

A natural classification of *Lasiosphaeria* based on nuclear LSU rDNA sequences

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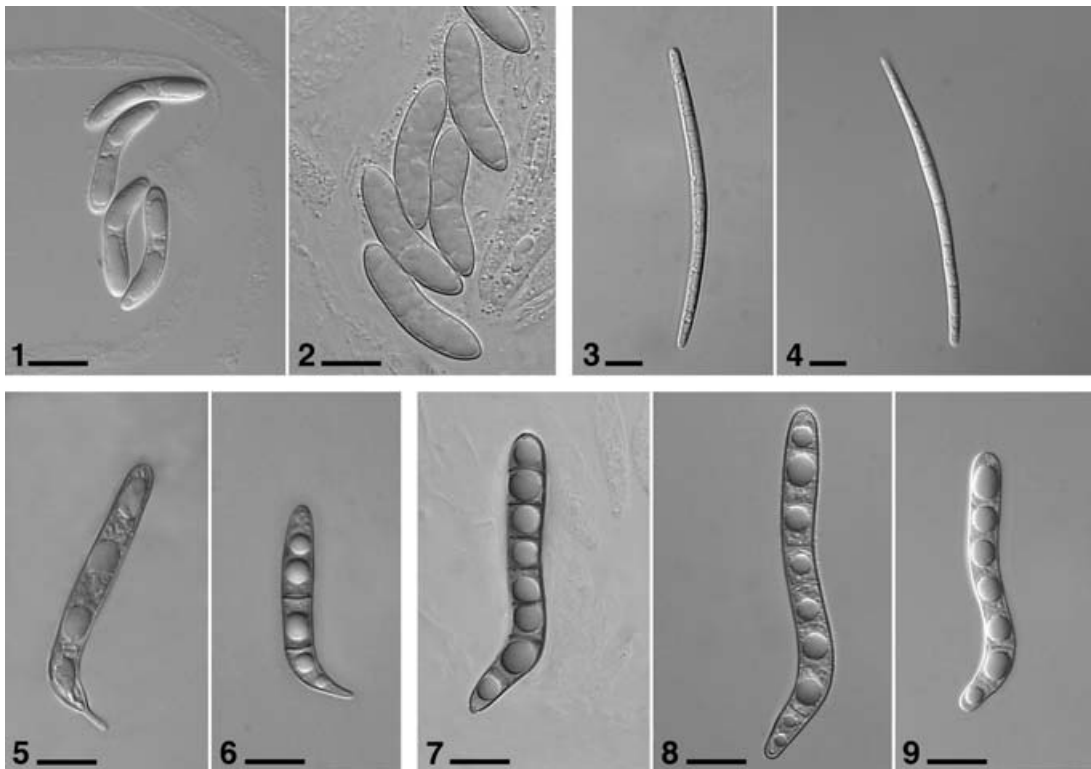
The current circumscription of *Lasiosphaeria* includes taxa with a wide variety of ascomatal walls, ascomatal wall vestitures, and ascospore morphologies and a broad range of putative anamorphs. Despite the complexity of morphological characters in the genus, species within *Lasiosphaeria* can be arranged into four groups based on ascospore morphology. Taxa which possessed ascospores in each of the four groups were used in phylogenetic analyses of partial nuclear large subunit (LSU) rDNA sequences to test the monophyly of the genus and determine relationships among its species. *Lasiosphaeria* was found to be highly polyphyletic in that species segregated into seven well-supported monophyletic clades dispersed among several orders. Three new genera, *Echinosphaeria*, *Hilberina*, and *Immersiella*, are erected for three of these clades while the genus *Lasiosphaeris* is reintroduced for a fourth clade. These data support *Ruzenia* as a previously established genus and the transfer of *Lasiosphaeria raciborskii* to *Chaetosphaeria*. The circumscription of *Lasiosphaeria* has been considerably narrowed to better reflect a natural classification. These taxonomic changes are additionally supported by a combination of morphological characters which are discussed in relation to the phylogenetic trees.

INTRODUCTION

The genus *Lasiosphaeria* is one of the most speciose in the *Sordariales*. It is composed of primarily lignicolous taxa which are commonly found throughout temperate regions. The genus was originally based on seven species along with five additional species, which were presumably considered to belong in the genus, but were not transferred (Cesati & De Notaris 1863). Four of these species (*L. ovina*, *L. hirsuta*, *L. rhacodium*, and *L. canescens*) currently remain in the genus. The history of *Lasiosphaeria* and its synonyms is quite complex and has already been elaborately discussed by Lundqvist (1972), who also explained why *L. ovina* should be recognized as its type species. Since its conception, the genus has continually been expanded to include an array of taxa with a broad range in ascomatal walls, ascomatal wall vestitures, and ascospore morphologies. *Lasiosphaeria* currently contains over 180 names (Reed & Farr 1993; *Index of Fungi*), most of which probably do not belong, and a monographic study to revise all type material is presently underway (Miller & Huhndorf, unpubl.). The generic concept in its broadest sense contains taxa with large, membranous ascomata that are immersed to erumpent, or superficial, globose

to obpyriform, glabrous, tomentose, villose, or setose, and possess an outer ascomal wall that is smooth or tuberculate. Ascospores can be hyaline to yellowish and usually turn pale brown with age, are allantoid, reniform, cylindrical, filiform, or fusiform, may bend or taper to a point in the lower third, are aseptate to multi-septate, and may possess hyaline, gelatinous appendages or mucilaginous tips at one or both ends. Species of *Lasiosphaeria* are also associated with a broad range of anamorphs ranging from *Phialophora* in *L. hirsuta* (Hughes 1951, Gams & Holubová-Jechová 1976), *L. hispida* (Gams 2000), and *L. ovina* (Gams & Holubová-Jechová 1976), to *Endophragmiella* and *Selenosporella*-like synanamorphs associated with *L. canescens* and *L. punctata* (Hughes 1979, Sivanesan 1983), to an unidentified anamorph with blastic-annellidic conidiogenesis associated with *L. dactylina* (Hilber, Webster & Hilber 1987).

