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Two new genera in the Magnaporthaceae, a new addition to *Ceratosphaeria* and two new species of *Lentomitella*

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Abstract: *Ceratosphaerella* is described as a new genus for *C. castillensis* and *C. rhizomorpha*. The genus is related to *Ophioceras* but distinguished by ascomata with a basal stroma and shorter, fusiform ascospores. *Muraeriata* is described for *M. collapsa* and *M. africana*, two species that are distinguished by having a vacuolate middle ascomal wall layer. The ascospores resemble those of *Ceratosphaerella* and ascospores in both genera are morphologically similar to those of *Ceratosphaeria lampadophora*. Both new genera are placed in the Magnaporthaceae based on LSU and SSU data. A species previously identified as *Ophioceras tenuisporum* was re-examined, found to fit the description of *Pseudohalonectria phialidica* and is transferred to *Ceratosphaeria* based on LSU data. *Lentomitella tropica* and *L. pallibrunnea* are described for two species that have long-necked ascomata with pale brown, ellipsoid ascospores and large ascus rings. Sequence data from the LSU places them in a clade with hyaline-spored *Lentomitella crinigera* and *L. cirrhosa*.

Key words: Ascomycota, *Ceratosphaerella*, *Harporhiza*, LSU and SSU rDNA, *Muraeriata*, *Ophioceras*, systematics

INTRODUCTION

Representatives of the Magnaporthaceae have been included in numerous phylogenetic studies (Shearer et al 1999, Réblová et al 2004, Réblová and Seifert 2004, Réblová 2006) but additions to the group based on molecular data have been few. *Ophioceras* Sacc.

and *Pseudohalonectria* Minoura & T. Muroi were included by Shearer et al (1999), and *Ceratosphaeria* Niessl was shown to belong to the clade by Réblová et al (2004). Members of the family were included in the study of the systematic position and phylogenetic relationships of *Ceratostomella* Sacc. and morphologically similar taxa (Réblová 2006). At that time the genus *Lentomitella* Höhn. was reinstated for three species that have hyaline, ellipsoidal ascospores, short-stipitate asci with a conspicuous, refractive apical ring and a *Phaeoisaria*-like anamorph in culture. The genus forms a strongly supported monophyletic clade based on sequences of the nuclear small and large subunit ribosomal DNA (SSU, LSU). These taxa were compared to the morphologically similar *Ceratosphaeria lampadophora* (Berk. & Broome) Niessl in the Magnaporthaceae clade and found to be separated genetically.

Surveys of wood-inhabiting Sordariomycetes in Costa Rica, Kenya and Puerto Rico have uncovered several species that appear to be morphologically similar to the taxa treated by Réblová (2006) and some species that fit the circumscription of the Magnaporthaceae. In addition a fungus matching the description of *Ceratosphaeria castillensis* C.L. Sm. also was found. To reveal the relationships of these unknown fungi and *C. castillensis* new sequences of the SSU and LSU were analyzed and compared to known datasets with maximum parsimony and Bayesian analyses.

MATERIALS AND METHODS

Taxon sampling.—Taxa sequenced in this study are listed under the examined specimens for each taxon along with their GenBank accession number. Representatives from families and orders within the Sordariomycetes were included to determine the phylogenetic position of *Lentomitella*. All voucher specimens are deposited in the Field Museum Mycology Herbarium (F). Ascomata were mounted in water and replaced with lactophenol containing azure A. Measurements were made and images were captured of material in both mounting fluids. Images were captured with photomacrography, bright field (BF), phase contrast (PH) and differential interference microscopy (DIC); photographic plates were produced following the methods of Huhndorf and Fernández (1998).

DNA extraction, PCR amplification and sequencing.—Genomic DNA was extracted from either mature fruiting bodies or from tissues grown in culture (Huhndorf et al 2004) and

purified with a QIAGEN DNeasy Plant Mini Kit (QIAGEN Inc., Hilden, Germany) according to the manufacturers specifications. PCR amplifications and sequencing reactions were carried out according to the methods of Fernández et al (1999), Huhndorf et al (2004) and Miller and Huhndorf (2005). For certain collections (GKM1084, GKM1262, SMH1865, SMH1888) primers LROR and LR3 (Vilgalys and Hester 1990) were used to amplify the nuclear LSU region with a MJ Research PTC 200 or PTC 220 Dyad thermo cycler set at these parameters: initial denaturation at 94 C for five min, followed by 34 cycles of denaturation at 94 C for 1 min, annealing at 50 C for 90 s and extension for 1 min at 72 C. A final extension step of 72 C for 10 min completed the reaction. PCR product was viewed on ethidium bromide-stained 1% TALE agarose gel and purified with GELase Agarose Gel-Digesting Preparation according to manufacturer specifications (Epicentre Biotechnologies, Madison, Wisconsin). Cycle sequencing reactions were carried out with the BigDye terminator cycle sequencing kit (Applied Biosystems, Foster City, California) with primers LROR, LR3, LR3R and LR6 (Vilgalys and Hester 1990), and amplicons were run on an ABI 3730 cycle sequencer (Amersham Pharmacia Biotech Inc., Piscataway, New Jersey). Amplification and sequencing of SSU was carried out with fungal universal primers NS1 and NS4 (White et al 1990).

