

A NEW LINEAGE OF LICHENIZED BASIDIOMYCETES INFERRED FROM A TWO-GENE PHYLOGENY: THE LEPIDOSTROMATACEAE WITH THREE SPECIES FROM THE TROPICS¹

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The lichen habit has apparently evolved independently in at least five major clades of mushroom-forming basidiomycetes (Agaricomycetes). Tracing the origin of lichenization in these groups depends on a clearer understanding of the phylogenetic relationships of basidiolichens to other fungi. We describe here a new family of basidiolichens made up of tropical, soil-inhabiting fungi that form lichenized, scale-like squamules and erect, coral-like fruiting structures. These structures are common to two basidiolichen genera, *Multiclavula* and *Lepidostroma*. Molecular studies have confirmed the phylogenetic position of *Multiclavula* species in the Cantharellales, but *Lepidostroma* species have never been sequenced. We obtained nuclear small and large subunit ribosomal sequences from specimens of *L. calocerum* collected in Costa Rica and Mexico and also from specimens of two *Multiclavula* spp. recently described from Rwanda. The phylogenetic placement of these fungi within the Agaricomycetes was investigated using likelihood and Bayesian analyses. Our results indicate that *L. calocerum* and the Rwandan species form a natural group unrelated to *Multiclavula* and sister to the Atheliales, members of which are neither lichen-forming nor clavarioid. The independent evolution of morphologically similar forms in so many groups of basidiomycetes is a remarkable example of convergence, indicating similar pathways to lichenization in these fungi.

Key words: Africa; Agaricales; Atheliales; basidiomycetes; *Lepidostroma*; lichens; *Multiclavula*; neotropics; rDNA sequence analyses; symbiosis.

In this paper, we report that two recently discovered terricolous basidiomycetes belong to a previously unknown lichenized lineage. In addition to clarifying relationships among lichenized basidiomycetes, our paper demonstrates the need for study of lichen biodiversity in tropical areas, which remain very much unexplored. Indeed, as shown by Nelsen et al. (2007) and Lücking (2008), uncertainty about the total number of lichen species on earth is due primarily to the lack of data in tropical taxa, especially the lack of modern taxonomic treatments. Further, savannas and open dry forests represent neglected biomes in tropical Africa, and their lichen flora is virtually unknown, especially saxicolous and terricolous species. Although representing a large proportion of the protected areas in the continent, they are currently under threat due to inappropriate management (Burgess et al., 2004).

Less than 0.5% of described lichen-forming fungi belong to the Basidiomycota (Honegger, 1996), and all are mushroom-forming basidiomycetes (Agaricomycetes) that associate with cyanobacteria or green algae. The morphological diversity of these lichens is assumed to derive from independent evolution of the lichen-forming habit from non-lichen ancestors, and recent molecular phylogenetic studies (Larsson et al., 2006;

Larsson, 2007; Lawrey et al., 2007; Nelsen et al., 2007; J. Lawrey et al., unpublished manuscript) now indicate that they can be placed in as many as five different major groups of Agaricomycetes (Table 1).

Figure 1 shows the diversity of forms among accepted genera of basidiolichens (Oberwinkler 1970, 1984, 2001; Redhead et al. 2002a, b). Basidiolichens can be crustose, squamulose (scale-like) or foliose. Some species are *Multiclavula*-like, resembling coral fungi, others are omphalinoid, producing mushrooms with decurrent gills, or appearing as bracket fungi, or crust-like resupinate basidiomata. The first molecular phylogeny that included basidiolichens (Gargas et al., 1995) confirmed a long-held hypothesis that the lichen habit has evolved several times in the basidiomycetes, and studies done since that time have begun to clarify the composition and evolutionary development of the lichen-forming groups. In the mushroom-forming Agaricales, two common and relatively diverse genera, the omphalinoid chlorolichen *Lichenomphalia* and the bracket-forming cyanolichen *Dictyonema*, are now confirmed as closely related members of the Hygrophoraceae in the Agaricales (Lutzoni and Vilgalys, 1995; Lutzoni, 1997; Redhead et al., 2002a; Lodge et al., 2006; J. Lawrey et al., unpublished manuscript). The genus *Multiclavula*, forming coral-like fruiting structures, has long been recognized as a core member of the order Cantharellales, which includes a wide variety of nutritional forms in addition to lichen-formers (Binder et al., 2005; Lawrey et al., 2007; Nelsen et al., 2007). In the Corticiales, the squamulose basidiolichen *Marchandiomphalina foliacea* is the only lichen-forming species in that order, nested within a diverse group of saprotrophs and pathogens (Diederich and Lawrey, 2007; Lawrey et al., 2008). Some associations, especially those of *Athelia*, *Athelopsis*, *Cyphellostereum*,

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TABLE 1. The genera of basidiolichens, updated from a summary originally published by Nelsen et al. (2007). Genera in bold are truly lichenized. The others are considered to be weakly or doubtfully lichenized.