The generic concept of *Lasiosphaeria* has traditionally been overly broad and several workers have discussed the complexity of the genus (Seaver 1912, Munk 1957, Carroll & Munk 1964, Lundqvist 1972, Hilber & Hilber 1979, Candoussau, Fournier & Magni 2001, Hilber & Hilber 2002). Although definite criteria for placing taxa into *Lasiosphaeria* have never been



Figs 1–9. Representative species of *Lasio-sphaeria* s. lat. divided into four groups (A–D) based on ascospore morphology: Figs 1–2 = Group A; Figs 3–4 = Group B; Figs 5–6 = Group C; Figs 7–9 = Group D. Bars = 10 µm. **Fig. 1.** *Ruzenia spermoides* (SMH4606). **Fig. 2.** *Echinosphaeria canescens* (SMH2627). **Figs 3–4.** *Chaetosphaeria raciborskii* (SMH2499, SMH2132). **Figs 5–6.** *Hilberina caudata* (SMH3156, SMH3918). **Fig. 7.** *Immersiella immersa* (SMH4104). **Fig. 8.** *Lasio-sphaeria hispida* (SMH3336). **Fig. 9.** *Lasio-sphaeria ovina* (SMH1538).

established (Carroll & Munk 1964, Candoussau *et al.* 2001), taxa have generally been added or transferred into the genus based on overall similarities in their ascomatal walls, ascomatal wall vestitures, and/or ascospore morphologies. Carroll & Munk (1964) pointed out that ‘radically different spore types may occur together with one and the same peridium type’. However, despite the wide range of ascospore types currently accepted in *Lasio-sphaeria*, taxa can be arranged into four generalized groups (Figs 1–9, Groups A–D) based on their ascospore morphologies as was previously shown by Candoussau *et al.* (2001). Although several species have been transferred to other genera over the years (*cf.* Candoussau *et al.* 2001), a number of new species with a variety of ascomal and ascospore morphologies have recently been added (Réblová 1997, Vasilyeva 1998, Candoussau *et al.* 2001, Taylor *et al.* 2001). The circumscription of *Lasio-sphaeria* will continue to expand and become increasingly heterogeneous as additional taxa are added until a clear delimitation is provided. Therefore, the objective of this study was to use an independent data set in the form of partial nuclear LSU rDNA sequences to: (1) test the monophyly of *Lasio-sphaeria*; (2) assess relationships among species of *Lasio-sphaeria* possessing different ascomal, ascospore, and anamorph morphologies; and (3) determine which morphological

characters are phylogenetically informative for delimiting *Lasio-sphaeria*.

MATERIALS AND METHODS

Taxon sampling

Taxa sequenced in this study are listed in Table 1 along with their source information, geographical locality, and GenBank accession number. Representatives from several families and orders within the *Sordariomycetes* were included to determine the phylogenetic position of putatively polyphyletic taxa currently included in *Lasio-sphaeria*. Four members of the *Xylariales*, *Daldinia concentrica*, *Diatrype disciformis*, *Eutypa sp.*, and *Xylaria hypoxylon*, were used as outgroups. All voucher specimens are deposited in the Field Museum Mycology Herbarium (F). Additional taxa obtained from GenBank are listed in Table 2. Cultures of multispore isolates were obtained following the techniques of Huhndorf, Miller & Fernández (2004). Ascomata were squash-mounted in water and images of micromorphological structures were captured with a Dage DC-330 video system mounted on a Zeiss Axioskop microscope using differential interference (DIC) microscopy. Images were processed using Adobe Photoshop 3.0 or 5.0 (Adobe Systems, Mountain View, CA).

Table 1. Taxa sequenced in this study.

Taxon	Source ¹	Geographical locality	GenBank accession nos.
<i>Chaetosphaeria raciborskii</i>	SMH3119	Puerto Rico	AY436402
<i>Echinosphaeria canescens</i>	SMH4791 (a) ²	Indiana	AY436403
<i>E. canescens</i>	TL5730 (a)	Denmark	AY436404
<i>Hilberina caudata</i>	SMH2107	Puerto Rico	AY436405 ³
<i>H. caudata</i>	SMH3918	Michigan	AY436406
<i>Immersiella caudata</i>	SMH3298	North Carolina	AY436407 ³
<i>I. immersa</i>	SMH2589	Michigan	AY436408 ³
<i>I. immersa</i>	SMH4104	Wisconsin	AY436409
<i>Lasiosphaeria glabrata</i>	TL4529 (a)	Denmark	AY436410
<i>L. glabrata</i>	SMH4617	Denmark	AY436411
<i>L. lanuginosa</i>	SMH3819	North Carolina	AY436412
<i>L. ovina</i>	SMH4605	Denmark	AY436413
<i>L. rugulosa</i>	SMH1518	Puerto Rico	AY436414 ³
<i>L. sorbina</i>	GJSL555	Louisiana	AY436415 ³
<i>L. sorbina</i>	CBS885.85	Germany	AY436416
<i>Lasiosphaeria hirsuta</i>	SMH1543	Wisconsin	AY436417 ³
<i>L. hirsuta</i>	JF02183 (a)	France	AY436418
<i>L. hispida</i>	SMH3336	North Carolina	AY436419 ³
<i>L. hispida</i>	TL6019	Denmark	AY436420
<i>Ruzenia spermoides</i>	CBS101621	Czech Republic	AY436421
<i>R. spermoides</i>	SMH4606	Denmark	AY436422

¹ Abbreviations: CBS, Centraalbureau voor Schimmelcultures; GJS, Gary J. Samuels; JF, Jacques Fournier; SMH, Sabine M. Huhndorf; TL, Thomas Læssøe.

² (a) = DNA extracted from ascomata; all others were extracted from cultures.

³ For these taxa, although 1100 bp were used in the analyses, 1300 bp were sequenced and deposited in GenBank.

DNA extraction, PCR amplification, sequencing and sequence alignment

Detailed protocols for the extraction, amplification, and sequencing of DNA and methods for the alignment of LSU sequences are fully described in Huhndorf *et al.* (2004).