Sequence alignment and phylogenetic analyses.—Sequence data for 13 species were aligned with sequences of 44 species retrieved from GenBank, representing major clades within the Sordariomycetes where LSU data was available, using Se-AL v2.0a11 Carbon (Rambaut 2002). The data matrix was assembled with Se-AL, then exported to Mesquite v1.12 (Madison and Madison 2006) where it was aligned automatically with ClustalX v 1.83 (Thompson et al 1997). The aligned matrix was imported back into Se-AL and edited manually. *Scutellinia scutellata* DQ247806 was used as an outgroup taxon. A maximum parsimony analysis was performed with PAUP v 4.0 b10 (Swofford 2002). A heuristic search consisting of 200 random stepwise addition replicates was conducted with parsimony as the optimality criterion. Gaps were treated as missing data. Tree bisection reconnection was chosen as the branch-swapping algorithm with MULTREES option in effect. Branch support was estimated by performing 2000 bootstrap replicates with a heuristic search consisting of 200 random stepwise addition replicates for each bootstrap replicate using the above settings. (Felsenstein 1985). The tree was visualized with PAUP (Swofford 2002). A Bayesian analysis was conducted with MrBayes 3.1.2 (Huelsenbeck et al 2001), which approximates posterior probabilities of clades with Markov chain Monte Carlo (MCMC) (Huelsenbeck and Ronquist 2001). The data matrix was analyzed with the general time reversible model of substitution including estimation of invariant sites and assuming a discrete gamma distribution (GTR + I + G) with six rate categories provided for the nucleotide substitution model and for priors, with the chain temp set at 0.2. The nucleotide substitution model was chosen with Modeltest 3.7 (Posada and Crandall 1998). Trees were sampled every 100 out of a total of 2 000 000

generations, with the first 5000 trees (25% of total) being discarded as burn-in of the chain. Only clades with posterior probability values $\geq 95\%$ were considered to be phylogenetically informative. The resultant consensus tree was viewed with PAUP (Swofford 2002).

A second analysis examining the relationship among the Magnaporthaceae was performed by aligning SSU sequences for the new taxa with representative sequences from the data matrix in Chen et al (1999). A maximum parsimony and Bayesian analysis was performed on this dataset with the same parameters above with the exception that *Xylaria hypoxylon* U32411 was used as the outgroup taxon and the bootstrap analysis was terminated after 500 replicates.

RESULTS

Sequence alignment and phylogenetic analyses.—The LSU alignment contained a total of 57 taxa and 1008 characters, 573 of which were constant and 309 were parsimony informative. The heuristic search produced 27 equally parsimonious trees (one of which is shown in FIG. 1) with a consistency index of 0.386 and a retention index of 0.614. The second alignment based on a subset of *Ophioceras* SSU sequences contained 32 taxa and a total of 1006 characters, 797 of which were constant and 114 that were parsimony informative. The analysis of the second alignment produced 960 most parsimonious trees (one of which is shown in FIG. 2) with a consistency index of 0.674 and a retention index of 0.830.

Species relationships.—The LSU and SSU genealogies both contain a clade representing the Magnaporthaceae, supported by both bootstrap and significant bayesian posterior probabilities. Both genes support a strong clade containing *Ophioceras* species and a proposed new genus. The LSU tree includes clades within the Magnaporthaceae containing two species of *Ceratosphaeria* and another proposed new genus in a sister clade of *Magnaporthe*. Two new species of *Lentomitella* are strongly supported as a sister group of the other two known species of *Lentomitella*, *L. cirrhosa* (Pers.) Réblová and *L. crinigera* (Cooke) Réblová. Ordinal or familial placement of the genus is not supported so it remains in the Sordariomycetidae incertae sedis.

TAXONOMY

Ceratosphaerella Huhndorf, M. Greif, Mugambi & A.N. Mill., gen. nov.

Ascomata lageniformia vel globosa longirostria, pagina aspera, subiculum tomentosum copiosum, hyphae vel rhizomorpha compositum. Paries pseudoparenchymaticus, e cellulis multangularibus vel elongatis compositus. Asci clavati, cum annulo apicali, elati, angusti. Ascosporae fusiformes, septatae.