Taxon	Systematic position	References
Acantholichen	Hygrophoraceae (Agaricales)	Jørgensen (1998), J. Lawrey et al. (unpublished)
<i>Athelia</i>	Atheliales, polyphyletic	Poelt and Jülich (1969), Larsson et al. (2004), Larsson (2007), loosely associated with green algae
<i>Athelopsis</i>	Atheliales, polyphyletic	Larsson et al. (2004), Larsson (2007), loosely associated with green algae
<i>Cyphellostereum</i>	Podoscyphaceae (Polyporales or polyporoid clade) or Hymenochaetales (hymenochaetoid clade)	Moncalvo et al. (2002), Redhead et al. (2002b); few weakly lichenized species (Aptroot and Sipman 2001)
Dictyonema	Hygrophoraceae (Agaricales)	Gargas et al. (1995), Hibbett and Thorn (2001), Chaves et al. (2004), Lawrey et al. (unpublished)
Lepidostroma	Lepidostromataceae	This paper
Lichenomphalia	Hygrophoraceae (Agaricales)	Hibbett and Thorn (2001), Redhead et al. (2002a)
Marchandiomphalina	Corticiales	Palice et al. (2005), Lawrey et al. (2007), Diederich and Lawrey (2007), Lawrey et al. (2008)
Multiclavula	Clavulinaceae (Cantharellales)	Gargas et al. 1995, Lawrey et al. (2007), Nelsen et al. (2007)
Semiomphalina	Agaricales or euagarics clade	Corner (1966), Redhead (1984), Hibbett and Thorn (2001), Redhead et al. (2002a)

Semiomphalina spp., are only weakly lichenized, but they are important because they provide evidence of possible transitional forms. All these basidiolichens have a wide diversity of saprobic, mycorrhizal, and lichen-associated relatives (Lawrey et al., 2007; Nelsen et al., 2007).

Recent fieldwork in Rwanda (eastern Africa) led to the discovery of two new species of basidiolichens growing on soil in frequently burned savannas or on lateritic soils. Both produce a typical persistent thallus and ephemeral coral-like fruiting bodies of the *Multiclavula* type. They were originally described in the genus *Multiclavula* (as *M. akagerae* and *M. rugaramae*) based on morphological and anatomical characters only (Fischer et al., 2007). LSU sequences obtained from these species were found to be related to the Atheliales, so distantly related to *Multiclavula* (Cantharellales) that they were assumed at the time to be contaminations.

However, since then, two of us (J.L. and M.S.) obtained sequences of *Lepidostroma calocerum*, a species mentioned by Fischer et al. (2007) as a possible relative of the Rwandan species. Phylogenetic analysis of these sequences and those of the two species from Rwanda showed that they all form a well-supported monophyletic group most closely related to the Atheliales. The generic name *Lepidostroma* is available for it, and we assign it to the new family Lepidostromataceae. The objective of this paper is to describe this new family and to discuss its taxonomy and evolutionary significance based on a two genes phylogeny.

MATERIALS AND METHODS

Taxon sampling—Well-preserved and freshly collected lichen specimens lacking any visible symptoms of fungal infection were used for DNA isolation. We obtained 12 new sequences from five specimens belonging to three taxa of *Lepidostroma* from continental Africa (Rwanda) and America (Costa Rica, Mexico) (Table 2). In addition, two sequences were obtained for a specimen of *Acantholichen pannarioides* from the Galápagos Islands. Eighty-three sequences were added from GenBank. The four outgroup species were chosen based on Larsson (2007) and Lawrey et al. (2007): *Exidiopsis calcea* (Auriculariales), *Exidia uvapsassa* (Auriculariales), *Dacrymyces* sp. (Dacrymycetales) and *Calocera cornea* (Dacrymycetales). In total, the data set included 54 specimens representing 50 species. For the specimen of *Lepidostroma rugaramae*, sequences were obtained from two different DNA extractions: one from the thallus (squamules) and one from the basidiocarps.

Molecular data—Genomic DNA was isolated from lichen specimens using the Puregene Genomic DNA Purification Kit (GENTRA Systems, Minneapolis, Minnesota, USA) using the manufacturer's plant tissue extraction protocol. Amplification reactions were prepared for a 50- μ L final volume containing 5 μ L 10 \times Taq Buffer (Roche, Basel, Switzerland), 2.5 μ L of each of the 20 μ M primers, 1 μ L of 10 mg-mL⁻¹ bovine serum albumin (#2616, Applied Biosystems/Ambion, Austin, Texas, USA), 1 μ L of 25 mM MgCl₂, 1.25 U Taq DNA polymerase (Roche) and 1 μ L of template genomic DNA. PCR was performed on Peltier Thermal Cyclers PTC-100 or PTC-150 (MJ Research-Biorad, Hercules, California, USA). A targeted fragment of about 0.9 kb at the 5' end of the nuclear large subunit (nucLSU) rDNA gene was amplified using primers LR0R (Rehner and Samuels, 1994), with LR5 (Vilgalys and Hester, 1990). A fragment of about 1.6 kb of the nuclear small subunit (nucSSU) rDNA gene was amplified and sequenced using primers NSSU97A (Kauff and Lutzoni, 2002) and NS24 (Gargas and Taylor, 1992). PCR products were purified using the QIAquick PCR Purification Kit (Qiagen, Hilden, Germany). The yield of the PCRs was verified by running the products on a 1% agarose gel and stained with ethidium bromide. Both strands of nucLSU and nucSSU were sequenced directly using the amplification primers. Additional primers for sequencing were used: LR3R, LR3, LR5, LR5R, NSSU1088, NSSU1088R, SR7 and SR7R (Kauff and Lutzoni, 2002; Vilgalys and Hester, 1990; <http://www.botany.duke.edu/fungi/mycolab>). Sequence fragments were assembled with Sequencher version 4.6 (Gene Codes, Ann Arbor, Michigan, USA). Sequences were subjected to BLAST searches to verify their closest relatives and to detect potential contaminations.

