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A new lichenicolous teleomorph is related to plant pathogens in *Laetisaria* and *Limonomyces* (Basidiomycota, Corticiales)

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Abstract: Molecular and morphological data were used to assess the taxonomic placement of an undescribed lichenicolous basidiomycete teleomorph collected in Luxembourg, Belgium and Germany. The new species is ecologically and morphologically similar to *Marchandiobasidium aurantiacum*, teleomorph of the common bulbilliferous lichen pathogen *Marchandiomyces aurantiacus*. However phylogenetic analysis of nuclear and mitochondrial rDNA sequences indicated a close relationship of the new species—not to *M. aurantiacum* but to turf grass pathogens in genera *Laetisaria* and *Limonomyces*, including the generic type of *Laetisaria*. We are including the new species in *Laetisaria* based on strong statistical support for the clade containing these teleomorphs and several *Marchandiomyces* anamorphs. The morphological and ecological diversity of this clade indicates a potentially significant evolutionary role played by lichen-associated species in the Corticiales.

Key words: *Corticium*, *Erythricium*, *Marchandiobasidium*, *Marchandiomyces*, mitosporic fungi, phylogeny, rDNA

INTRODUCTION

Lichenicolous fungi are obligately lichen-associated organisms that have evolved many times throughout the Ascomycota and Basidiomycota. Ecologically they include host-specific parasites, broad-spectrum pathogens, commensals, and necrotrophic forms. More than 1500 species have been described, and it is estimated that 3000 species eventually will be described (Lawrey and Diederich 2003). Fewer than 5% of lichenicolous species are basidiomycetes, but they represent eight orders and four classes (Lawrey and Diederich 2003 Web site http://www.lichenicolous.net), and recent phylogenetic studies are beginning to clarify the evolutionary roles played by these unusual fungi.

A number of common lichenicolous fungi are known to be members of the Corticiales, an ecologically diverse order of entirely resupinate fungi that has been resolved in molecular studies (Boidin et al. 1998, Langer 2002, Larsson et al. 2004, Binder et al. 2005, Larsson 2007, Hibbett et al. 2007). Lichenicolous forms in the Corticiales include anamorphic species in the form genus *Marchandiomyces* Diederich & D. Hawksw. and the teleomorph *Marchandiobasidium aurantiacum* Diederich & Schultheis. All these species are characterized by production of orange or coral bulbils on a variety of lichen hosts. A comprehensive phylogenetic study of these fungi and their relatives (Lawrey et al. 2008) indicated that they are distributed among several clades of species exhibiting a wide diversity of nutritional modes in addition to the lichenicolous habit.

The genus concept of *Marchandiomyces* has changed significantly since it first was established for the bulbilliferous lichen pathogens *M. corallinus* (Roberge) Diederich & D. Hawksw. (Diederich 1990) and *M. aurantiacus* (Lasch) Diederich & Etayo (Diederich 1996). At that time the genus was considered to be strictly lichenicolous and anamorphic. Lignicolous and foliicolous species eventually were discovered (DePriest et al. 2005, Diederich and Lawrey 2007, Lawrey et al. 2008), indicating a considerably greater ecological diversity for the group. A presumptive teleomorph for *M. aurantiacus* also was discovered and named *Marchandiobasidium aurantiacum* (Diederich et al. 2003). However it later was found to be unrelated to the other *Marchandiomyces* species leading Diederich and Lawrey (2007) to suggest that it no longer should be considered a member of *Marchandiomyces* s.str. The only indication of a possible teleomorph for these species emerged from molecular studies (DePriest et al. 2005; Lawrey et al. 2007, 2008) that consistently placed *Marchandiomyces* species together in a clade containing plant pathogens in genera *Laetisaria* Burds. (with three named species) and *Limonomyces* Stalpers & Loer. (two species).

We recently had the opportunity to study a lichenicolous fungus collected from various locations in Luxembourg, Belgium and Germany that is similar

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in color to *Marchandiomyces corallinus* but is fertile with basidiome morphology similar to the teleomorph *Marchandiobasidium aurantiacum*. We were able to isolate one of the specimens and obtained from it nuclear small (nuSSU), large (nuLSU), and mitochondrial small (mtSSU) ribosomal sequences, which we analyzed to determine the phylogenetic position of the new species and its relationship to *Marchandiomyces* and *Marchandiobasidium*. We had available an alignment of nuclear rDNA sequences representing various members of the Corticiales (Lawrey et al. 2008) to which we added mtSSU sequences not previously available. Our objectives were (i) to determine the phylogenetic position of the unknown species and (ii) to consider possible teleomorph connections involving described *Marchandiomyces* anamorphs.