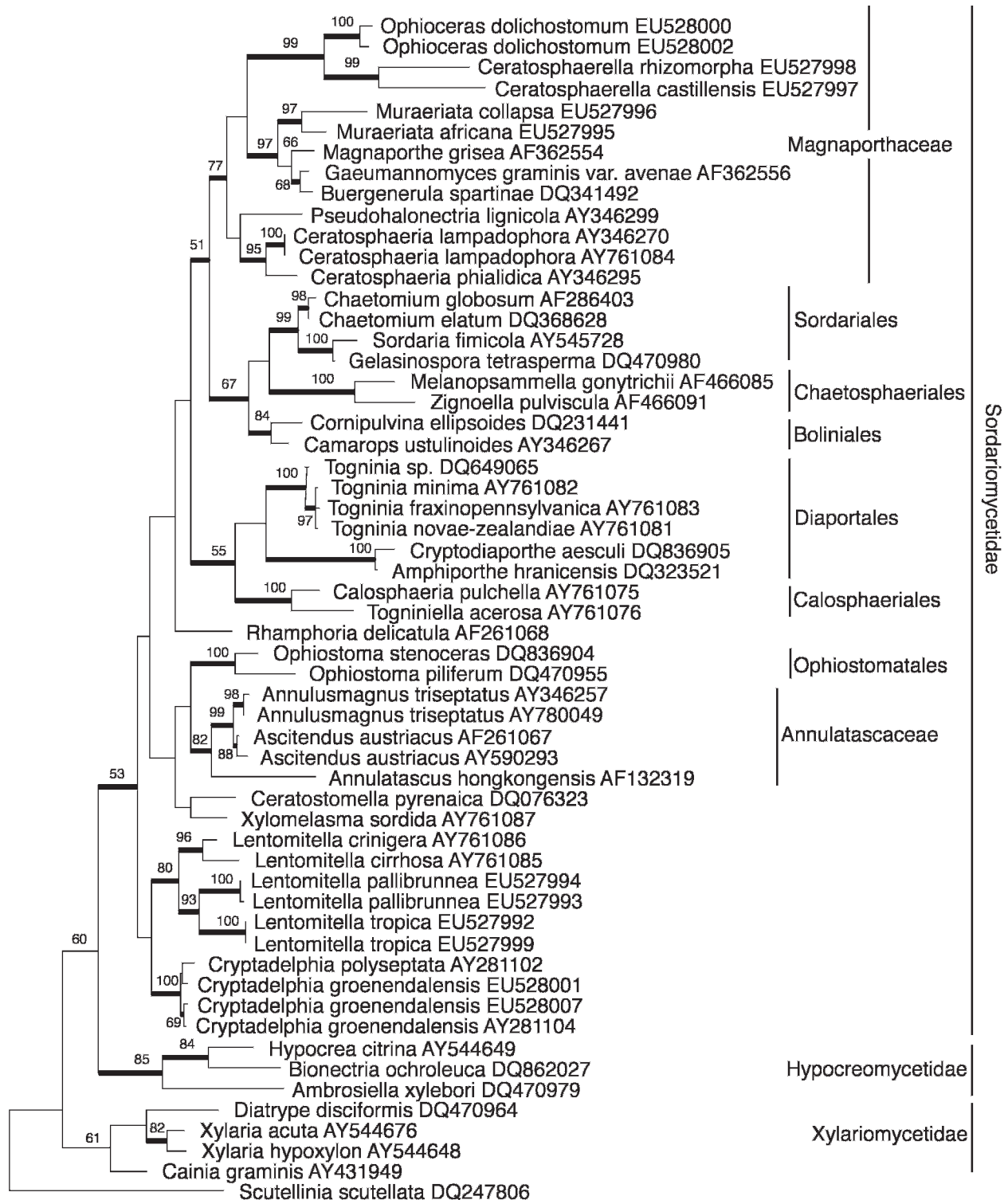


FIG. 1. Phylogenetic tree obtained from maximum parsimony showing the position of *Ophioceras*, *Ceratosphaeria* and other taxa based on large subunit rDNA sequence data. *Ophioceras*, *Ceratosphaerella*, *Ceratosphaeria*, and *Muraeriata* form strongly supported clades within the Magnaporthaceae, and *Lentomitella* forms a strongly supported clade of uncertain position within the Sordariomycetidae. Bootstrap support values $\geq 50\%$ are shown above branches and Bayesian support $\geq 95\%$ is indicated by thickened branches.