Phylogenetic analyses

Equally and unequally weighted maximum parsimony (MP) analyses were performed using PAUP* 4.0b10 (Swofford 2002). Thirteen ambiguously aligned regions were delimited and characters in these regions along with two introns, portions of the 5' and 3' ends, and constant characters were excluded from all MP analyses. Characters were unordered, gaps were treated as missing, 1000 random-addition replicates were implemented with TBR branch-swapping, MULTREES option was in effect, and zero-length branches were collapsed in the equally weighted analyses. Unequally weighted analyses were conducted in which changes among transitions, transversions, and gaps were

subjected to a symmetric stepmatrix generated using STMatrix ver. 2.2 (François Lutzoni & Stefan Zoller, Biology Department, Duke University). This program calculates the costs for changes among these character states based on the negative natural logarithm of the percentages of reciprocal changes between any two character states. The phylogenetic signal from twelve of the thirteen ambiguous regions was recovered using INAASE (Lutzoni *et al.* 2000) and also analyzed in the unequally weighted analyses. The remaining ambiguous region was excluded because its recoded character contained more than 32 character states, which is not allowed in PAUP* 4.0b10. Branch support for all MP analyses was estimated by performing 1000 bootstrap replicates (Felsenstein 1985) with a heuristic search consisting of 100 random addition replicates for each bootstrap replicate using the above settings.

The best-fit model of evolution was determined by MODELTEST 3.06 (Posada & Crandall 1998). While maximum likelihood analyses were not computationally possible due to the large size of the data set, analyses of Bayesian inference were performed using MrBayes 3.0b4 (Huelsenbeck & Ronquist 2001) (<http://morphbank.ebc.uu.se/mrbayes3>) as an additional assessment of branch support. Constant characters were included, the above model of evolution was implemented, and 10 million generations were sampled every 1000th generation resulting in 10 000 total trees. The first 2000 trees, which represented the burn-in phase of the analysis, were discarded. Posterior probabilities were determined from a consensus tree generated using the remaining 8000 trees. This analysis was repeated five times starting from different random trees to insure trees from the same tree space were being sampled during each analysis.

RESULTS

Sequence alignment

The final alignment included 77 taxa and 1306 bp. The first 59 bp of the 5' end and the last 17 bp of the 3' end were excluded from all analyses due to missing data in most taxa. Thirteen ambiguous regions representing 222 characters were also excluded from all analyses due to uncertainty in the alignment. Two taxa (*Lasiosphaeria caudata*, *Linocarpon appendiculatum*) possessed spliceosomal introns (56 bp and 65 bp, respectively), which contained the highly conserved donor, branch and acceptor sites known to occur in these introns in ascomycetes (Bhattacharya *et al.* 2000). Of the remaining 887 characters, 486 were constant and 96 were parsimony-uninformative for a total of 305 parsimony-informative characters in the equally weighted analyses. Twelve additional parsimony-informative characters derived from the unequivocally-coded ambiguous regions were also included in the unequally weighted analyses.

Table 2. Sequences used in this study obtained from GenBank.

Taxon	GenBank accession nos.
<i>Apiosordaria verruculosa</i>	AY346258
<i>Aporothielavia leptoderma</i>	AF096186
<i>Bombardia bombardia</i>	AY346263
<i>Bombardioidea anartia</i>	AY346264
<i>Camarops petersii</i>	AY346265
<i>C. tubulina</i>	AY346266
<i>C. ustulinoides</i>	AY346267
<i>Caudatispora biapiculata</i>	AY346269
<i>Ceratocystis virescens</i>	CVU47824
<i>Cercophora aff. mirabilis</i>	AY346271
<i>Chaetomium globosum</i>	AF286403
<i>C. microascoides</i>	AY346273
<i>Chaetosphaerella phaeostroma</i>	AY346274
<i>Chaetosphaeria innumera</i>	AY017375
<i>C. ovoidea</i>	AF064641
<i>C. raciborskii</i>	AF466078
<i>Coniochaeta ligniaria</i>	AY346275
<i>Coniochaetidium savoryi</i>	AY346276
<i>Copromyces</i> sp.	AY346277
<i>Daldinia concentrica</i>	DCU47828
<i>Diaporthe phaseolorum</i>	AY346279
<i>Diatrype disciformis</i>	DDU47829
<i>Eutypa</i> sp.	AY346280
<i>Farrowia longicollea</i>	AF286408
<i>F. seminuda</i>	AF286410
<i>Gelasinospora tetrasperma</i>	AY346281
<i>Helminthosphaeria carpathica</i>	AY346282
<i>H. clavarium</i>	AY346283
<i>H. hyphodermatis</i>	AY346284
<i>Hypomyces luteovirens</i>	AF160237
<i>Jugulospora rotula</i>	AY346287
<i>Lasiosphaeria ovina</i>	AF064643
<i>Lasiosphaeriella nitida</i>	AY346289
<i>Leptospora gregaria</i>	AY346290
<i>Linocarpon appendiculatum</i>	AY346291
<i>Melanochaeta hemipsila</i>	AY346292
<i>Microascus trigonosporus</i>	MTU47835
<i>Nectriopsis violacea</i>	AF193242
<i>Neurospora crassa</i>	AF286411
<i>Nitschkia grevillii</i>	AY346294
<i>Petriella setifera</i>	PSU48421
<i>Plectosphaerella cucumerina</i>	PCU17399
<i>Podospora fimiseda</i>	AY346296
<i>Poroconiochaeta discoidea</i>	AY346297
<i>Rimaconus jamaicensis</i>	AF335326
<i>Schizoparme botrytidis</i>	AF408383
<i>Schizothecium curvisporum</i>	AY346300
<i>Sordaria macrospora</i>	AY346301
<i>Strattonia carbonaria</i>	AY346302
<i>Striatosphaeria codinaeaphora</i>	AF466088
<i>Thielavia cephalothecoides</i>	AF286413
<i>Triangularia mangenotii</i>	AY346303
<i>Valsa ceratosperma</i>	AF408387
<i>Xylaria hypoxylon</i>	XHU47841
<i>Zopfiella ebriosa</i>	AY346305
<i>Zygopleuroge zygospora</i>	AY346306

Phylogenetic analyses

The equally weighted analysis generated four equally MP trees, which differed only in the relationships among taxa in the *Chaetomiaceae* (data not shown). The unequally weighted analysis generated a single MP

tree (Fig. 10), which did not differ significantly from the trees produced in the equally weighted analysis. The best-fit model determined by MODELTEST was the TrN+I+G model (Tamura & Nei 1993). Therefore, Bayesian analyses were conducted with the number of rate categories set to six, rates set to invgamma, and all remaining parameters estimated from the default prior probabilities.