MATERIALS AND METHODS

Anatomical methods.—Specimens of the unknown species were collected Feb 2010 in Luxembourg by the first author, and two additional herbarium specimens from Belgium and Germany were obtained on loan. The fungus was growing along with the lichenicolous pathogen *Marchandiobasidium aurantiacum* on lichens *Physcia tenella* (Scop.) DC. and *P. adscendens* (Fr.) H. Olivier on the bark of *Platanus*. Herbarium specimens are deposited in BR, GMUF, STU and in the private collections of P. Diederich and D. Van den Broeck. The microscopical examination (including all microscopical measurements) was carried out with a Zeiss Photomikroskop III with thin hand-made sections or squash preparations mounted in water or in a mixture of 5% KOH, Congo red and phloxine. Amyloidity of basidiospores was tested with Lugol's reagent without and with pretreatment with 5% KOH and Melzer's reagent.

Isolation of fungal culture.—Cultures of the type specimen of the unknown species were isolated from basidiospores obtained from herbarium material with methods discussed in Lawrey (2002). Spores germinated within 7 d on potato dextrose agar (PDA, Difco, Detroit, Michigan) without antibiotics, and outgrowths were isolated for liquid culture in either potato dextrose or Sabouraud's (Difco) medium with dextrose. Using the same media we also obtained a culture from bulbils of *Marchandiobasidium aurantiacum* collected together with the unknown species. For both isolates approximately 2 µg dry mycelial mass was harvested from liquid cultures after 2 wk and extracted for DNA analysis.

Molecular techniques.—Genomic DNA was extracted from isolated fungal tissue with the Bio 101 Fast DNA Spin Kit for tissue (Qbiogen) according to the manufacturer's protocol with slight modifications. About 10 ng extracted DNA were subjected to a standard PCR in a 25 µL reaction volume with either Taq Gold polymerase (Applied Biosystems) or Bio-X-Act Long Mix (Bioline) according to manufacturer's protocols. We amplified approximately 1700 bp of the nuLSU with primers LR0R, LR3R, LR16, LR8R, LR5, LR7, LR9 and ITS rDNA with ITS5 and ITS4 primers available from the Duke University Mycolab Website (http://www.biology.duke.edu/fungi/mycolab/primers.htm). nuSSU with an approximate length of 1750 bp for most species was sequenced with NS17UCB, NS19UCB, NS3, NS21UCB, NS23UCB, NS24UCB, NS22UCB, NS20UCB, NS2 (White et al. 1990, Gargas and Taylor 1992). Primers for amplification and sequencing of the mtSSU rDNA were mrSSU1 and mrSSU3R (Zoller et al. 1999). After viewing the PCR product with ethidium bromide on a 1% agarose gel and confirming its size the products were purified with magnetic beads (Agencourt Biosciences). The purified PCR products were used in standard sequencing reactions with BigDye Terminator Ready Reaction Mix 3.1 (Applied Biosystems). The sequencing reactions were purified with Sephadex G-50 (Sigma-Aldrich), dried in a speedvac, denatured in HiDi Formamide (Applied Biosystems) and run either on a SCE-9610 capillary machine (SpectruMedix LLC) or an ABI3130-xl capillary machine (Applied Biosystems). The data were analyzed with the program BaseSpectrum (for SpectruMedix) or ABI software, and 500–700 bases were collected for each primer used. These sequences were analyzed with Sequencher 4.7 (Gene Codes Corp.) software for manual base calling and to make contiguous alignments of overlapping fragments.

We obtained nuLSU, nuSSU, and mtSSU sequences from the unknown isolate and new mtSSU sequences from several potentially related fungi studied by Lawrey et al. (2008). These included *Laetisaria arvalis* Burds., *Limonomyces culmigenus* (R.K. Webster & D.A. Reid) Stalpers & Loer., *L. roseipellis* Stalpers & Loer., and a recently isolated culture of *Marchandiobasidium aurantiacum* collected from the type locality of the unknown. Other mtSSU sequences in the dataset were obtained from GenBank.