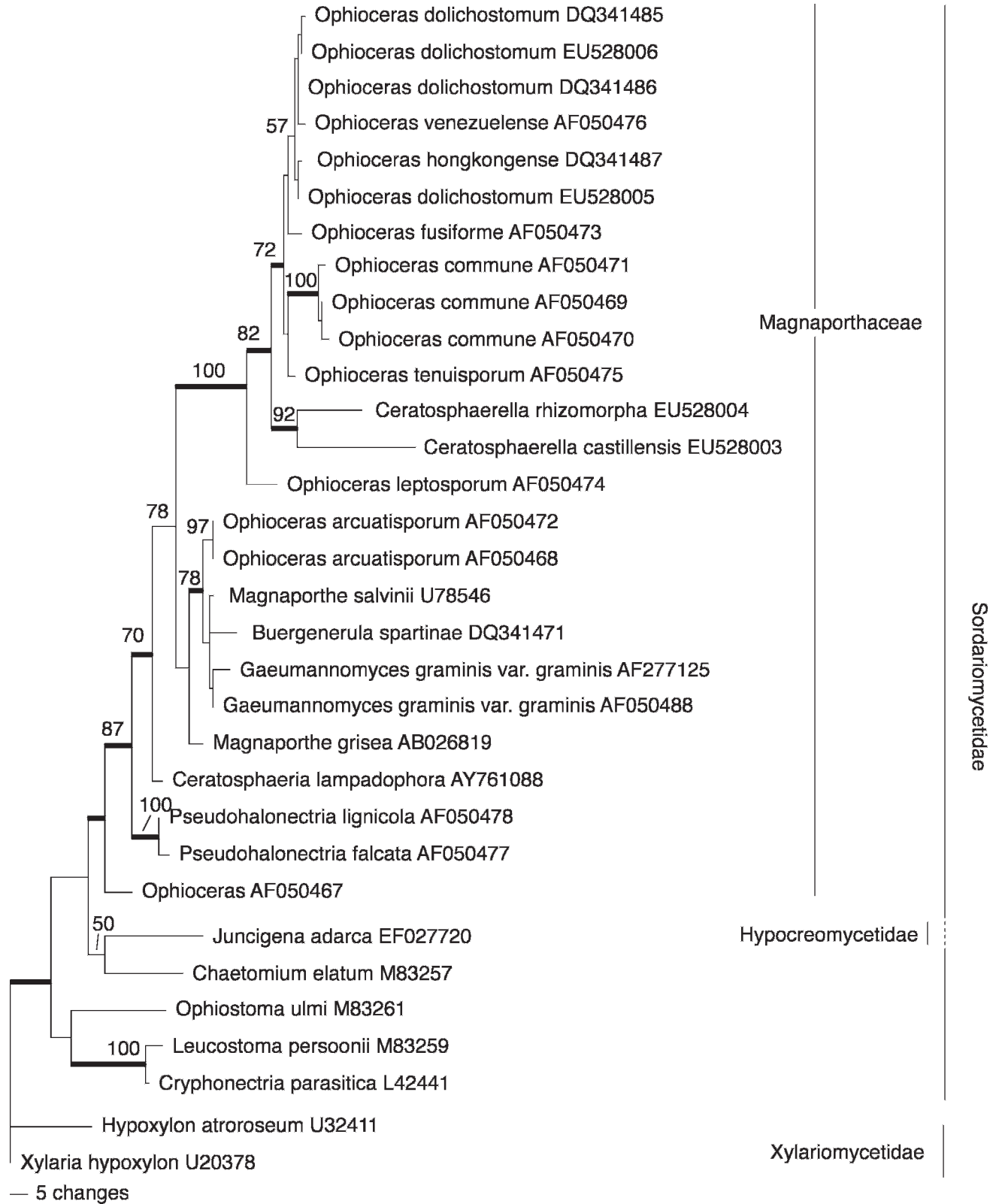
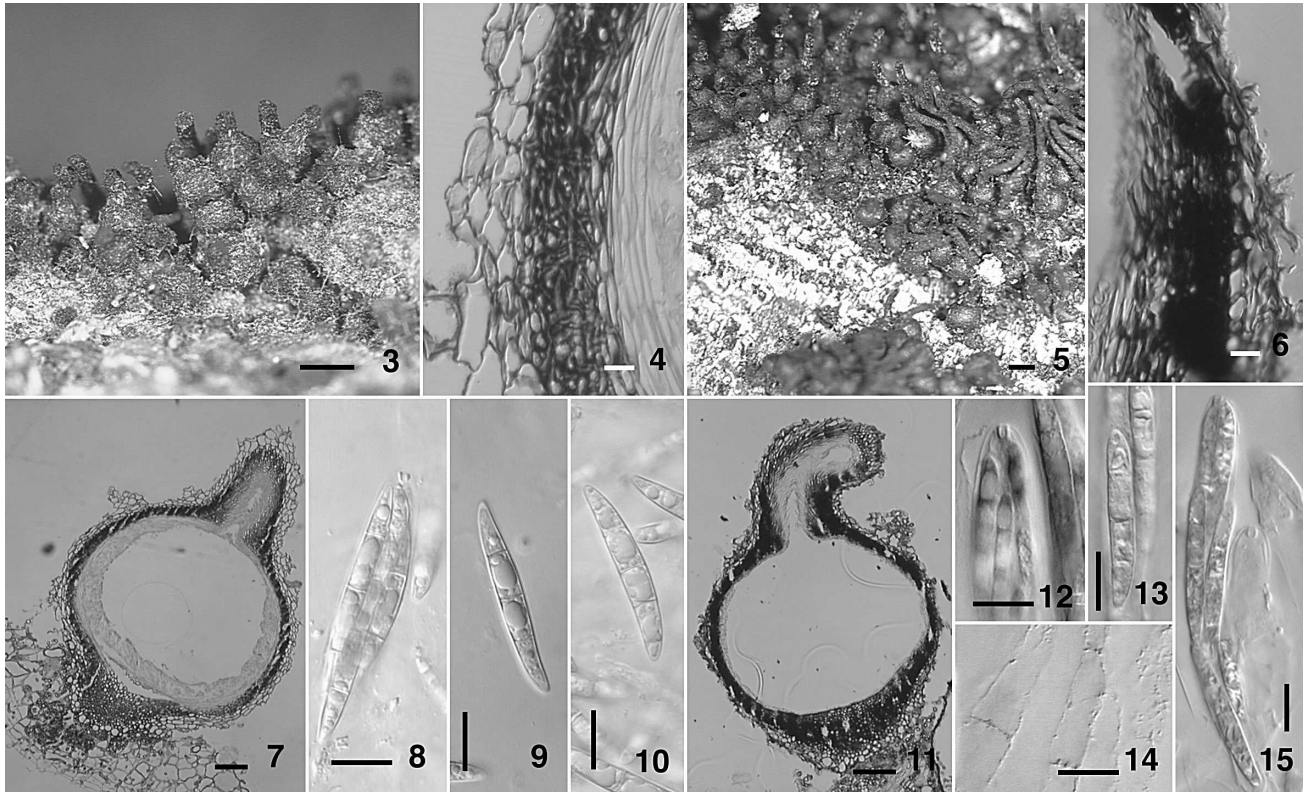