The genus *Lasiosphaeria* as currently circumscribed was found to be highly polyphyletic in that species segregated into seven distinct monophyletic clades dispersed throughout several orders (Fig. 10). As expected, species of *Lasiosphaeria* possessing ascospores in each of the four different groups segregated into different clades, which were scattered throughout the tree. In fact, even species possessing similar ascospore types (i.e. A and D) segregated into multiple clades. All clades were well supported by both high bootstrap support and significant Bayesian posterior probabilities (Fig. 10), except the clade containing *L. hirsuta* and *L. hispida*, which had low bootstrap support. The circumscription of *Lasiosphaeria* is considerably narrowed for the clade containing the type species of the genus, *L. ovina*. A new genus is proposed for the clade containing *Cercophora caudata* and *Lasiosphaeria immersa*, while the generic name *Lasiosphaeris*, is re-introduced to accommodate *L. hirsuta* and the type species of the genus, *L. hispida*. A new genus in the *Helminthosphaeriaceae* is proposed to accommodate *Lasiosphaeria canescens*. *Ruzenia* is accepted as a previously established genus, and a valid transfer of its type species is made below (Hilber & Hilber 2002). These data support the transfer of *Ruzenia* from the *Lasiosphaeriaceae* to the *Helminthosphaeriaceae*. *L. raciborskii* occurred in the well-supported *Chaetosphaeriales* clade and is transferred to *Chaetosphaeria*¹. A new genus is proposed for *L. caudata*, which occurred on a separate branch as a sister clade to the *Sordariomycetidae/Hypocreomycetidae* clade.

TAXONOMY

Echinosphaeria A. N. Mill. & Huhndorf, gen. nov.

Etym.: Refers to the spiny appearance of the ascomata, which are covered with thick-walled setae.

Ascomata superficialia, subglobosa vel ovoidea, setosae; setae brunnea, parietibus crassis; collum papillatum glabratum. Asci unitunicati, cylindrici, apice annulo refractili, iodo noncoerulescenti provisi, sine globulo sub-apicali. Ascosporae allantoideae, hyalinae vel pallide brunneae, sine appendicibus.

Ascomata superficial, subglobose to ovoid, covered with thick-walled, brown setae; neck papillate, glabrous.

¹ *Chaetosphaeria raciborskii* (Penz. & Sacc.) F. A. Fern. & Huhndorf, **comb. nov.** Basionym: *Ophiochaeta raciborskii* Penz. & Sacc., *Malpighia* 11: 406 (1897).