Phylogenetic analyses.—An alignment (TreeBASE S2054) of nuLSU and nuSSU sequences from a study of the Corticiales (Lawrey et al. 2008) was used as a starting point to which we added mtSSU sequences to produce a three-locus dataset containing 24 terminals (TABLE I). This included three outgroup sequences based on Binder et al. (2005), Larsson (2007), Lawrey et al. (2007, 2008) representing the Gloeophyllales (*Heliocybe sulcata* [Berk.] Redhead & Ginns) and the Thelephorales (*Sarcodon imbricatus* [L.] P. Karst., and *Thelephora* sp.). All sequences were aligned with MacClade 4.08 (Maddison and Maddison 2005). Sequence positions representing primer sites and missing data at the terminal ends of sequences were trimmed and ambiguous regions were excluded to produce an approximately 3000 bp alignment, which was submitted to TreeBASE. Because it was not possible to complete the nuLSU, nuSSU and mtSSU sequences for the same set of 24 samples the dataset was tested for positive conflict with maximum likelihood (ML) with RAxML 7.0.4 (Stamatakis 2006). Models of evolution were selected for each partition with the Akaike information criterion (AIC) as implemented in Modeltest 3.06 (Posada and Crandall 2001). Maximum likelihood bootstrap (BS) proportions were calculated with 1000 BS replicates implementing the GTRMIX model

TABLE I. Species, cultures and GenBank accession numbers of fungi in this study, including the new species *Laetisaria lichenicola*. Sequences acquired in this study are indicated in boldface

Species name	Strain	GenBank accession numbers		
		NuLSU	NuSSU	mtSSU
Corticium roseum	CBS 205.91	EF537893	—	—
Cytidia salicina	CBS 727.85	DQ915478	—	AF214458
Dendrocorticium roseocarneum	FPL1800	AF393053	AF334910	AF334875
Erythricium laetum	NH14530	AY586655	—	—
Erythricium salmonicolor	—	AF506709	—	—
Galzinia incrustans	HHB-12952-Sp.	AF518617	AF518578	AF518679
Heliocybe sulcata	D.797	AF518619	AF334915	AF334881
Laetisaria arvalis	CBS 131.82 (type strain)	EU622842	EU622843	**HQ168390**
Laetisaria fuciformis	NJ-2 Jackson	AY293192	AY293139	AY293232
Laetisaria lichenicola	CBS 128705 (type strain)	**HQ168400**	**HQ168399**	**HQ168389**
Limonomyces culmigenus	ATCC 22523	EU622848	EU622847	—
Limonomyces roseipellis	CBS 299.82	EU622844	EU622845	**HQ168396**
Marchandiobasidium aurantiacum	CBS 128706	**HQ168397**	**HQ168398**	**HQ168388**
Marchandiomphalina foliacea	Palice 2509	AY542864	AY542864	—
Marchandiomyces buckii	ATCC MYA 2992 (type strain)	DQ915472	DQ915462	**HQ168392**
Marchandiomyces corallinus	ATCC MYA-3182	—	DQ915464	**HQ168393**
Marchandiomyces lignicola	ATCC MYA 3674	—	DQ915465	**HQ168391**
Marchandiomyces marsonii	ATCC MYA 4210 (type strain)	EU622839	EU622838	**HQ168395**
Marchandiomyces nothofagicola	JL261-04	DQ915474	DQ915466	**HQ168394**
Punctularia strigoso-zonata	HHB-11897-Sp.	AF518642	AF518586	—
Sarcodon imbricatus	REG Sim1	AF518646	AY293157	—
Thelephora sp.	DSH 96-010	AF287890	AF026627	AF026670
Tretopileus sphaerophorus	JCM10092	—	AB006005	—
Vuilleminia comedens	T-583	AF518666	AF518594	AF518699

with gamma distribution, approximated with four categories. We found no evidence of topological conflict between partitions > 70% (Mason-Gamer and Kellogg 1996) and therefore combined the data for final analysis.

