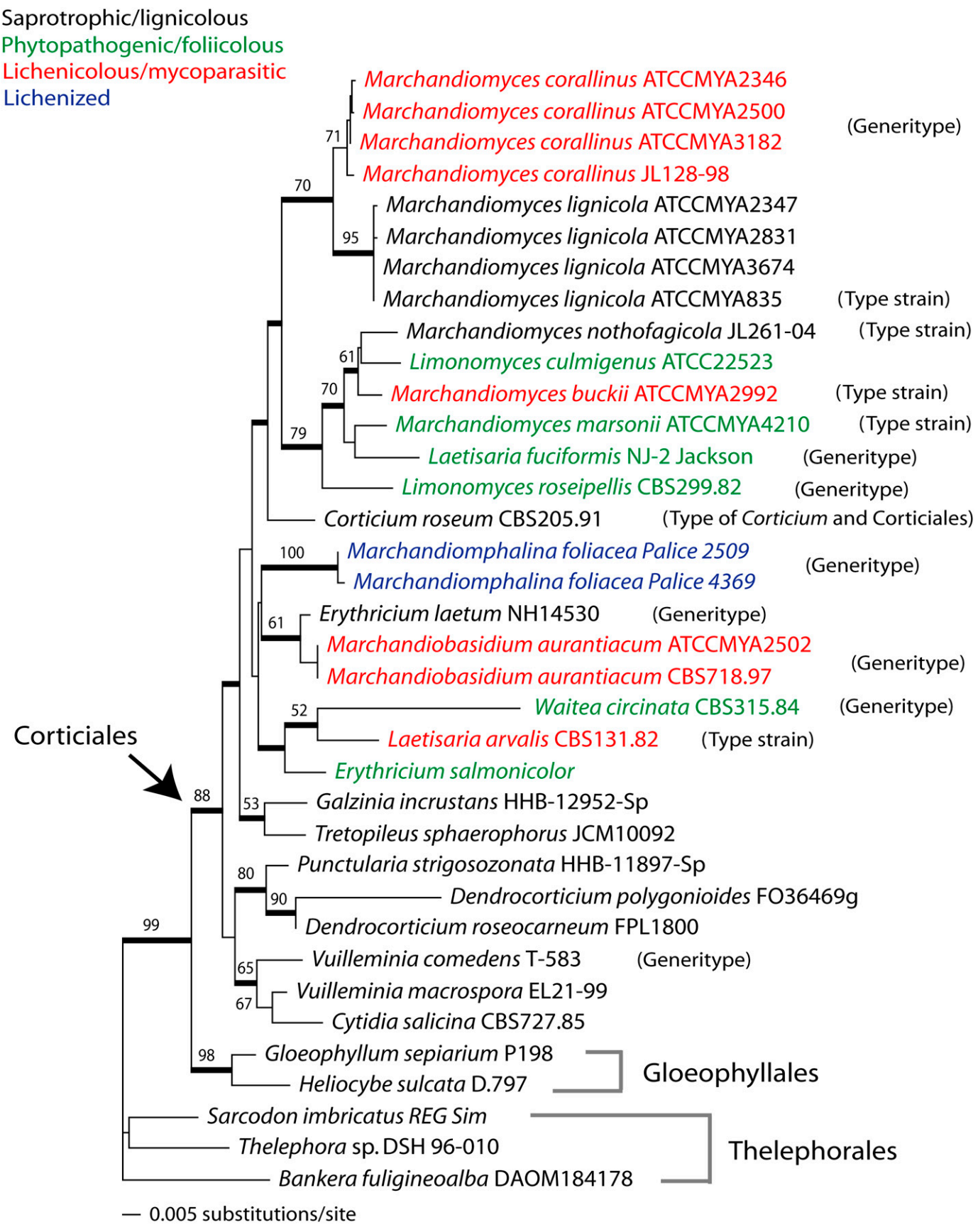
Fig. 1. Phylogenetic relationship of *Marchandiomyces marsonii* to other fungi in the Corticiales inferred from nuclear small and large subunit rDNA sequences using maximum likelihood. Branches in boldface indicate posterior probability values >0.95, ML bootstrap support values (in %) are provided along nodes. The generic types are indicated for the clades containing *Marchandiomyces* spp. and relatives. Nutritional and/or substrate ecology is indicated by color. The labeling of the major clades of Agaricomycetes follows the Assembling the Fungal Tree of Life (AFTOL) initiative to provide a unifying classification for the kingdom Fungi (Hibbett et al., 2007).