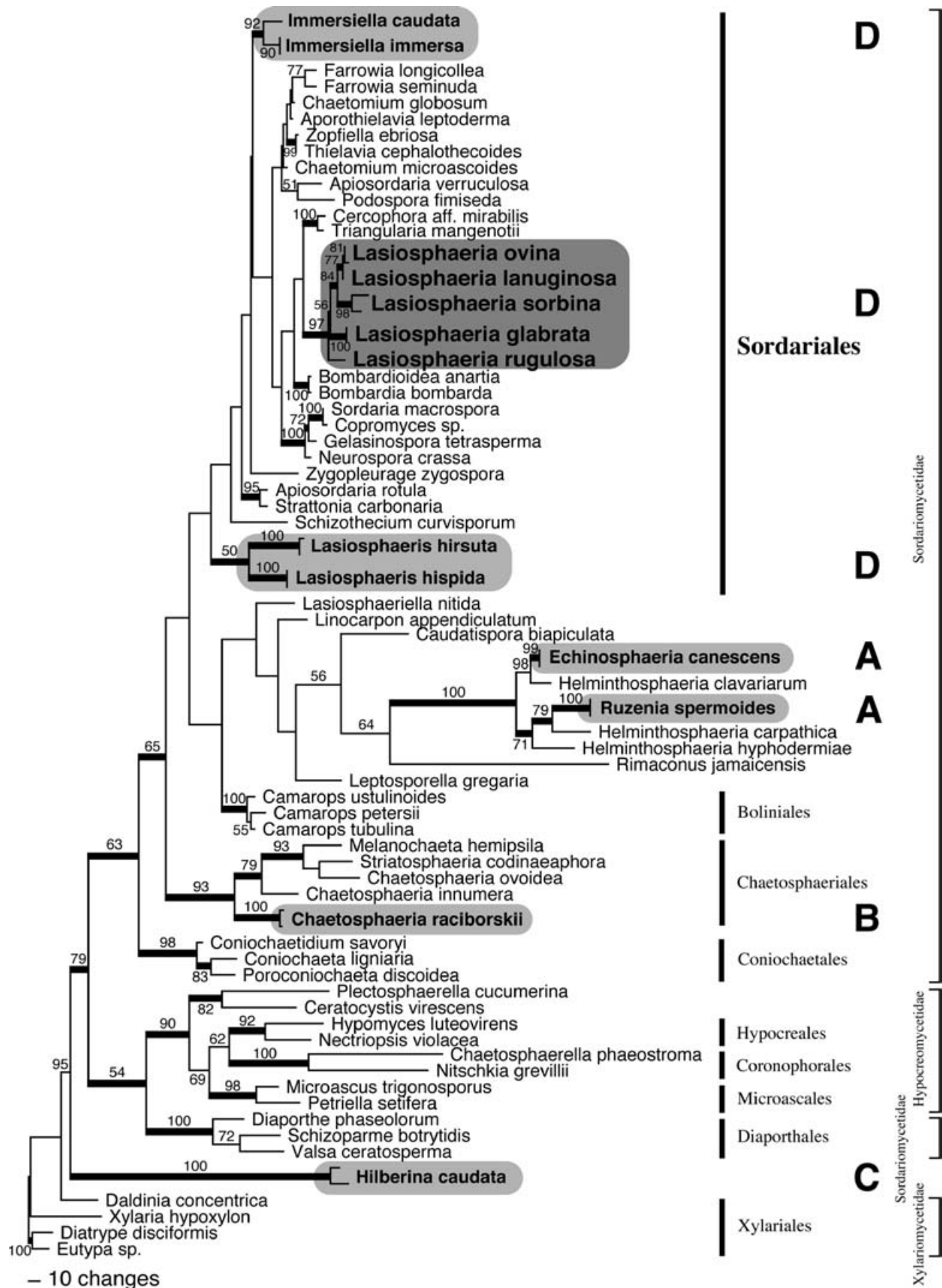


Fig. 10. Phylogram of the single most parsimonious (MP) tree generated from the unequally weighted analysis of 1306 bp of the 5' end of nuclear LSU rDNA for 77 ascomycete sequences; length = 3636.56 steps, CI=0.4, RI=0.671, RC=0.269. Bootstrap values $\geq 50\%$ are shown above or below branches. Thickened branches indicate posterior probabilities $\geq 95\%$. Taxa in *Lasiosphaeria* sensu lato are in bold in lightly shaded boxes while taxa retained in *Lasiosphaeria* sensu stricto are in larger font in a heavily shaded box. Subclass and order designations following Huhndorf *et al.* (2004) are given along the right side. Letters refer to the four ascospore groups (A–D) referred to in the text and Figs 1–9.

Asci unitunicate, cylindrical, with a refractive, inamyloid apical ring, subapical globule absent. *Ascospores* allantoid, hyaline, turning pale brown with age, without gelatinous appendages.

Type species: **Echinosphaeria canescens** (Pers: Fr.) A. N. Mill. & Huhndorf, **comb. nov.** (Basionym: *Sphaeria canescens* Pers., *Syn. Meth. Fung.*: 72, 1801).

Hilberina Huhndorf & A. N. Mill., **gen. nov.**

Etym.: Named in honour of Oswald Hilber.

Ascomata superficialia, subglobosa vel ovoidea, leviter tuberculata, setosae; setae brunnea, parietibus tenuibus vel crassis. *Asci* unitunicati, cylindrici, apice annulo refractili, iodo non-coerulescenti provisi, sine globulo sub-apicali. *Ascospores* cylindricae, geniculateae, cum extrema apiculata, hyalinae vel pallidae-brunneae, sine appendicibus.

Ascomata superficial, subglobose to ovoid, occasionally tuberculate, covered with thin- to thick-walled brown setae; neck papillate, glabrous. *Asci* unitunicate, cylindrical, with a refractive, inamyloid apical ring, subapical globule absent. *Ascospores* cylindrical, geniculate, one end tapering to a distinct point, hyaline, turning pale brown with age, without gelatinous appendages.

Type species: **Hilberina caudata** (Fuckel) Huhndorf & A. N. Mill., **comb. nov.** (Basionym: *Leptospora caudata* Fuckel, *Jahrb. Nassauischen Vereins Naturk.* 23–24: 144 (1870).

Immersiella A. N. Mill. & Huhndorf, **gen. nov.**

Etym.: Refers to the immersed nature of the ascomata, which are always found growing in very loose, porous wood.

Ascomata immersa, erumpentia, ovoidea vel obpyriformia, cum hyphae longae, flexuosae, brunneae; collum papillatum, longitudinaliter sulcatum, ex cellulis inflatis, parietibus crassis compositum. *Asci* unitunicati, cylindrici, apice annulo refractili, iodo noncoerulescenti provisi, cum globulo sub-apicali. *Ascospores* cylindricae, sigmoideae vel geniculateae, hyalinae vel pallide brunneae, cum vel sine appendice, cum vel sine cellula superiore inflata, brunnea.

Ascomata immersed becoming erumpent, ovoid to obpyriform, covered with flexuous, thin-walled, brown hairs below neck; neck papillate, usually sulcate, usually covered with short, thick-walled, swollen cells. *Asci* unitunicate, cylindrical, with a refractive, inamyloid apical ring and subapical globule. *Ascospores* cylindrical, sigmoid or geniculate, hyaline, turning pale brown with age, with or without gelatinous appendages, one end may become swollen and turn brown.

Type species: **Immersiella immersa** (P. Karst.) A. N. Mill. & Huhndorf, **comb. nov.** (Basionym: *Lasiosphaeria immersa* P. Karst., *Bid. Känn. Finlands Natur Folk* 23: 162 (1873).

Additional species: **Immersiella caudata** (Curr.) A. N. Mill. & Huhndorf, **comb. nov.** (Basionym: *Sphaeria caudata* Curr., *Trans. Linn. Soc. London* 22: 320 (1859).

Lasiosphaeria Ces. & De Not., *Comment. Soc. Crittog. Ital.* 1: 229 (1863).

Ascomata superficial, subglobose to ovoid, covered with variously colored tomentum below neck; neck indistinct to papillate, glabrous; ascomal contents yellow or rarely pinkish to orange. *Asci* unitunicate, cylindrical to cylindro-clavate, with a refractive, inamyloid apical ring and usually with a subapical globule. *Ascospores* cylindrical, sigmoid, geniculate, or curved, hyaline to yellowish, sometimes turning pale brown with age, with or without gelatinous appendages, one end may become swollen and turn brown.

Type species: *Lasiosphaeria ovina* (Pers: Fr.) Ces. & De Not. 1863.

Additional species: **Lasiosphaeria lanuginosa** (H. Crouan & P. Crouan) A. N. Mill. & Huhndorf, **comb. nov.** (Basionym: *Sordaria lanuginosa* H. Crouan & P. Crouan, *F. Fin.*: 22 (1867); **Lasiosphaeria rugulosa** (A. N. Mill. & Huhndorf) A. N. Mill. & Huhndorf, **comb. nov.** (Basionym: *Cercophora rugulosa* A. N. Mill. & Huhndorf, *Sydowia* 53: 215 (2001).

Lasiosphaeris Clem., *Gen. Fungi*: 173 (1909).

Ascomata superficial, subglobose to obpyriform, usually tuberculate, covered with thin- to slightly thick-walled, brown to black setae; neck indistinct to conical, usually sulcate, glabrous. *Asci* unitunicate, cylindrical, with a refractive, inamyloid apical ring and subapical globule. *Ascospores* cylindrical, sigmoid or geniculate, hyaline, turning pale brown with age, usually with short gelatinous appendages, one end may become swollen and turn brown.