RAxML 7.0.4 (Stamatakis 2006) was used for maximum likelihood (ML) analysis with 1000 ml bootstrap replicates and the same settings as used for the single-locus datasets but in this case with an alignment partition file to specify the three data partitions (nuLSU, nuSSU and mtSSU). Individual shape parameters, GTR rates and base frequencies are estimated and optimized for each partition. Clades with bootstrap values of 70% or greater were considered to be significantly supported. In addition Bayesian phylogenetic analyses were carried out with the Metropolis-coupled Markov chain Monte Carlo method (MCMCMC) in MrBayes 3.0b4 (Ronquist and Huelsenbeck 2003). Analyses were run with 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 with 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* 4.0b10 (Swofford 2002) with the proportion of trees gathered after convergence of likelihood scores, and clades with PP ≥ 0.95 were considered to be significantly supported.

RESULTS

Phylogenetic placement of unknown.—The Bayesian consensus tree (FIG. 1) resulting from analysis of a combined alignment of nuLSU, nuSSU and mtSSU sequences from the Corticiales, Gloeophyllales and Thelephorales was identical to the optimal maximum likelihood (ML) tree (tree score of −lnL = 11495.191). Bayesian runs converged after 200 000 generations, and 23 534 trees were used to compute posterior probability (PP) values; ML bootstrap values (BS) were calculated from 1000 replicates. In the resulting phylogeny the Corticiales and the clade containing described *Marchandiomyces* species both were supported strongly by PP and BS values, a result observed in DePriest et al. (2005) and Lawrey et al. (2007, 2008). The new species was found to be sister to the foliicolous anamorph *Marchandiomyces marsonii* Diederich & Lawrey (PP = 100%, BS = 100%) in a strongly supported (PP = 100%, BS = 100%) clade made up of the plant pathogens *Laetisaria fuciformis* (McAlpine) Burds., *Limonomyces culmigenus*, *L.*