Saprotrophic/lignicolous
Phytopathogenic/foliicolous
Lichenicolous/mycoparasitic
Lichenized
Marchandiomyces corallinus ATCCMYA2346
Marchandiomyces corallinus ATCCMYA2500
Marchandiomyces corallinus ATCCMYA3182
Marchandiomyces corallinus JL128-98
(Generitype)
Marchandiomyces lignicola ATCCMYA2347
Marchandiomyces lignicola ATCCMYA2831
Marchandiomyces lignicola ATCCMYA3674
Marchandiomyces lignicola ATCCMYA835 (Type strain)
Marchandiomyces nothofagicola JL261-04 (Type strain)
Limonomyces culmigenus ATCC22523
Marchandiomyces buckii ATCCMYA2992 (Type strain)
Marchandiomyces marsonii ATCCMYA4210 (Type strain)
Laetisaria fuciformis NJ-2 Jackson (Generitype)
Limonomyces roseipellis CBS299.82 (Generitype)
Corticium roseum CBS205.91 (Type of Corticium and Corticiales)
Marchandiomphalina foliacea Palice 2509
Marchandiomphalina foliacea Palice 4369
(Generitype)
Erythricium laetum NH14530 (Generitype)
Marchandiobasidium aurantiacum ATCCMYA2502
Marchandiobasidium aurantiacum CBS718.97
(Generitype)
Waitea circinata CBS315.84 (Generitype)
Laetisaria arvalis CBS131.82 (Type strain)
Erythricium salmonicolor
Corticiales
Galzinia incrustans HHB-12952-Sp
Tretopileus sphaerophorus JCM10092
Punctularia strigosozonata HHB-11897-Sp
Dendrocorticium polygonioides FO36469g
Dendrocorticium roseocarneum FPL1800
Vuilleminia comedens T-583 (Generitype)
Vuilleminia macrospora EL21-99
Cytidia salicina CBS727.85
Gloeophyllum sepiarium P198
Heliocybe sulcata D.797
Gloeophyllales
Sarcodon imbricatus REG Sim
Thelephora sp. DSH 96-010
Bankera fuligineoalba DAOM184178
Thelephorales
71
70
95
61
70
79
100
61
52
88
53
80
90
99
65
67
98
0.005 substitutions/site

out that Index Fungorum (http://www.indexfungorum.org) lists the current name of *E. salmonicolor* as *Phanerochaete salmonicolor* (Berk. & Broome) Jülich, but given our results and the likelihood that *Phanerochaete* is polyphyletic (Larsson, 2007), we decided to retain the name *E. salmonicolor* in this paper. It is probable that neither name is appropriate.

We propose to describe the unknown here as a species of *Marchandiomyces* s.l. within the clade containing two other *Marchandiomyces* spp., and *Laetisaria fuciformis* and *Limonomyces roseipellis*. Further taxonomic resolution of these groups will require additional study of more species. A species we hope to include soon is *Laetisaria agaves* Burds. & Gilb., which was described from Arizona and produces coral, cartilaginous basidiocarps on leaf bases of *Agave* species (Burdsall and Gilbertson, 1982).

New species description*—*Marchandiomyces marsonii Diederich & Lawrey, sp. nov. (Figs. 2–11)

Marchandiomyces species insignis bulbillis foliicola, rotundatis, applanatis, corallinis, 160–260 μm diameter, hyphis elongatis, interdum ramosis, raro septatis, 1.5–3.5 μm diameter.

Type: Australia: Queensland: Cairns, on trees along road near Convention Centre, 16°55′40″S, 145°46′45″E, alt. 6 m a.s.l. (coordinates obtained by GPS), on dead, hanging leaves of *Pandanus oblatus* (Pandanaceae), 22 August 2006, *G. Marson & M.-L. Wu s.n.* (BRI–holotype; herb. Diederich–isotype). Type culture ATCC MYA-4210.