Type species: *Lasiosphaeris hispida* (Tode: Fr.) Clem. 1909.

Additional species: **Lasiosphaeris hirsuta** (Fr.: Fr.) A. N. Mill. & Huhndorf, **comb. nov.** (Basionym: *Sphaeria hirsuta* Fr., *Syst. Mycol.* 2: 449 (1823).

Ruzenia O. Hilber, *Gen. Lasiosphaeria*: 7 (2002).

Ascomata superficial, subglobose to obovoid, slightly roughened, glabrous; neck indistinct. *Asci* unitunicate, cylindrical, with a refractive, inamyloid apical ring, subapical globule absent. *Ascospores* allantoid, hyaline, without gelatinous appendages.

Type species: **Ruzenia spermoides** (Hoffm.: Fr.) O. Hilber ex A. N. Mill. & Huhndorf, **comb. nov.** (Basionym: *Sphaeria spermoides* Hoffm., *Veg. Crypt* 2: 12 (1790). *Typus*: ‘Hannoverae leit?’, 1783, Herb. Ehrhart (GOET 211200015 – **lectotypus, hic designates**).

When Hilber & Hilber (2002) described *Ruzenia*, the new species combination was not correctly published because the authors failed to cite the place of original publication of the basionym. The type material they cited was also incorrect. The material selected here as lectotype was chosen from eight collections at GOET

under the name '*Sphaeria globosa*'. It was the only one with a putative date earlier than the date of publication and it was in good condition.

DISCUSSION

The genus *Lasiosphaeria* has been widely conceived to include a diverse array of taxa possessing a broad range of ascomatal walls, ascomatal wall vestitures, and ascospore morphologies. Over the years, several workers have attempted to divide this heterogeneous genus into more natural groups. The first was Fuckel (1870) who transferred species with aseptate ascospores (i.e. *L. ovina*, *L. spermoides*) into *Leptospora*, while retaining species with septate ascospores (i.e. *L. hirsuta*, *L. rhacodium*) in *Lasiosphaeria*. Von Höhnelt (1918) and Chenantais (1919) later used differences in ascomatal wall vestitures and ascospore morphologies, respectively, to delimit genera. Finally, Hilber & Hilber (2002) divided *Lasiosphaeria* into five sections based on differences in ascomatal walls, ascomatal wall vestitures, ascospore morphology, and anamorphs. Four of these sections correspond to genera recognized above.

As suggested by Candoussau *et al.* (2001), most species of *Lasiosphaeria* can be divided into one of four groups based on ascospore morphology (Figs 1–9, Groups A–D). Species in Group A possess ascospores which are short and allantoid to reniform (Figs 1–2), whereas ascospores in Group B are long and fusiform to filiform (Figs 3–4). Groups C and D contain taxa which possess similar ascospores that are cylindrical and sigmoid or geniculate. However, ascospores in Group C taper to a distinct point at one end (Figs 5–6), whereas those in Group D are rounded or taper only slightly (Figs 7–9). Species possessing each of these ascospore types segregated into several distinct well-supported monophyletic groups (Fig. 10). However, ascospore morphology alone proved to be phylogenetically uninformative for delimiting genera since species possessing ascospores in Groups A and D were polyphyletic.

It has recently been suggested that a more natural classification of *Lasiosphaeria* should be based on anamorphic data (Candoussau *et al.* 2001). *Lasiosphaeria hirsuta*, *L. hispida*, and *L. ovina* possess *Phialophora* anamorphs (Hughes 1951, Gams & Holubová-Jechová 1976, Gams 2000), while *C. raciborskii* possesses a *Conioscypha*-like anamorph (unpubl. data). *Endophragmiella* and *Selenosporella*-like synanamorphs have been associated with *E. canescens* and *L. punctata* (Hughes 1979, Sivanesan 1983) and Gams (1973) illustrated what appears to be a *Selenosporella*-like anamorph germinating from ascospores of *R. spermoides*. We have also observed a *Selenosporella*-like anamorph in several cultures of *R. spermoides* (data not shown). Finally, an unidentified anamorph with blastic-annellidic conidiogenesis has been associated with *L. dactylina* (Hilber *et al.* 1987). Although anamorphs are known or have been suggested for most species of

Lasiosphaeria included in this study, the majority of *Lasiosphaeria* spp. not represented in this study lack anamorphic data. Interestingly, species possessing each of the four different ascospore types also possess different anamorphs. However, as with ascospores above, anamorphs alone are not phylogenetically informative for delimiting taxa since species possessing similar anamorphs were polyphyletic.

A simple one-character taxonomy based on a single teleomorph or anamorph character cannot be used for delimiting these genera. While monophyly is the criterion used for delineating taxa in a phylogenetic context, establishing morphological character-based criteria for delimiting these genera is more complex and is based on a combination of teleomorph and/or anamorph characters as discussed below for each genus.

Species of *Lasiosphaeria* possessing similar ascospores in Group D segregated into three distinct well-supported clades within the Sordariales (Fig. 10). The first clade is *Lasiosphaeria*, which is much more narrowly defined by restricting it to the clade containing the type species of the genus, *L. ovina*. This clade contained members of *Lasiosphaeria* and *Cercophora*, genera which have traditionally been distinguished by their ascospore morphology (Lundqvist 1972). While species in both genera possess cylindrical ascospores, those in *Cercophora* eventually develop a swollen, pigmented head (Lundqvist 1972). However, as was first pointed out by von Höhnelt (1918) and later discussed by Lundqvist (1972), certain species of *Lasiosphaeria* and *Cercophora* possess similar ascomatal walls and vestitures. All five members of this clade possess similar ascomatal walls in which the outer wall layer is composed of hyphae (which constitutes the vestiture in this case). While the tomentose vestiture is well-developed and quite obvious in *L. lanuginosa*, *L. ovina*, and *L. sorbina*, it is reduced to a sparse covering of short, brown hairs in *L. glabrata* and *L. rugulosa* (Miller & Huhndorf 2001). These species also possess yellow (or rarely pinkish to orange) centrum pigments, and it is the combination of these ascomatal wall and centrum characters which distinguish *Lasiosphaeria* from all other genera. While *Cercophora coprophila* possesses a similar ascomatal wall with tomentose vestiture and *C. areolata* possesses a yellow centrum, neither belong in *Lasiosphaeria* since the centrum contents of the former are hyaline and the ascomatal wall of the latter is distinctly areolate and covered with long, flexuous, brown hairs. Both of these species occur within the Sordariales, but outside the *Lasiosphaeria* clade, in analyses of the LSU, β -tubulin, and RPB2 genes which included increased taxon sampling within the Sordariales (Miller & Huhndorf, unpubl.). *Cercophora sparsa* and *C. sulphurella* also possess similar ascomatal walls with tomentose vestiture and yellow centra. However, these species occur as unsupported sister taxa to the highly supported *Lasiosphaeria* clade in the expanded phylogenetic analyses discussed above (Miller & Huhndorf, unpubl.). Until these species are found

to be supported in the *Lasiosphaeria* clade, we take a conservative approach by not including them in the genus.