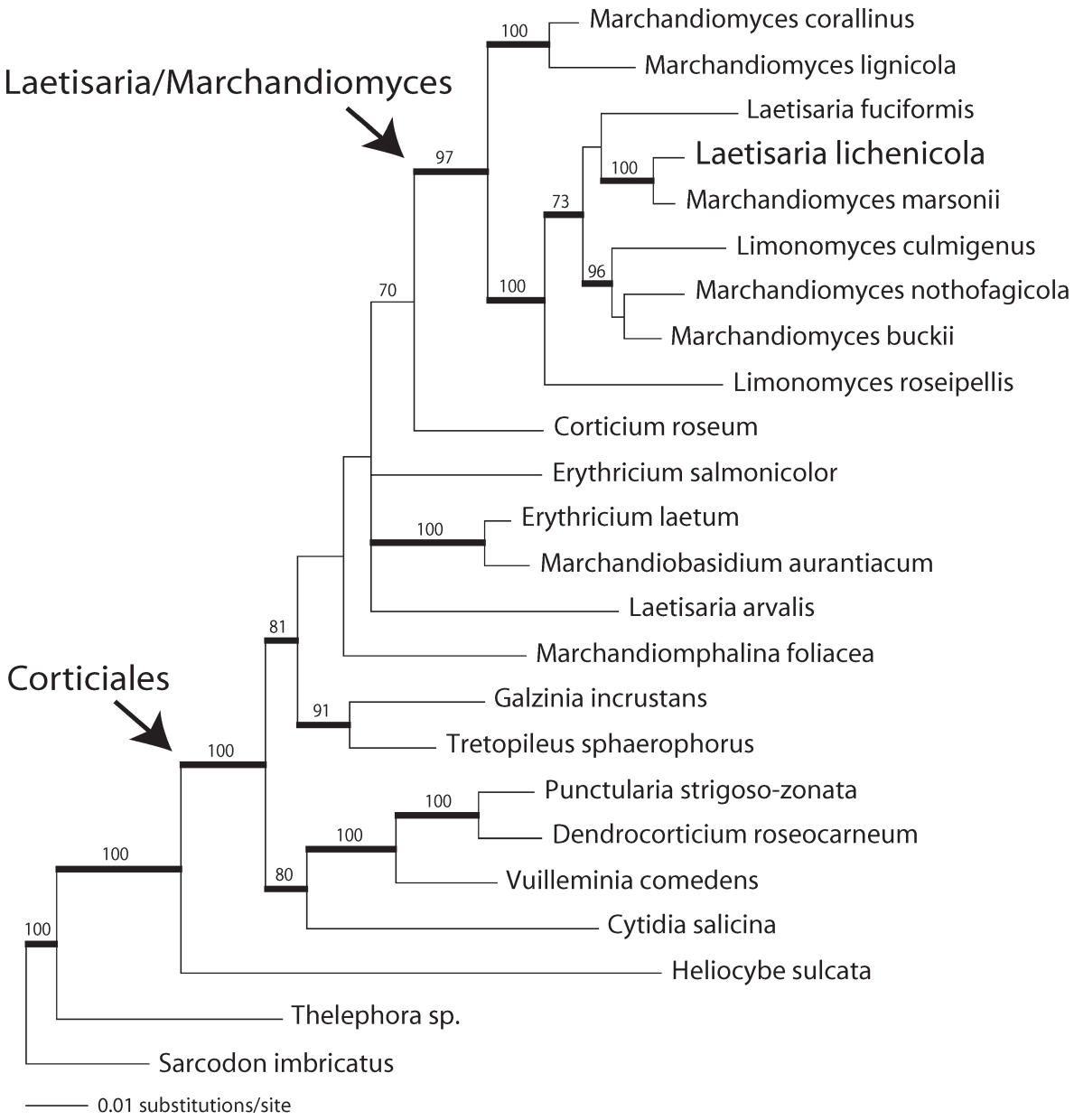

FIG. 1. Phylogenetic relationship of *Laetisaria lichenicola* to other fungi in the Corticiales inferred from nuclear small and large subunit and mitochondrial small subunit rDNA sequences. Bayesian tree with branches in boldface indicating posterior probability values ≥ 0.95; RAxML bootstrap percentages ≥ 70% are indicated at nodes.

roseipellis, and two lichenicolous *Marchandiomyces* species, *M. buckii* Diederich & Lawrey and *M. nothofagicola* Diederich & Lawrey. This cluster of species is sister to another strongly supported (PP = 95%, BS = 100%) clade containing the lichenicolous *Marchandiomyces corallinus* and the lignicolous *M. lignicola* Lawrey & Diederich. The two groups together form a well supported (PP = 95%, BS = 97%) ''Laetisaria/Marchandiomyces'' clade sister to *Corticium roseum* Pers., the type species of the Corticiales. The close relationship we expected between the new species and the lichen pathogen *Marchandiobasidium aurantiacum* was not supported by our phylogeny. Consistent with (Lawrey et al. (2008) and Ghobad-Nejhad and Hallenberg (2010), *M. aurantiacum* was found to be sister to the saprotrophic/pathogenic fungus *Erythricium laetum* (P. Karst.) J. Erikss. & Hjortstam in a poorly resolved group of fungi including the plant pathogen *Erythricium salmonicolor* (Berk. & Broome) Burdsall, the facultative mycoparasite *Laetisaria arvalis* and the basidiolichen *Marchandiomphalina foliacea*.

FIG. 2. *Laetisaria lichenicola*. A–C. Basidiomata developing on the thallus of *Physcia adscendens* and *P. tenella*; note the granular surface in C representing large basidiospores attached to the sterigmata. D. Thallus of *P. tenella* after being attacked by *L. lichenicola* (left: host thallus killed by the fungus, keeping its characteristic color, basidiomata absent) and *Marchandiobasidium aurantiacum* (right: orange bulbils, basidiomata absent); note the smooth, shiny surface of the host after being attacked by *L. lichenicola* and the more disintegrated, mat surface during an invasion by *M. aurantiacum*. E. Thallus remnants of *P. tenella* and *Xanthoria parietina* fusing after being attacked by *L. lichenicola* and covered by a thin, shiny, varnish-like layer. F, G. Mature basidia with two sterigmata, with an additional immature basidium (G bottom right). H. Two hyphal septa with dolipores. I–L. Basidiospores. A, B, D–F, H–J: holotype; C, G, K: *Heklau* s.n.; L: *Van den Broeck* 3431. G, I, K, L show living cells observed in water; F, H, J have been stained with a mixture of 5% KOH, Congo red and phloxin. Bars: A, B, D, E = 1 mm; C = 0.2 mm; F, G, I–L = 5 µm; H = 1 µm.