Phylogenetic analyses—NucLSU and nucSSU sequences for taxa listed in Table 2 were aligned using the program MacClade version 4.05 (Maddison and Maddison, 2002). Because it was not possible to complete the nucLSU and nucSSU sequences for the same set of 50 taxa, analyses for incongruence among loci were carried out on data sets with 39 taxa for which all two genes were sequenced. Topological incongruence among data sets was examined using 1000 replicates of neighbor-joining bootstrapping (NJ-bs) with the distance measure estimated by maximum likelihood under a six-parameter (general time reversible [GTR], Rodríguez et al., 1990) "best-fit" evolutionary model for nucleotide substitution (Yang et al., 1994; Cunningham et al., 1998; Liò and Goldman, 1998) using PAUP* version 4.0b10 (Swofford, 2002). Best-fit evolutionary models were estimated for all NJ analyses using hierarchical likelihood ratio tests (LRTs) as implemented in the program Modeltest version 3.06 (Posada and Crandall, 1998). All topological bipartitions with NJ-bs values $\geq 70\%$ were compared for all three loci. A conflict was assumed to be significant if two different relationships (one being monophyletic and the other being non-monophyletic) for the same set of taxa were both supported with bootstrap values $\geq 70\%$ (Mason-Gamer and Kellogg, 1996). Based on this criterion, no conflict was detected so that the LSU and SSU data sets were concatenated. A combined two-locus data set was assembled: a 50-taxon combined data set (supermatrix approach) with 11 missing sequences of the nucSSU gene.

For maximum likelihood analyses the best-fit model of DNA evolution was chosen using the Akaike information criterion (AIC) as implemented in