Basidiomata and conidiomata unknown. *Colonies* foliicolous, appearing as numerous bulbils. *Mycelium* not observed. *Bulbils* developing superficially over leaves of *Pandanus oblatus*, dispersed, rarely touching each other, roundish, strongly applanate, 160–260 μm in diameter, pastel red (Kornerup and Wanscher, 1984: 8A4–5) (Figs. 3, 4); surrounded by a thin, necrotic, hyaline layer 3–4.5 μm thick that does not stain with lactophenol cotton blue (Fig. 9) and that remains as an extremely thin, white, subspherical envelope after the disappearance of the interior of old bulbils (Fig. 5), sometimes giving the impression of white, empty "bulbils" (Fig. 8); external portions of bulbils with dispersed crystalline granules best visible in polarized light (Fig. 7) but without specialized cells (Fig. 10); bulbils composed of a dense agglomeration of thin-walled, smooth, hyaline, irregular, elongate, occasionally branched and septate hyphae (Fig. 11), cells mainly 1.5–3.5 μm diameter, clamps not observed in squash preparations.

Colonies in liquid culture pale pinkish orange, with aerial hyphae from which dispersed bulbils occasionally develop. Hyphae hyaline, septate, straight or curved, frequently branched, 2–3(−3.5) μm diameter, septa with clamps. Bulbils rare, leaving white, empty "bulbils" when disappearing (Fig. 8).

Marchandiomyces marsonii named after Guy Marson (Luxembourg) who discovered and collected the new taxon while searching for new *Orbilia* species in Australia.

Additional specimen examined—Australia: Queensland: Cairns, 4 km north of city center, Flecker Botanical Gardens, 16°54′01″S, 145°44′57″E, 14 m a.s.l. (coordinates obtained by global positioning system), on dead, hanging leaves of *Pandanus oblatus*, August 2006, *G. Marson & M.-L. Wu s.n.* (herb. Diederich).