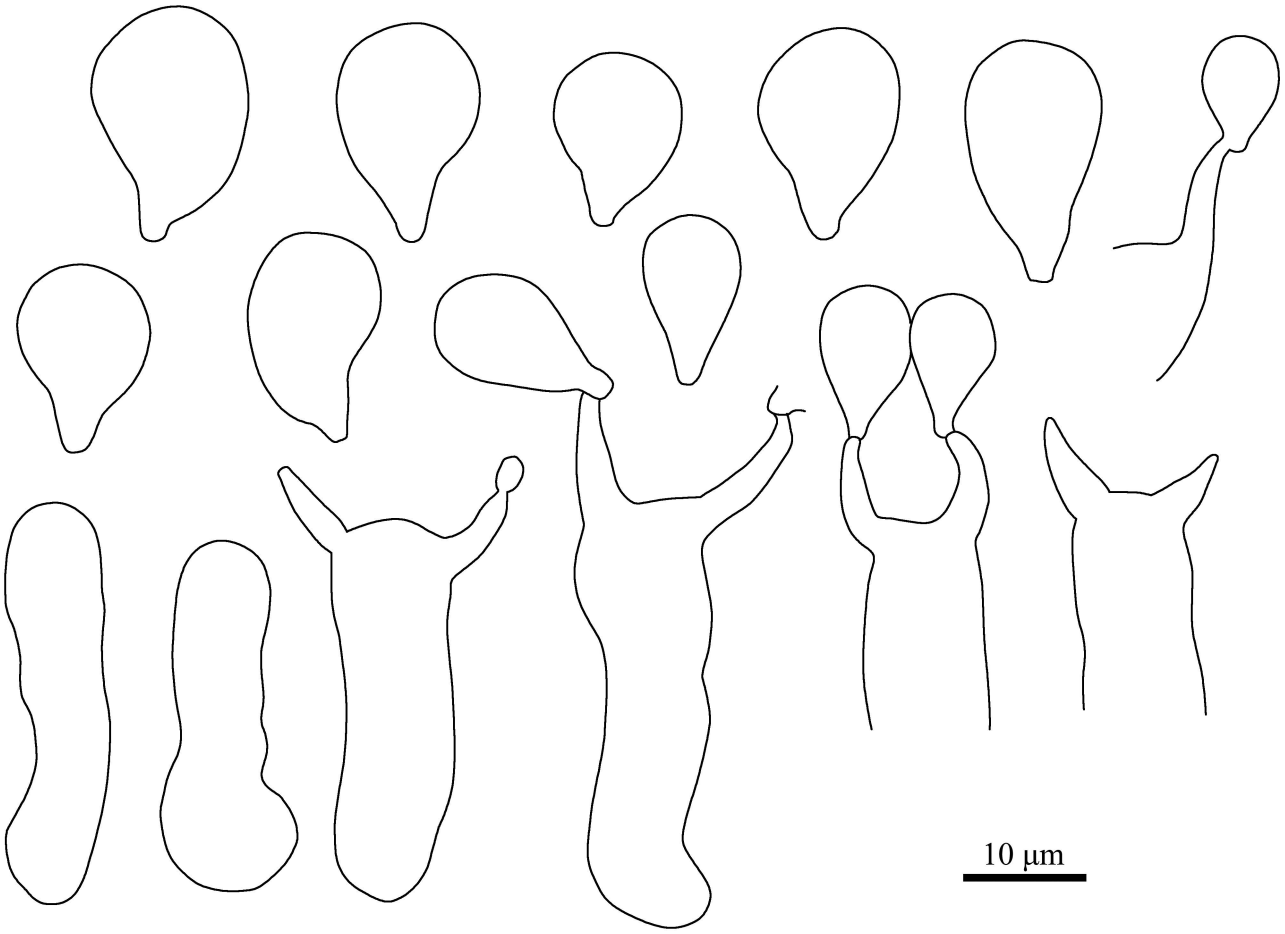

FIG. 3. *Laetisaria lichenicola* (holotype). Immature and mature basidia and basidiospores.

New species description.—The clade containing the new species included teleomorphs in two genera, *Laetisaria* and *Limonomyces*, and several *Marchandiomyces* anamorphs. We are describing the new species in *Laetisaria*, a decision based on its close relationship to the generic type *Laetisaria fuciformis* and the strong probability that this clade ultimately will be recognized at the genus level with *Laetisaria* (1979) having priority over *Limonomyces* (1982). However, given the unusual ecological and morphological diversity of this group, we think it is premature to form additional new combinations at this point.