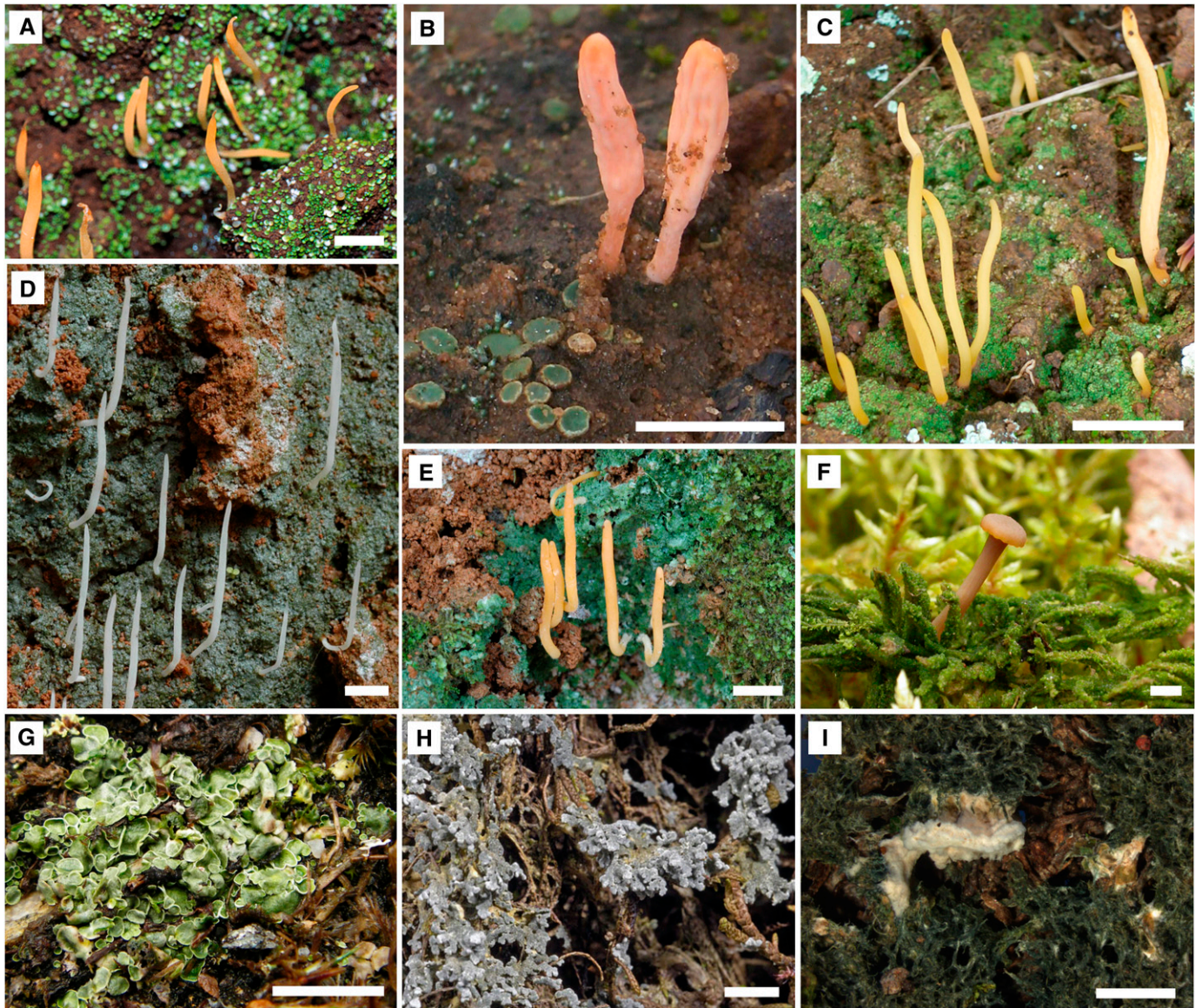


Fig. 1. Diversity of basidiolichens. (A–F) Thallus and basidiocarps of (A) *Lepidostroma calocerum* (G. W. Martin) Oberw. (Costa Rica, photograph by R. Lücking), (B) *Lepidostroma rugaramae* (Rwanda, photograph by E. Fischer), (C) *Lepidostroma akagerae* (Rwanda, photograph by E. Fischer), (D) *Multiclavula* cf. *mucida* (Pers.) R. H. Petersen (Thailand, photograph by R. Walley), (E) *Multiclavula* cf. *clara* (Berk. & M. A. Curtis) R. H. Petersen (Thailand, photograph by R. Walley), and (F) *Lichenomphalia umbellifera* (L.) Redhead, Lutzoni with its *Botrydina*-type thallus overgrowing mosses and an omphalinoid basidiocarp (Belgium, photograph by J.-P. Duvivier). (G) *Coriscium*-type thallus of *Lichenomphalia hudsoniana* (H. S. Jenn.) Redhead (Scotland, photograph by D. Genney). (H) *Acantholichen pannarioides* P. M. Jørg. (Galapagos, photograph by F. Bungartz). (I) Thallus and basidiocarp of *Dictyonema interruptum* (Carmich. ex Hook.) Parmasto (Madeira, photograph by D. Ertz). Bars: (A–G) 5 mm; (H–I) 2 mm.

Modeltest version 3.06 (Posada and Crandall, 2001). Maximum likelihood (ML) searches were performed with the program GARLI version 0.95 (Zwickl, 2006). Each run was repeated three times from random starting trees using the auto-terminate setting and default parameters. The ML bootstrap replicates (significant at 70%) were used to calculate a majority rule consensus tree in PAUP* to assess clade support. Bayesian 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 under the GTR model using a gamma-distributed rate parameter and a proportion of invariable sites. Two parallel MCMCMC runs were performed each using four chains and 5 million 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 de-

termined by calculating a 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 lineage—For the 50-taxon combined data set, the maximum likelihood analysis resulted in one optimal tree (Fig. 2) with a score of $-\ln L = 17400.195$ using the GTR+I+I model with nucleotide frequencies estimated ($A = 0.26$,

TABLE 2. Specimens and DNA sequences for nuclear small (Nuc-SSU) and large subunit (Nuc-LSU) used in this study, with respective voucher information from GenBank. GenBank accession numbers (in boldface) refer to sequences (14) generated by this project. All other sequences (83) were obtained directly from GenBank. Vouchers generated by this study are deposited in the following herbaria: Charles Darwin Research Station (CDS), National Botanic Garden of Belgium (BR), Field Museum of Natural History (F), and University of Nebraska Omaha (OMA).