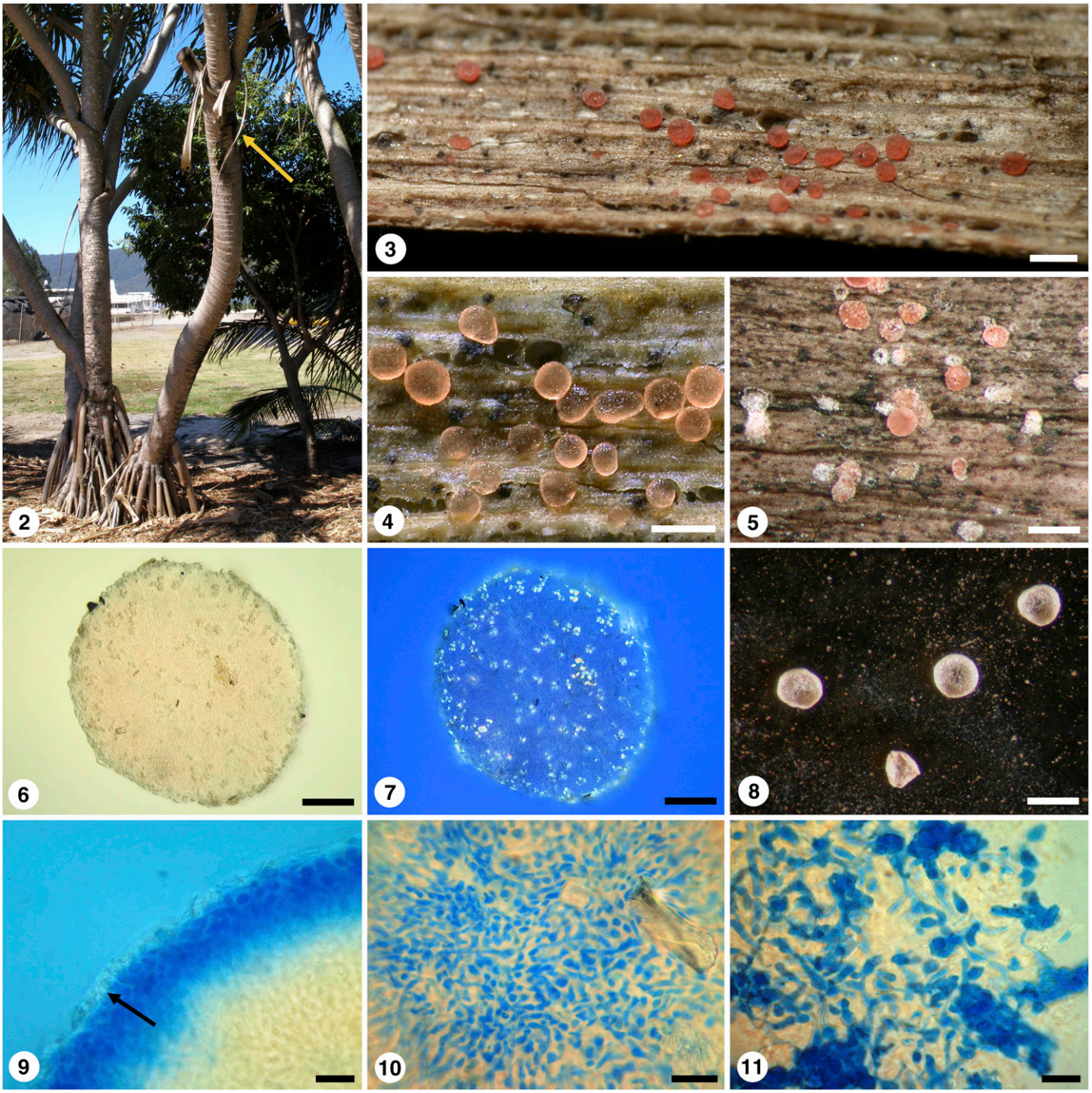
DISCUSSION

The new species *Marchandiomyces marsonii* differs from others in the genus by its foliicolous habit and its large, reddish, flattened bulbils (Fig. 3). These resemble apothecia of species in the ascomycete genus *Orbilia* so closely that they were initially collected and labeled as such, and only subsequent microscopic examination revealed them to be basidiomycete bulbils. These bulbils presumably develop superficially from mycelium growing on *Pandanus* leaves without indication that they invade or degrade the leaves. Because the two known collections are from dead leaves still hanging on the tree (Fig. 2), it is not known if they are also present on living leaves. We therefore assume the species is saprobic/foliicolous.

The large, flattened bulbils of *M. marsonii* are similar to those of other members of the genus in color; production of pink, red or coral-colored pigments appears to be a common characteristic of many members of the clade. Formation of bulbils (or sclerotia) is also relatively common among the corticioid fungi and is a characteristic of all members of the genus *Marchandiomyces*. These are best regarded as resting or dispersal structures capable of surviving unfavorable conditions for long periods of time. In the case of *M. marsonii*, 5-month-old herbarium specimens were still viable, and other bulbilliferous species remain viable even longer. For example, the lichenicolous fungus *Burgoa angulosa* Diederich, Lawrey & Etayo (Cantharellales) could be cultured after five years in a herbarium.

Bulbils may themselves sometimes disintegrate and disappear from the surface of the host. We have repeatedly observed that in older herbarium specimens of *Marchandiomyces corallinus* and *Marchandiobasidium aurantiacum* the fungus apparently disappeared, even in rich specimens originally containing numerous bulbils. Some of this change in appearance can be attributed to mechanical loss because bulbils can be dislodged from the host lichen relatively easily. However, we believe that some loss takes place because bulbils remain viable for extended periods of time and eventually disintegrate in the herbarium. Our observations of *Marchandiomyces marsonii* provide unexpected support for this idea. Unlike other *Marchandiomyces* species, bulbils of *M. marsonii* are surrounded by a thin, hyaline, necrotic layer (Fig. 9). As bulbils age, they entirely disintegrate, leaving only this outer layer. In specimens from *Pandanus* leaves, older bulbils appear to get paler, turn white, and eventually disappear, leaving only the outer, necrotic layer (Fig. 5). Similarly, bulbils in isolated cultures entirely disappear as they age, leaving a surrounding layer in the form of a white, ball- or cuplike, empty structure with a large irregular opening (Fig. 8). The disintegration of bulbils in *M. marsonii*, and by implication other *Marchandiomyces* species as well, indicates these structures may remain viable for long periods under highly unfavorable conditions, but once they exhaust their resources they die and disintegrate, leaving little evidence of their presence on the host.