TAXONOMY

Laetisaria lichenicola Diederich, Lawrey & Van den Broeck sp. nov. FIGS. 2–3

MycoBank MB518789

A Marchandiobasidio aurantiaco basidiomatibus roseis, basidiis duobus sterigmatibus et bulbillis nullis differt.

Basidiomata light pink to coral, effused, thin, adnate, granulose, floccose, margin indeterminate, reaching 2 mm diam (FIG. 2A–E). Basal hyphae hyaline, straight or occasionally contorted, lacking clamps, smooth, 2.5–3 µm wide, wall ca. 0.5 µm; dolipores ca. 0.5 µm (FIG. 2H). Subhymenial hyphae hyaline, thin-walled, lacking clamps, septate, smooth, 2.5–4 µm wide. Hymenium comprising one layer of basidia on vertically branching, thin-walled hyphae. Hyphidia, cystidia and other sterile hymenial elements lacking. Basidia initially elongate cylindrical, ca. 25–40 × 7.5–9 µm, basally up to 11.5 µm wide, without basal, lateral probasidial bladder; when mature, generally becoming clavate to suburniform, aseptate, ca. 30–35 × 9.5–12 µm, distinctly wider than the supporting hyphae; wall 0.6–1.0 µm; basal clamps lacking (FIGS. 2F, G; 3); sterigmata two per basidium, 6.5–11.5 µm long and 3–3.5 µm wide at the base, curved. Basidiospores hyaline, smooth, aseptate, nonamyloid, pyriform or lacriform, one side frequently flattened or slightly concave, with a prominent truncate apiculus of 1.5–3 µm diam, 14.5–18.5(–20.0) × (8–)10.5–12.5 µm; wall 0.4–0.7 µm (FIGS. 2I–L, 3). Conidial and bulbilliferous morphs unknown.

Type. LUXEMBOURG: Dudelange, center of city, trees on parking along Schwaarze Wee, on *Platanus*, on *Physcia tenella* and *P. adscendens*, 15 Feb 2010, *P. Diederich 16900* (BR–HOLOTYPUS; GMUF, herb. Diederich–ISOTYPI). Type culture: JL393-10, CBS 128705.

The new species differs from *Marchandiobasidium aurantiacum* (FIG 2D, E) by the color of the basidiomata (pink versus orange), the basidia with only two sterigmata (instead of four) and the absence of bulbils.

Additional specimens examined. BELGIUM. PROV. ANTWERPEN: Klein Vorst of Vorst-Meerlaar (Laakdal), Geelsebaan, kerkhof (aan kruispunt met Lindestraat), 51°05′06″N, 5°04′35.8″E, on *Physcia tenella*, 31 Jan 2010, *D. Van den Broeck 3431* (herb. Van den Broeck). GERMANY: Württemberg, Neckarland, Stuttgart, Kräherwald, Wegkreuzung 210 m SW Pkt. 379.5, on *Fraxinus*, on *P. tenella*, 4 Mar 2009, *M. Heklau* (STU).

DISCUSSION

Our main objective was to infer the phylogenetic position of an unknown lichenicolous species with characteristics similar to the teleomorphic *Marchandiobasidium aurantiacus* and many described *Marchandiomyces* anamorphs. Based on anatomy, morphology and ecology, our unknown would almost certainly have been classified as a new lichenicolous species in *Marchandiobasidium*. However all genetic evidence supports its placement in a clade containing the generitypes of *Laetisaria* and *Limonomyces*, along with several *Marchandiomyces* anamorphs. This clade was recovered by us in Lawrey et al. (2008) with moderate support, and it received considerably greater support in the present study with the addition of sequences of the new species and new mtSSU sequences. Given the close relationship of the unknown to the generitype *Laetisaria fuciformis* and the strong likelihood that all of these species eventually will be placed in a single genus, we are electing to name it in *Laetisaria*.