FIG. 2. Phylogenetic tree obtained from maximum parsimony showing the relationship between species of *Ophioceras* based on small subunit rDNA sequence data. A majority of *Ophioceras* isolates along with *Ceratosphaerella* form a strongly supported clade within the Magnaporthaceae. Bootstrap support values $\geq 50\%$ are shown above branches and Bayesian support $\geq 95\%$ is indicated by thickened branches.



FIGS. 3–15. *Ceratosphaerella castillensis*. 3, 5. Ascomata on the substrate. 4, 6. Longitudinal section through ascomatal wall. 7, 11. Longitudinal section through ascus. 8, 15. Ascus. 9, 10, 13. Ascospores. 12. Ascus apex. 14. Paraphyses. 3, 5 by photomicrography; 4, 6–15 by DIC. Bars: 3, 5 = 500 μm ; 7, 11 = 100 μm ; 4, 6, 8–10, 12–15 = 10 μm . 3, 4, 7–10 from SMH1865; 5, 6, 11–15 from NY isotype.

Ascomata lageniform to globose, long-beaked, with a sterile base, collapsing or not when dry, surface roughened, subiculum abundant, hyphal or rhizomorphic. Ascomal wall with an outer layer of large, pale brown, polygonal to globose, pseudoparenchymatic cells. Asci clavate, with a tall, narrow, apical ring. Ascospores fusiform, septate.

Typus generic. *Ceratosphaerella castillensis* (C.L. Sm.) Huhndorf, M. Greif, Mugambi et A.N. Mill.

Etymology. smaller than *Ceratosphaeria*. “ella” meaning diminutive.

Ceratosphaerella castillensis (C.L. Sm.) Huhndorf, M. Greif, Mugambi & A.N. Mill., comb. nov. FIGS. 3–15 \equiv *Ceratosphaeria castillensis* C.L. Sm., Bull. Lab. Nat. Hist. Univ. Iowa 2:403. 1893. Basionym.

Ascomata globose, long beaked, with a sterile base, not collapsing when dry, dark brown, surface roughened, venter 525–650 μm diam, neck 250–400 μm long, occurring in large clusters, superficial on sparse, subicular hyphae. *Ascomal wall* of *textura angularis-globosa* in surface view; in longitudinal section 65–75 μm thick, composed of an inner layer of flattened hyaline cells, a middle layer of small, polygonal to irregular, dark brown cells, an outer layer of large,

pale brown, polygonal to globose, pseudoparenchymatic cells. *Ascomatal apex* elongate beak-like, 250–400 μm high, 190–220 μm wide, composed of cells similar to the ascomal wall cells, ostiole circular, with periphyses. *Paraphyses* abundant, ca. 7–9 μm wide just above the asci, long tapering above. *Asci* clavate, 70–90 \times 10–14 μm ; with a tall, narrow, apical ring, 1.5–2.5 μm wide, 1.5–2.5 μm high. *Ascospores* fusiform, hyaline to pale brown, 3-septate, 29–40 (–45) \times 4–5.5 μm .