Our results revealed an unanticipated link between *Marchandiomyces* and certain members of the genera *Laetisaria* and *Limonomyces*, most of which are pathogens or endophytes of grasses that form orange, red, or coral sclerotia (Burdsall, 1979; Burdsall et al., 1980; Stalpers and Loerakker, 1982). The genera *Laetisaria* and *Limonomyces* have been assumed to be related, based largely on disease symptoms and anatomical characters of isolated cultures (Burdsall, 1979; Burdsall et al., 1980; Stalpers and Loerakker, 1982), but support of this relationship from genetic studies has been sparse. A recent phylogenetic study of the sterile red fungus pathogen of grasses (Andjic et al., 2005) using nuc-ITS1 sequences indicated that *Limonomyces roseipellis*, *L. culmigenus*, *Laetisaria arvalis*,



Figs. 2–11. *Marchandiomyces marsonii* Diederich & Lawrey (holotype). **2.** *Pandanus oblatus* tree with dead, hanging leaf (arrow) on which the holotype grows. **3.** Dry and **4.** rehydrated bulbils of *M. marsonii* over *Pandanus* leaf. **5.** Old, dying bulbils, becoming white (dry). Bulbil examined in **6.** water and **7.** in polarized light, showing crystals (Figs. 6, 7 are from a series of photographs at different focal planes, combined into one sharp picture). **8.** White remnants of disintegrated bulbils in culture. Figs. 9–11. Bulbils stained briefly with lactophenol cotton blue. **9.** Optical section (stain partly penetrating bulbil) showing hyaline, nonstained, necrotic, outer layer (arrow). **10.** Surface view, only outer cells stained. **11.** Squash preparation. Scale bars: Figs. 3–5, 8 = 500 µm; Figs. 6, 7 = 50 µm; Figs. 9–11 = 10 µm. Figs. 2–4. Photo credits Guy Marson.

and *L. fuciformis* were all closely related, a result partially (but not completely) supported by our analysis. Because we were not able to align GenBank ITS1 sequences from Andjic et al. (2005) with ITS sequences that we obtained from our cultures of *Limonomyces* or *Laetisaria*, we could not replicate their results. Nevertheless, our results using nuc-SSU and nuc-LSU sequences indicate a close relationship among *Limonomyces* spp., certain *Marchandiomyces* spp. (*M. buckii*, *M. nothofagicola*, *M. marsonii*) and *Laetisaria fuciformis*, with *Limonomyces roseipellis* occupying a basal position in the clade. The group has diverse substrate ecologies, with the use of monocot vascular plants a notable theme, suggesting an important influence

on the evolution of the group. Contrary to Andjic et al. (2005), our results do not support the hypothesis that *L. arvalis* is closely related to either *Limonomyces* or to the generitype *Laetisaria fuciformis*. Given the apparent relationship between *Erythricium salmonicolor* and *L. arvalis*, taxonomic reevaluation of these species is needed.

The Corticiales is one of the most ecologically diverse groups of Agaricomycetes, containing saprobes, plant and fungal pathogens, and lichens. Members of the genus *Marchandiomyces* s.l. and their close relatives are especially diverse ecologically, with lichenicolous (*M. corallinus*, *M. buckii*, *Marchandiobasidium aurantiacum*), lignicolous (*Marchandiomyces lignicola*, *M. nothofagicola*), lichen-forming (*Marchandiomphalina foliacea*), and foliicolous (*Laetisaria* and *Limonomyces* spp., *Marchandiomyces marsonii*) members represented in the group. Because of the wide range of ecological conditions in the group, determining the ecology of the common ancestor would be of interest. Many members of the Corticiales form fruiting structures on recently deceased branches or grow on decorticated wood. These include *Corticium roseum*, *Galzinia incrustans* (Höhn. & Litsch.) Parmasto, *Punctularia strigosozonata* (Schwein.) P.H.B. Talbot, *Dendrocorticium* spp., *Vuilleminia* spp., strongly suggesting a lignicolous and possibly saprotrophic ancestor, an idea mentioned before (DePriest et al., 2005). Because many species have been described only recently and additional species are likely to be discovered in the future, discussions about ancestral ecology may be premature at this point. In *Marchandiomyces* s.l., however, the close association of lichenized, lichenicolous, and various plant-associated species indicates an unusually marked tendency for ecological transitions among these particular fungi.

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