Species	Order ^{a,b}	Strain or herbarium accession	GenBank accession number	
			Nuc-SSU	Nuc-LSU
<i>Acantholichen pannarioides</i>	Agaricales	Bungartz 5593 (CDS)	FJ171726	EU825953
<i>Albatrellus skamanius</i>	Russulales	DAOM 220694	AF287829	AF393044
<i>Athelia arachnoidea</i> (1)	Atheliales	MYA-3672	DQ915454	—
<i>Athelia arachnoidea</i> (2)	Atheliales	815	—	AF518601
<i>Athelia decipiens</i>	Atheliales	JS4930	—	AY586632
<i>Athelia pyriformis</i>	Agaricales	Hjm 18581 (GB)	—	EU118605
<i>Athelopsis subinconspectua</i>	Atheliales	KHL8490	—	AY586634
<i>Bondarcevomyces taxi</i>	Boletales	Dai2524	DQ534677	DQ534672
<i>Byssocorticium pulchrum</i>	Atheliales	KHL11710	—	AY586639
<i>Calocera cornea</i>	Dacrymycetales	FP-102602-Sp.	AY293121	AY293170
<i>Dacrymyces</i> sp.	Dacrymycetales	FPL8953	AY705954	AY691892
<i>Dendrocorticium roseocarneum</i>	Corticiales	FPL1800	AF334910	AF393053
<i>Dictyonema glabratum</i>	Agaricales	JFA 11953a	DQ917663	DQ917660
<i>Exidia uvapsassa</i>	Auriculariales	TA s.n. JAPAN	AY654890	AY645056
<i>Exidiopsis calcea</i>	Auriculariales	HHB-15059-Sp.	AY293130	AY293180
<i>Echinodontium tinctorium</i>	Russulales	DAOM 16666	AF026578	AF393056
<i>Fibulorhizoctonia</i> sp.	Atheliales	LA082103L	AY654887	AY635779
<i>Fomitiporia mediterranea</i>	Hymenochaetales	3/22.7	AY662664	AY684157
<i>Galerina marginata</i>	Agaricales	PBM 2518	DQ440635	DQ457669
<i>Gautieria othii</i>	Gomphales	REG636	AF393043	AF336249
<i>Gloeophyllum sepiarium</i>	Gloeophyllales	DAOM 137861	AF026608	AF393059
<i>Hydnum albidum</i>	Cantharellales	MB11–6024/2	AY293135	AY293186
<i>Hydnum rufescens</i>	Cantharellales	MB18–6024/1	AY293136	AY293187
<i>Hygrophoropsis aurantiaca</i>	Boletales	MB 03–127	AY662663	AY684156
<i>Hygrophorus flavodiscus</i>	Agaricales	PBM 2509	DQ444860	AY635769
<i>Hygrophorus pudorinus</i>	Agaricales	PBM 2721	DQ444861	DQ457678
<i>Junghuhnia subundata</i>	Polyporales	LR-38938	AF518582	AF518625
<i>Kuehneromyces rostratus</i>	Agaricales	PBM 2703	DQ457624	DQ457684
<i>Lachnella villosa</i>	Agaricales	CBS 609.87	AY705959	DQ097347
<i>Laetisaria fuciformis</i>	Corticiales	NJ-2 Jackson	AY293139	AY293192
<i>Lepidostroma akagerae</i> (1)	Lepidostromataceae *	holotype (BR)	FJ171729	FJ171733
<i>Lepidostroma akagerae</i> (2)	Lepidostromataceae *	Ertz 7673 et al. (BR)	FJ171730	FJ171734
<i>Lepidostroma calocerum</i> (1)	Lepidostromataceae *	R05 (F)	FJ171727	FJ171737
<i>Lepidostroma calocerum</i> (2)	Lepidostromataceae *	Egan 18705 (OMA)	FJ171728	FJ171738
<i>Lepidostroma rugaramae</i> (B)	Lepidostromataceae *	Ertz 8544 et al. (BR), basidiocarp	FJ171731	FJ171735
<i>Lepidostroma rugaramae</i> (S)	Lepidostromataceae *	Ertz 8544 et al. (BR), squamules	FJ171732	FJ171736
<i>Leptosporomyces galzinii</i>	Atheliales	KHL 11079 (GB)	—	EU118642
<i>Multiclavula corynoides</i>	Cantharellales	Lutzoni 930804–2	—	U66440
<i>Multiclavula mucida</i>	Cantharellales	DSH96–056	AF026613	AF287875
<i>Paxillus vernalis</i>	Boletales	MB-062	AY662662	AY645059
<i>Piloderma byssinum</i>	Atheliales	KHL8456	—	AY586699
<i>Piloderma lanatum</i>	Atheliales	JS24861	—	AY586700
<i>Piloderma olivaceum</i>	Atheliales	EL 34/99 (GB)	—	DQ469289
<i>Plicaturopsis crispa</i>	Agaricales	FP-101310-Sp.	AY293148	AY293203
<i>Polyporoletus sublividus</i>	Russulales	DAOM 221078	AF287840	AF393066
<i>Polyporus squamosus</i>	Polyporales	FPL-6846	AF026573	AF393069
<i>Pterula echo</i>	Agaricales	DJM 302S58	DQ092911	AY629315
<i>Sarcodon imbricatus</i>	Thelephorales	REG Sim1	AY293157	AF518646
<i>Serpula himantioides</i>	Boletales	P99	AJ440948	AJ440943
<i>Sistotrema eximum</i>	Cantharellales	Thorn429	AF334935	AF393076
<i>Stephanospora caroticolor</i>	Agaricales	IOC-137/97	AF518591	AF518696
<i>Thelephora</i> sp.	Thelephorales	DSH 96–010	AF026627	AF287890
<i>Tylospora asterophora</i> (1)	Atheliales	KHL8566	—	AY463480
<i>Tylospora asterophora</i> (2)	Atheliales	lw79 (TUB)	—	AF325323
<i>Xerocomus chrysenteron</i>	Boletales	TDB-635	M94340	AF071537

^a Order names are based on Matheny et al. (2006) and Larsson (2007).

^b The family without order affiliation is labeled Lepidostromataceae*.

C = 0.20, G = 0.28, T = 0.26), a rate matrix of substitutions (A-C = 0.95, A-G = 3.59, A-T = 1.24, C-G = 0.92, C-T = 7.78, G-T = 1.0), proportion of invariable sites = 0.55, and α = 0.55. Bayesian runs converged after 400000 generations and 44700 trees were used to

compute posterior probability values. The topology of the Bayesian consensus tree was fully consistent with that of the ML tree.

Sequences representing the four Rwandan specimens were found to combine with those of *Lepidostroma calocerum* in a