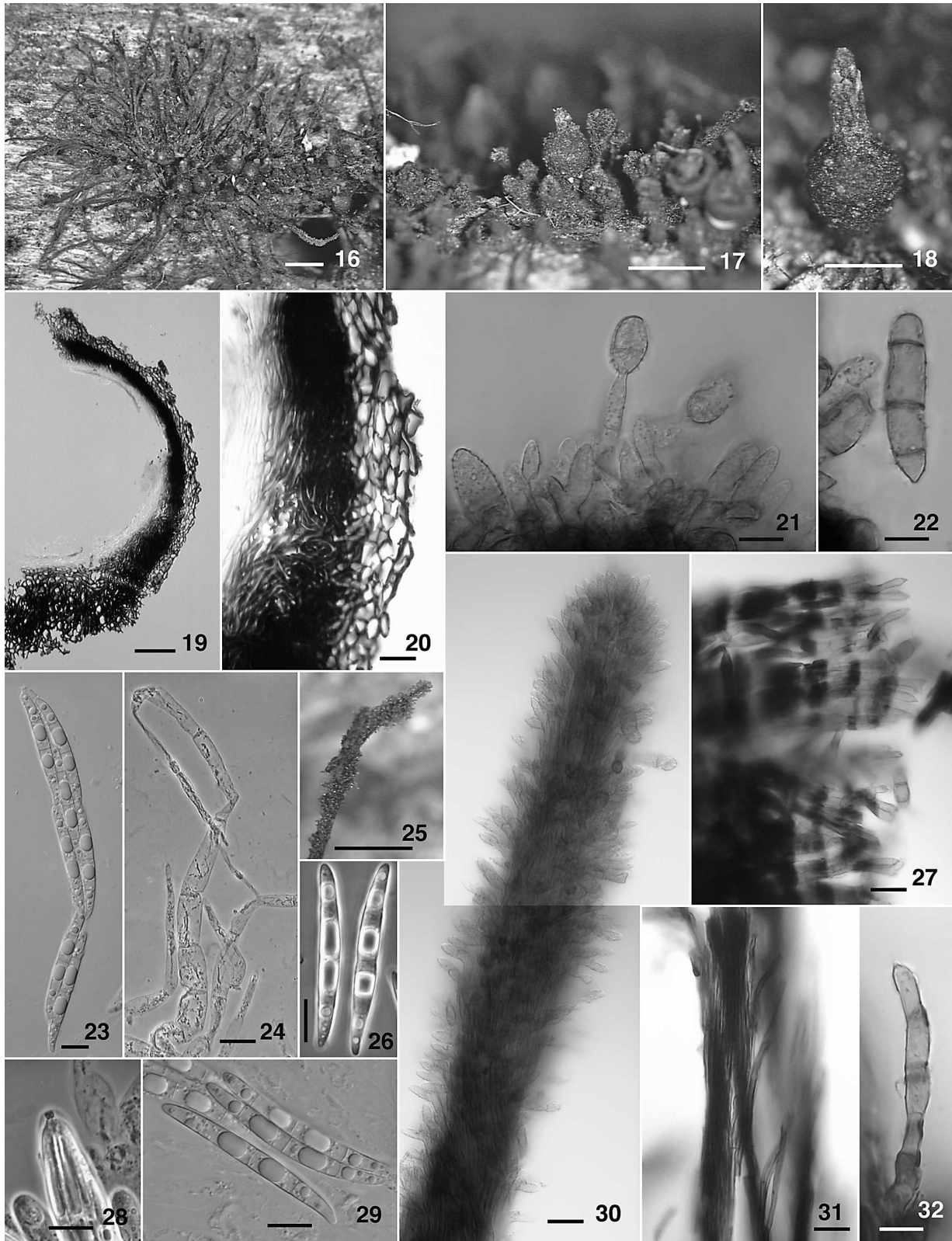
Habitat. On bark or wood.

Known distribution. Costa Rica, Nicaragua, Puerto Rico.

Specimens examined: COSTA RICA. Provincia Limon, Estacion RB Hitoy Cerare, near creek close to station, elev 120 m, on wood on the ground, 20-I-1999, *Milton Umana*, SMH4044. NICARAGUA. Castillo Viejo, Feb–Mar 1893, *C.L. Smith*, Central American Fungi 13, (ISOTYPE, NY). PUERTO RICO. Caribbean National Forest, El Verde Research Area, 16 ha Grid, Luquillo Mts., on 38 cm diam log, on bark, 10-X-1995, *S.M. Huhndorf*, SMH1865, GenBank EU527997, EU528003 (F).

Ceratosphaerella rhizomorpha Huhndorf & Mugambi, sp. nov. FIGS. 16–32

Ascomata lageniformia vel globosa longirostria, 500–



FIGS. 16–32. *Ceratosphaerella rhizomorpha*. 16–18. Ascomata on the substrate. 19, 20. Longitudinal section through ascomatal wall. 21. Phialide and conidia. 22. Conidium. 23. Ascus. 24. Paraphyses. 25, 30, 31. Rhizomorphic strand. 26, 29. Ascospores. 27, 32. Conidiophores. 28. Ascus apex. 16–18, 25 by photomicrography; 19, 20 by BF; 21–23, 27, 29–32 by DIC; 24, 26, 28 by PH. Bars: 16, 17 = 1 mm; 18, 25 = 500 μ m; 19 = 100 μ m; 20, 24, 27, 30, 31 = 20 μ m; 21–23, 26, 28, 29, 32 = 10 μ m. All from Mugambi 1262.

750 µm diam, 500–900 µm alta sine rostro, 800–1600 µm alta cum rostro, pagina aspera, subiculum tomentosum copiosum, rhizomorpha compositum. Paries pseudoparenchymaticus, e cellulis multangularibus vel elongatis compositus, 110–130 µm crassus. Asci clavati, 115–145 × 13–16 µm, cum annulo apicali, elati, angusti. Ascosporae fusiformes, 39–49 × 3.5–4.5 µm, triseptatae, hyalinae vel pallide brunneae.

Etymology. referring to the subicular threads.