The new genus *Immersiella* was established for a second well-supported clade within the *Sordariales*. It is represented by two species, *I. immersa* and *I. caudata*, which also possess ascospores from Group D and *Cercophora*, respectively. However, *Immersiella* is distinguished from *Lasiosphaeria* and *Lasiosphaeris* in having immersed to erumpent ascomata covered with long, flexuous, brown hairs (Hilber & Hilber 1983). *Lasiosphaeria* and *Lasiosphaeris* have superficial ascomata covered with either tomentum or setae, respectively. *Immersiella* can also be distinguished from *Lasiosphaeria* in having hyaline centrum contents.

The genus *Lasiosphaeris* is reintroduced for the third clade in the *Sordariales*. It is represented by two species, *L. hispida* and *L. hirsuta*, which also possess ascospores in Group D. *Lasiosphaeris* was erected by Clements (1909) with *L. hispida* as its type species, and despite its obvious undesirable quality of nearly being a homonym to *Lasiosphaeria*, it is a validly published and acceptable genus. Although this clade has low bootstrap support in these analyses (Fig. 10), it contains high bootstrap support in the expanded analyses discussed above (Miller & Huhndorf, in unpubl.). *Lasiosphaeris* can be distinguished from other genera in having ascomatal walls which are usually tuberculate, setose ascomatal vestitures, hyaline centrum contents, and cylindrical, sigmoid or geniculate ascospores. These morphological characters are also found in several species of *Cercophora* and *Lasiosphaeria* not included in this study (e.g. *C. ambigua*, *C. appalachianaensis*, *C. arenicola*, *Lasiosphaeria moseri*, *L. porifera*, *L. rhacodium*, *L. tuberculosa*), which most likely also belong in *Lasiosphaeris*. The taxonomy of the genus is currently under revision (Miller & Huhndorf, unpubl.).

Two species previously placed in *Lasiosphaeria* occurred on a long, well-supported branch in the *Helminthosphaeriaceae* (Fig. 10). *Echinospaeria canescens* and *R. spermoides* occurred as two distinct well-supported clades interspersed among three species of *Helminthosphaeria*. Although both species possess a *Selenosporella*-like anamorph and similar ascospores in Group A, *E. canescens* possesses thick-walled setae, while *R. spermoides* is glabrous. Members of *Helminthosphaeria* possess setose ascomatal vestitures, ellipsoid ascospores, and a putative *Diplococcium*-like anamorph. Additional work is needed in this family to determine which, if any, morphological characters unite these three genera. *Ruzenia* was originally distinguished from other taxa in *Lasiosphaeria* in having a basal stroma (Hilber & Hilber 2002). However, the genus is better delimited by a combination of glabrous ascomata and allantoid ascospores, and is distinguished from *Echinospaeria* by its lack of an ascomatal vestiture. *L. punctata* also has setose ascomatal vestitures, ascospores in Group A, and an associated

Selenosporella-like anamorph. However, attempts to sequence two different collections were unsuccessful. A further attempt to obtain sequences from cloned copies using the TOPO TA cloning kit (Invitrogen, Carlsbad, CA) was successful, but, unfortunately, multiple LSU copies were found. A putatively single-copy gene such as RPB2 will need to be sequenced to determine whether *L. punctata* belongs in *Echinospaeria*. Additional taxa, which may also belong in the *Helminthosphaeriaceae* such as *L. coacta*, *L. stuppea*, and *Phaeotrichosphaeria* spp., need to be studied to determine their phylogenetic affinities.

Rimaconus jamaicensis which was recently removed from *Lasiosphaeria* (Huhndorf *et al.* 2001), was weakly-supported as the sister taxon to the *Helminthosphaeriaceae* clade. *Rimaconus jamaicensis* was presumably originally placed in *Lasiosphaeria* (Seaver 1912) due to its ascospores, which resemble those in Group C. However, its conical, immersed ascomata with heavily melanized ascomatal walls are quite different from those found in other species of *Lasiosphaeria* s. lat. Our data support its removal from *Lasiosphaeria* and its placement in a new genus.

Two representatives of *C. raciborskii*, which possess ascospores in Group B, were included in these analyses. Our data place this species in the strongly-supported *Chaetosphaeriales* clade, and analyses of LSU with increased taxon sampling within this order along with data from the β -tubulin gene suggest that it should be transferred to *Chaetosphaeria* (Fernández, pers. comm.).

Two representatives of *H. caudata*, which possess ascospores in Group C, occurred on a separate branch as a sister clade to the *Sordariomycetidae/Hypocreomycetidae* clade. This clade lacked affinities to any other group and therefore, the new genus, *Hilberina*, is proposed. *Hilberina* is distinguished in having setose ascomatal vestitures and cylindrical ascospores which taper to a distinct point at one end. Several additional species also possess similar vestitures and ascospores. Most of these are difficult to germinate in culture and only *H. caudata* and *L. alexandrae* have been reported to germinate, but neither produced an anamorph (pers. obs., Taylor *et al.* 2001). Additional representatives need to be included in phylogenetic analyses to determine whether ascospore morphology is phylogenetically informative for placing taxa in this genus.

Lasiosphaeria, as previously circumscribed, contained taxa which possessed a wide range of variation in their teleomorphs and anamorphs, and, as expected, was shown to be highly polyphyletic. With the removal of several taxa with disparate characters, *Lasiosphaeria* is much more naturally defined to include taxa with tomentose ascomatal vestitures and yellow centrum pigments. Additional species remaining in *Lasiosphaeria* most likely belong elsewhere and, after careful study, should be transferred to other genera based on morphological and/or molecular data.