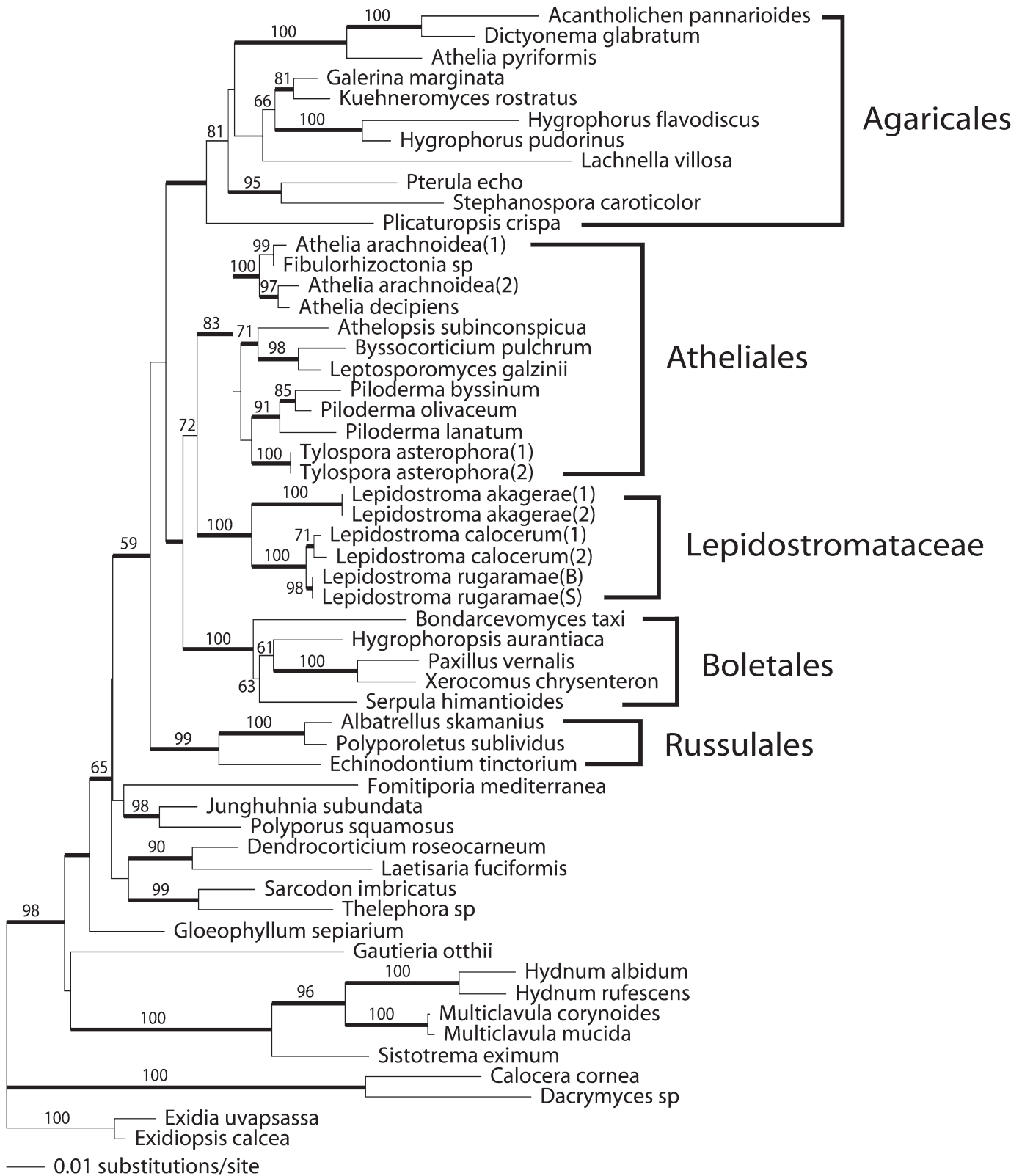


Fig. 2. Phylogenetic relationship of *Lepidostroma* species and their relatives to other fungi in the Agaricomycetes inferred from nuclear small and large subunit rDNA sequences using maximum likelihood. Branches in boldface indicate posterior probability values >0.95, maximum likelihood bootstrap support values (in %) are provided along nodes. 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).

well-supported clade (PP = 100; ML-bs = 100) that we are calling the ‘Lepidostromataceae’. Two well-supported sister groups were revealed within the Lepidostromataceae. One is represented by two specimens of *L. calocerum* and one specimen of *L. rugaramae*, and the second one by two specimens of *L. akagerae*. The Lepidostromataceae is sister to a well-supported clade represented by members of the Atheliales. The ‘Lepidostromataceae’ + Atheliales clade has only moderate support (PP = 63; ML-bs = 72) and is sister to a well-supported clade (PP = 100; ML-bs = 100) represented by various members of the Boletales. The Lepidostromataceae + Atheliales + Boletales are sister to representatives of the Agaricales. Relationships within the Agaricales are well supported (PP almost always \geq 95; MP-bs often \geq 97). Except for the new group containing *Lepidostroma* spp., all these sister-group relationships are similar to those obtained by Larsson et al. (2004), Binder and Hibbett (2006), Matheny et al. (2006), and Lawrey et al. (2007).

Representatives of the more basal remaining groups were generally well supported. The Cantharellales, with five species, including the lichenized *Multiclavula corynoides* and *M. mucida*, form a monophyletic and quite well-supported group (PP = 100; ML-bs = 100) at the base of the tree. The relationships between these taxa are well resolved and supported. Different smaller groups are present between the Cantharellales and the Russulales, including the Gomphales, the Gloeophyllales, and the Hymenochaetales with one species each, the Thelephorales, the Corticiales, and the Polyporales with two species each. The relationships between them are poorly resolved. The Russulales, including three species from different genera, form a monophyletic well-supported group (PP = 98; ML-bs = 99).

Taxonomy—*Family*—Lepidostromataceae Ertz, Eb. Fisch., Killmann, Sérus. & Lawrey fam. nov.

Mycobank no.—MB 512318

Type species—*Lepidostroma calocerum* (G. W. Martin) Oberw.; *Basionym*: *Clavaria calocera* G. W. Martin, Lilloa 5: 196 (1940).

Diagnosis—Fungi lichenisati. Thalli squamulosi, cortex ex strato singuli cellularum in visione frontali forma profunde lobata. Photobiontes chlorococcoidei. Basidiomata clavariiformes, simplices. Basidia subclavata, clavato-cylindriformes, 4 sterigmatis instructis. Sporae hyalinae, tenuitunicatae, guttulateae et planae.

Specimens examined and sequenced—Costa Rica, Coto Brus County, Las Cruces Biological Station, on exposed roadside soilbank, 8°47'N, 82°57'W, 1200 m a.s.l., ix, 2007, *R. Lücking* R05 (F); Mexico, Estado de Veracruz, Sierra Madre Oriental south of Xico above the Coyopolan River, on roadside rocks and trees, 19°25'01"N, 97°00'44"W, 1308 m, viii, 2007, *R. Egan* 18705 (OMA).