Ascomata lageniform to globose, long-beaked, with a sterile base, sometimes collapsing when dry, dark brown, surface roughened, sometimes with white flakes, venter 500–750 µm wide, 500–900 µm high including the sterile base, neck 300–600 µm long, occurring in large clusters, superficial on abundant, subicular hyphae. *Subiculum* extensive, forming large, dense, radiating patches, composed of brown to black, septate hyphae, aggregating into 100–125 µm wide, rhizomorphic strands, dichotomously branched, forming synnematus hyphomycete at the branch ends. *Ascomal wall* of *textura angularis-globosa* in surface view; in longitudinal section 110–130 µm thick, composed of an inner layer of flattened hyaline cells, a middle layer of small, polygonal to irregular, dark brown cells, an outer layer of large, pale brown, polygonal to globose, pseudoparenchymatic cells. *Ascomatal apex* elongate beak-like, 300–600 µm high, 150–250 µm wide, wall composed of cells similar to the ascomal wall cells, ostiole circular, with periphyses. *Paraphyses* abundant, ca. 12–17 µm wide just above the asci, long tapering above. *Asci* clavate, 115–145 × 13–16 µm; with a tall, narrow, apical ring, 1.5–2.5 µm wide, 1.5–2.5 µm high; with 8 biseriata ascospores. *Ascospores* fusiform, hyaline to pale brown, 3-septate, 39–49 × 3.5–4.5 µm. *Anamorph* *Didymobotryum*-like, on the substrate. Colony dark brown to black, composed of rhizomorphic-like threads radiating from a central cluster of ascomata. *Synnemata* straight or flexuous, stipe composed of brown threads, appearing black in mass, threads branching at the apical region forming a lighter brown head composed of conidiophores. *Conidiophores* elongate, septate forming darker brown bands at the septations, verrucose. *Conidiogenous cells* tretic, integrated and terminal, pale brown, verrucose, cylindrical, 10–14 × 4–6 µm. *Conidia* ellipsoid, pale brown, 1-septate when young, conidia on older synnemata cylindrical, 3-septate, with darker brown septa, 27–46 × 9–11 µm, verrucose.

Habitat. On bark or wood.

Known distribution. Kenya.

Specimens examined: KENYA. Western Province: Kagamega National Park, 0°17'30"N, 34°51'22"E, elev 1500 M, on decaying wood on the ground, 17-I-2007, G.K. Mugambi GKM1262, GenBank EU527998, EU528004 (HOLOTYPE EA, ISOTYPE F).

Ceratosphaeria lampadophora (Berk. & Broome) Niessl, Verhandl. naturforsch Vereines Brünn. 14:43 (1876). FIGS. 33–40

Ascomata depressed globose, long-beaked, brown to yellow brown, beak surface smooth, at times with yellow crystals, venter ca. 1000 µm diam, neck 850–900 µm long, occurring solitary, or in small groups, immersed becoming erumpent or only beaks erumpent. *Ascomal wall* in longitudinal section composed of an inner layer of flattened hyaline cells, a middle layer of small, polygonal to irregular, pale brown cells, an outer layer of irregular, yellow brown, pseudoparenchymatic cells. *Ascomatal apex* elongate beak-like, 850–900 µm high, 225–240 µm wide. *Paraphyses* abundant, ca. 4.5–5.5 µm wide just above the asci, long tapering above. *Asci* cylindrical, 150–175 × 8–9.5 µm; with a tall, narrow, apical ring, 2–3 µm wide, 3–3.5 µm high; with 8 biseriata ascospores. *Ascospores* fusiform, hyaline, 5–7 septate, 52–72 × 3.5–4.5 µm. For description of the type specimen see Hyde et al (1997).

Habitat. On bark or wood.