Species in genera *Laetisaria* and *Limonomyces* have been assumed to be closely related. They are mostly pathogens and endophytes of grasses that form orange, red or coral sclerotia (Burdsall 1979, Burdsall et al. 1980, Stalpers and Loerakker 1982). *Laetisaria* species differ from those of *Limonomyces* by having hyphidia, hyphae lacking clamps and multinucleate cells (Stalpers and Loerakker 1982). The new species *L. lichenicola* is similar in color to *Laetisaria* and *Limonomyces* spp. However it lacks both hyphidia and clamps and has basidiospores different in appearance from either *Laetisaria* or *Limonomyces* (we do not have information on the number of nuclei per cell). As a lichenicolous form it also differs ecologically from any described *Laetisaria* or *Limonomyces* species. Because phylogenetic analysis of molecular data consistently places all of these species in a single clade (Andjic et al. 2005, Lawrey et al. 2008, Ghobad-Nejhad and Hallenberg 2010) it is obvious that new generic concepts must be established. At present we are viewing the entire clade informally as a single presumptive genus with the oldest teleomorph name *Laetisaria* along with its presumed *Marchandiomyces* anamorphs serving as a useful label.

It should be mentioned that *Isaria fuciformis* Berk. is listed as the anamorph of *Laetisaria fuciformis* in older literature, leading one to wonder whether *Isaria* and *Marchandiomyces* might be synonyms with *Isaria* having priority. However these presumptions are clearly incorrect inasmuch as the form genus *Isaria* is ascomycetous. The nomenclatural and taxonomic problems associated with this presumed anamorph were discussed by Burdsall (1979), and the nomenclatural status of *Isaria* was discussed more recently by Hodge et al. (2005). Given that *Isaria* cannot be used as the anamorph for *Laetisaria fuciformis*, we suggest that *Marchandiomyces* is the only available anamorphic name for the ''Laetisaria/Marchandiomyces'' clade.

Another teleomorph in this clade was discovered recently in a molecular study of *Vuilleminia* and its relatives (Ghobad-Nejhad et al. 2010). The saprotrophic species *Laeticorticium quercinum* J. Erikss. & Ryvarden was found to be nested in the *Marchandiomyces* clade, prompting the authors to erect the new genus *Marchandiopsis* Ghobad-Nejhad & Hallenb. to accommodate this species. This becomes the third teleomorphic genus in the clade and the only teleomorph sister to the *Laetisaria* clade.

Our phylogeny confirms results of DePriest et al. (2005), Lawrey et al. (2007, 2008) and Ghobad-Nejhad and Hallenberg (2010) that *Marchandiobasidium aurantiacum* is not closely related to any described species in *Marchandiomyces* s. str. but instead is related most closely to *Erythricium laetum*, a salmon lignicolous fungus that grows on decayed wood, moist leaves and possibly living mosses (Binder et al. 2005). Our phylogeny suggests other fungal relatives might include *Erythricium salmonicolor*, *Laetisaria arvalis* and the basidiolichen *Marchandiomphalina foliacea*. Morphological data do not support a relationship of *M. aurantiacum* to species in genus *Erythricium*, which is characterized by resupinate, pink basidiomata, lack of clamps and basidiospores with cyanophilous walls. However *Erythricium* is seldom resolved as monophyletic in phylogenies based on molecular data. Ghobad-Nejhad and Hallenberg (2010) found some support for a monophyletic *Erythricium* with nuITS sequences, but they also reported difficulties aligning some of their

sequences and, perhaps as a consequence, detected significant topological conflict between nuITS and nuLSU gene trees. Although we had nuITS sequences available from our cultures and we obtained 5.8S gene trees identical those shown (FIG. 1), we had difficulties aligning ITS1 and ITS2 sequences and did not include them in our analyses. Because it appears that *Erythricium* is probably not monophyletic it is impossible at present to resolve the relationship of *Erythricium* species to other corticioid fungi, including the lichenicolous *M. aurantiacum*, without more data.

The new species *Laetisaria lichenicola* emphasizes the importance of lichenicolous species in the evolution of fungi in the Corticiales. These fungi now feature prominently in several major clades of the order, all of which include complex combinations of saprotrophic, pathogenic or symbiotic forms. The ''Laetisaria/Marchandiomyces'' clade is especially diverse ecologically, featuring as it does numerous independent lineages of lichenicolous forms. At present this diversity appears to be derived from saprotrophic ancestors represented by *Corticium roseum*, but the role played by lichen-associated fungi is not clear. We anticipate that as new species are discovered, isolated and sequenced the evolutionary transitions that gave rise to this complexity will become more evident.

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