New combination—*Lepidostroma akagerae* (Eb. Fisch., Ertz, Killmann & Sérus.) Ertz, Eb. Fisch., Killmann, Sérus. & Lawrey comb. nov.

Mycobank no.—MB 512321

Basionym—*Multiclavula akagerae* Eb. Fisch., Ertz, Killmann & Sérus., Bot. Journ. Linn. Soc. 155: 458 (2007).

Specimens examined and sequenced—Rwanda, province Kibungo, Akagera National Park, foot of Mt. Mutumba, open savannas and wooded gallery thickets along a small intermittent river, on open lateritic soil in burnt savanna, 01°38'51.6"S, 30°39'53.7"E, 1450 m a.s.l., iv.2005, *D. Ertz* 8556, *E. Fischer*,

D. Killmann & E. Sérusiaux (holotype: BR); ibidem, prov. Butare, Butare, IRST park with isolated trees on regularly cut meadows and plantations, on earth embankment. 02°37'0.20"S, 29°44'0.45"E, c. 1690 m a.s.l., iii.2005, *D. Ertz* 7673, *E. Fischer*, *D. Killmann & E. Sérusiaux* (BR).

New combination—*Lepidostroma rugaramae* (Eb. Fisch., Ertz, Killmann & Sérus.) Ertz, Eb. Fisch., Killmann, Sérus. & Lawrey comb. nov.

Mycobank no.—MB 512322

Basionym—*Multiclavula rugaramae* Eb. Fisch., Ertz, Killmann & Sérus., Bot. Journ. Linn. Soc. 155: 461 (2007).

Specimen examined and sequenced—Rwanda, province Kibungo, quartzitic outcrops at Nyarubuye with scattered trees and vegetation, on soil, 2°08'54.0"S, 30°44'44.1"E, 1800 m a.s.l., iv.2005, *D. Ertz* 8544, *E. Fischer*, *D. Killmann & E. Sérusiaux* (BR).

To summarize the morphological diagnostic differences between the species of *Lepidostroma*, we present an identification key at the end of the discussion.

DISCUSSION

The most striking result of this study was the extent of the phylogenetic gulf between the three species of *Lepidostroma* and the core group of *Multiclavula* species [type species: *M. corynoides* (Peck) R. H. Petersen] (Fig. 2). Although morphological convergence is relatively common in the Agaricomycetes, finding that the basidiolichens forming coral-like fruiting structures are not monophyletic was entirely unexpected. To our knowledge, this is the only case of two unrelated basidiolichen clades with morphologically indistinguishable fruiting structures. Even more remarkable is the finding that they form a distinct, strongly supported lineage apparently related to the Atheliales and the Boletales, two orders that do not contain obligate lichens or fungi with coral-like basidiocarps. This lineage is so distinct and strongly supported within the Agaricomycetes that we consider it to represent a new family consisting entirely of basidiolichens, which we are calling the Lepidostromataceae. Our phylogeny indicates that this family represents an origin of lichens separate from those of other recognized basidiolichens, especially those in the Agaricales, Cantharellales, and Corticiales, which appear to have evolved independently from different non-lichenized ancestors.

The species here assigned to *Lepidostroma* are characterized by typical coral-like basidiocarps with hymenium tissue covering the whole upper part of the basidiocarps and a permanent thallus composed of squamules with a distinct cortex (Fig. 1A–C). The cortex is composed of a single layer of cells that have a deeply lobate shape in front view. The basidia are subclavate in *L. akagerae*, subclavate or clavate in *L. rugaramae*, or clavate-cylindrical in *L. calocerum*, and the spores are ovoid, usually with a distinct eccentric apiculus, colorless, thin-walled, and guttulate (Mägdefrau and Winkler, 1967; Oberwinkler, 1970, 2001; Fischer et al., 2007).

The members of the Lepidostromataceae are very similar to the genus *Multiclavula* (Fig. 1D, E), with coral-like basidiocarps, a simple, monomitic hyphal system, septa with clamps, a dense subhymenium, and a thickening hymenium (Oberwinkler, 2001). Petersen (1967) described the genus *Multiclavula* and recognized 13 species including *Clavaria calocera* (= now

Lepidostroma calocerum). However, according to Oberwinkler (2001), the basidia of *Lepidostroma* are not suburniform and 4–6(–8)-spored as in *Multiclavula*, but subclavate to clavate-cylindrical and always four-sterigmate. *Multiclavula ichthyiformis* (Nelsen et al., 2007) differs from other species assigned to *Multiclavula* in several important characters, including the shape and structure of the basidiocarps and in the globose rather than ellipsoid basidiospores. It also has the extranumerary (4–6) stigmata, suburniform basidia, and the lichenized bulbils characteristic of the genus *Multiclavula* (Poelt and Obermayer, 1990) suggesting that those characters might be more important to characterize the genus.