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REFERENCES

- Bhattacharya, D., Lutzoni, F., Reeb, V., Simon, D., Nason, J. & Fernandez, F. (2000) Widespread occurrence of spliceosomal introns in the rDNA genes of ascomycetes. *Molecular Biology and Evolution* **17**: 1971–1984.
- Candoussau, F., Fournier, J. & Magni, J. (2001) New and rare species of *Lasiosphaeria* in southwestern France. *Mycotaxon* **80**: 201–240.
- Carroll, G. & Munk, A. (1964) Studies on lignicolous *Sordariaceae*. *Mycologia* **56**: 77–98.
- Cesati, V. & De Notaris, G. (1863) Schema di classificazione degli Sferiacei italici aschigeri. *Commentario della Societa Crittogamologica Italiana* **1**: 177–240.
- Chenantais, J.-E. (1919) Études sur les pyrenomycètes. *Bulletin de la Société Mycologique de France* **35**: 46–98.
- Clements, F. E. (1909) *The Genera of Fungi*. H. W. Wilson, Minneapolis.
- Felsenstein, J. (1985) Confidence intervals on phylogenies: an approach using the bootstrap. *Evolution* **39**: 783–791.
- Fuckel, L. (1870) *Symbolae mycologicae. Jahrbücher des Nassauischen Vereins für Naturkunde* **23–24**: 1–459.
- Gams, W. (1973) Phialides with solitary conidia? Remarks on conidium ontogeny in some hyphomycetes. *Persoonia* **7**: 161–169.
- Gams, W. (2000) *Phialophora* and some similar morphologically little-differentiated anamorphs of divergent ascomycetes. *Studies in Mycology* **45**: 187–199.
- Gams, W. & Holubová-Jechová, V. (1976) *Chloridium* and some other dematiaceous hyphomycetes growing on decaying wood. *Studies in Mycology* **13**: 1–99.
- Hilber, O. & Hilber, R. (1983) Neue und seltene Arten der Gattung *Lasiosphaeria* Ces. & de Not. *Sydowia* **36**: 105–117.
- Hilber, O., Webster, J. & Hilber, R. (1987) *Lasiosphaeria dactylina* sp. nov., a grass-inhabiting species. *Transactions of the British Mycological Society* **89**: 589–593.
- Hilber, R. & Hilber, O. (1979) Einige Anmerkungen zu der Gattung *Cercophora* Fuckel (*Lasiosphaeriaceae*). *Zeitschrift für Mykologie* **45**: 209–233.
- Hilber, R. & Hilber, O. (2002) *The Genus Lasiosphaeria and allied taxa*. O. Hilber, Kelheim.
- Höhnelt, F. von (1918) Mykologische Fragmente CCXV. Über die gattung *Lasiosphaeria* Cesati et de Notaris. *Annals of Mycology* **16**: 73–75.
- Huelsenbeck, J. P. & Ronquist, F. R. (2001) MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* **17**: 754–755.
- Hughes, S. J. (1951) Studies on microfungi. XI. Some hyphomycetes which produce phialides. *Mycological Papers* **45**: 1–36.
- Hughes, S. J. (1979) Relocation of species of *Endophragma* auct. with notes on relevant generic names. *New Zealand Journal of Botany* **17**: 139–188.
- Huhndorf, S. M., Fernández, F. A., Taylor, J. E. & Hyde, K. D. (2001) Two pantropical ascomycetes: *Chaetosphaeria cylindrospora* sp. nov. and *Rimaconus*, a new genus for *Lasiosphaeria jamaicensis*. *Mycologia* **93**: 1072–1080.
- Huhndorf, S. M., Miller, A. N. & Fernández, F. A. (2004) Molecular systematics of the *Sordariales*: the order and the family *Lasiosphaeriaceae* redefined. *Mycologia* **96**: in press.
- Lundqvist, N. (1972) Nordic *Sordariaceae* s. lat. *Symbolae Botanicae Upsalienses* **20** (1): 1–374.
- Lutzoni, F., Wagner, P., Reeb, V. & Zoller, S. (2000) Integrating ambiguously aligned regions of DNA sequences in phylogenetic analyses without violating positional homology. *Systematic Biology* **49**: 628–651.
- Miller, A. N. & Huhndorf, S. M. (2001) Neotropical ascomycetes 10. New and interesting *Cercophora* species. *Sydowia* **53**: 211–226.
- Munk, A. (1957) Danish pyrenomycetes. *Dansk Botanisk Arkiv* **17**: 1–491.
- Posada, D. & Crandall, K. A. (1998) Modeltest: testing the model of DNA substitution. *Bioinformatics* **49**: 817–818.
- Réblová, M. (1997) Fungal diversity in the Czech Republic. New species of *Apiorhynchostoma*, *Capronia*, *Ceratospheeria* and *Lasiosphaeria*. *Sydowia* **50**: 229–251.
- Reed, C. F. & Farr, D. F. (1993) *Index to Saccardo's Sylloge Fungorum Volumes I–XXVI in XXIX 1882–1972*. [Contribution No. XXXI.] Reed Library and Herbarium, Darlington, MD.
- Seaver, F. J. (1912) The genus *Lasiosphaeria*. *Mycologia* **4**: 115–124.
- Sivanesan, A. (1983) Studies on ascomycetes. *Transactions of the British Mycological Society* **81**: 313–332.
- Swofford, D. L. (2002) *PAUP*: phylogenetic analysis using parsimony (*and other methods)*. Version 4. Sinauer Associates, Sunderland, MA.
- Tamura, K. & Nei, M. (1993) Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution* **10**: 512–526.
- Taylor, J. E., Fröhlich, J. & Hyde, K. D. (2001) *Lasiosphaeria* and a similar new genus from palms. *Mycoscience* **42**: 369–377.
- Vasilyeva, L. N. (1998) *Pyrenomycetidae et Loculoascomycetidae*. [Plantae non vasculares fungi et Bryopsidae, orientis estremi Rossica. Fungi, V. 4.] Nauka, St Petersburg.

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