The thallus of *Multiclavula* species (especially the generic type: *M. corynoides*) is a gelatinous film with flattened to globular structures, either distinct or aggregated, containing coccomyxoid algae (Fig. 1D, E). This thallus type differs from the squamules of the three members of the Lepidostromataceae by the photobiont (coccomyxoid in *Multiclavula* and chlorococcoid in *Lepidostroma*), the absence of a distinct cortex with deeply lobate cells, and the squamule size (less than 100 μm in *Multiclavula*; 200–500 μm in *L. akagerae*; 1.5–2 mm in *L. rugaramae*; 0.5–1.5 mm in *L. calocerum*). Such relatively minor differences in photobiont and thallus structure usually do not warrant family or even generic distinction in basidiomycetes. Indeed, in omphalinoid lichenized species (*Omphalina* Quél. s.l.), the *Botrydina*-type thallus (Fig. 1F) is composed of spherical glomerules with a pseudoparenchymatous outer layer, whereas the *Coriscium*-type thallus (Fig. 1G) is composed of flattened, tiny and fragile, corticated squamules. Molecular studies of the genus *Omphalina* as a whole (Lutzoni and Vilgalys, 1995; Lutzoni, 1997), clearly demonstrated that the lichenized species with *Botrydina*- and *Coriscium*-type thalli (now assigned to the separate genus *Lichenomphalia*) form a well-supported monophyletic clade. However, the situation is not analogous to the group *Lepidostroma* + *Multiclavula*, as both structures in the omphalinoid lichenized species enclose coccomyxoid algae, whereas different photobionts are present in *Lepidostroma* (chlorococcoid) vs. *Multiclavula* (coccomyxoid).

Together, our results therefore provide strong support for two notions that are now being discussed widely in mycology: (1) that fruit body types in Basidiomycota are much more plastic than implied by traditional classifications (Hibbett and Thorn, 2001; Moncalvo et al., 2002; Nelsen et al., 2007; Hibbett, 2007), and (2) that thallus forms must be used with great care in lichen taxonomy, as demonstrated in many ascomycetous lichens (Grube and Hawksworth, 2007).

Interestingly, the two African species of *Lepidostroma* do not cluster together separate from the neotropical species, as might be expected on geographical grounds. Indeed, the phylogenetic analysis shows that *L. rugaramae* is sister to *L. calocerum*, which is presently known only from the neotropics. These two species also share a similar thallus morphology consisting of large and conspicuous squamules, whereas *L. akagerae* has much smaller ones. However, many tropical biomes, especially in Africa, are poorly studied and the habitat of both African species (on soil in regularly burnt savannas or laterite soils) is rarely studied by lichenologists. It is therefore premature to propose final conclusions, but the African origin of the genus is a hypothesis that should be tested in the future.

The polyphyly of the genus *Athelia* is here confirmed, as the species do not always cluster with members of the Atheliales, which we sampled extensively in our analyses. *Athelia arachnoidea* and *A. decipiens* are nested within the Atheliales, but

A. pyriformis is sister to the group in the Hygrophoraceae represented by the basidiolichens *Acantholichen pannarioides* (Fig. 1H) and *Dictyonema glabratum*, suggesting a possible non-lichenized ancestral condition for *Dictyonema*, a large tropical group of cyanolichens (J. Lawrey et al., unpublished manuscript). However, little is known about the biological status of *A. pyriformis*, making it premature to be overly speculative about its phylogenetic position or evolutionary significance in the clade. In his study of the phylogenetic distribution of corticioid fungi, Larsson (2007) discussed the confusing taxonomic history of *A. pyriformis*, which has been assigned to three different genera at various times in the past, on each occasion illustrated again and again (Christiansen, 1960; Oberwinkler, 1965; Jülich, 1972). Larsson (2007) suggested that the best solution is to establish a separate genus in the Hygrophoraceae for this taxon.

Given the separate phylogenetic position of the *Lepidostroma* group in the Agaricomycetes, it is difficult to infer its ancestral condition at this point. Basal relationships among the Agaricomycete lineages, including our new family, are still poorly resolved using sequences of ribosomal genes. However, as new data are collected and these relationships are clarified, it may be possible to investigate more thoroughly the apparent convergence of characters in many unrelated basidiolichens. For example, production of lichenized granules/squamules and *Multiclavula*-like fruiting structures appears to have evolved separately at least twice (Cantharellales and Lepidostromataceae). Basidiolichen lineages also commonly have lichenicolous (lichen parasites) and/or sclerotial/bulbilliferous members, suggesting possible preexisting conditions for the evolution of the lichen habit (Lawrey et al., 2007). We expect that a combined approach involving phylogenetic analysis of additional representatives of basidiolichen lineages and more thorough study of the structure, ecology, and reproductive biology of existing forms will help to elucidate both the unique pathways to lichenization taken by each lineage and the common ancestral conditions that make such transitions possible.

Identification key to the basidiolichens that form coral-like fruiting structures—

1. Thallus made of a gelatinous film, sometimes with flattened to globular structures less than 100 μm in diameter; cortex with deeply lobate cells lacking; photobiont coccomyxoid; basidia suburniform *Multiclavula* (see identification key in Petersen [1967])

1. Thallus made of distinct squamules of 200 μm to 2 mm in diam.; distinct cortex with deeply lobate cells present; photobiont chlorococcoid; basidia subclavate to clavate-cylindrical **2** [*Lepidostroma*]

2. Thallus composed of discrete, turgescens, and slightly convex squamules, contiguous or irregularly lobulate, up to 0.5 mm in diameter **L. akagerae**

2. Thallus composed of dispersed, rounded to reniform squamules, 0.5–2 mm in diameter **3**

3. Squamules reniform to deeply lobate, rarely rounded, without swollen whitish margin, upper surface dark green, without any paler spots or maculae, 0.5–1.5(–2.5) mm in diameter; algal cell layer in the lower part of medulla, never forming pyramidal columns projecting upward **L. calocerum**

3. Squamules rounded, with a conspicuous raised and swollen margin at least when young, upper surface pale to deep green with conspicuous paler spots or maculae, 1.5–2 mm in diameter; algal cell layer in the lower part of medulla and projecting upwards in pyramidal columns **L. rugaramae**

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