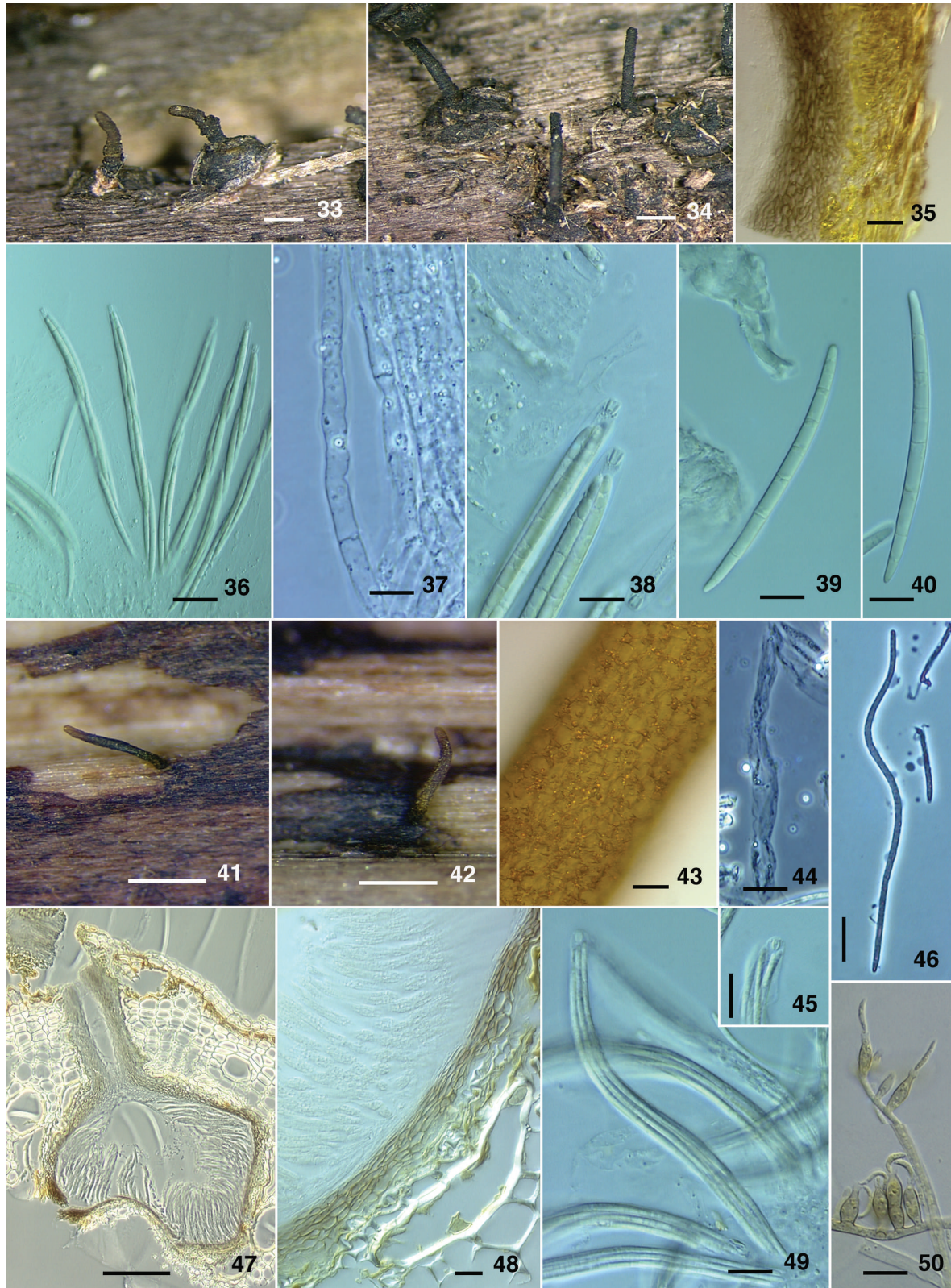
Known distribution. Europe.

Specimens Examined: FRANCE. Midi-Pyrénées, Ariège, Las Muros, Rimont, elev 450 m, on 3 cm branch on the ground, 25-IX-2002, A.N. Miller, J. Fournier, A.M. Stchigel, M. Caldusch, SMH4822, GenBank AY346270 (F).

Ceratosphaeria phialidica (Shearer) Huhndorf, M. Greif, Mugambi & A.N. Mill., comb. nov. FIGS. 41–50
= *Pseudohalonectria phialidica* Shearer, Can. J. Bot. 67(7): 1950. 1989. Basionym. (Type: USA. Illinois. Vermilion County. n.v.)

Ascomata globose, long-beaked, brown to yellow brown, beak surface smooth, at times with yellow crystals, venter 275–335 µm diam, neck 575–700 µm long, occurring solitary, widely scattered, deeply immersed, only beaks erumpent. *Ascomal wall* of *textura angularis-globosa* in surface view; in longitudinal section 22–26 µm thick, composed of an inner layer of flattened hyaline cells, a middle layer of small, polygonal to irregular, pale brown cells, an outer layer of irregular, yellow brown, pseudoparenchymatic cells. *Ascomatal apex* elongate beak-like, 575–700 µm high, 65–85 µm wide, wall composed of cells similar to the ascomal wall cells, ostiole circular, with periphyses. *Paraphyses* abundant, ca. 4–7 µm wide just above the asci, long tapering above. *Asci* cylindrical, 90–100 × 5.5–6.5 µm; with a tall, narrow, apical ring, 1.8 µm wide, 2.3–2.5 µm high; with 8 fasciculate ascospores. *Ascospores* filiform, hyaline, nonseptate, 65–85 × 1.3–1.7 µm. *Anamorph* *Harpophora*-like, in culture. *Conidiogenous cells* phialidic, ampulliform to lageniform, micronematous, hyaline, 9–12 × 3–4 µm. Collarete 2–3 µm wide. *Conidia* 8–13 × 1–2.2 µm. (For colony characteristics see Shearer 1989.)

Habitat. On herbaceous stems.



FIGS. 33–40. *Ceratosphaeria lampadosphora*. 41–50. *Ceratosphaeria phialidica*. 33, 34, 41, 42. Ascomata on the substrate. 35, 48. Longitudinal section through ascomatal wall. 36, 49. Asci. 37, 44. Paraphyses. 38, 45. Ascus apex with ring. 39, 40, 46. Ascospores. 43. Ascomatal neck surface. 47. Longitudinal section through ascus. 50. Phialides and conidia. 33, 34, 41, 42 by photomicrography; 35, 36, 38–40, 43, 45, 49, 50 by DIC; 37, 44, 46–48 by PH. Bars: 33, 34, 41, 42 = 500 μ m; 47 = 100 μ m; 35, 43 = 20 μ m; 36–40, 44–46, 48–50 = 10 μ m. 33–40 from SMH4822; 41–50 from SMH1643.

Known distribution. USA (Illinois, Puerto Rico)

Specimens examined: PUERTO RICO. Caribbean National Forest, El Verde Research Area, 16 ha Grid, Luquillo Mts., on 1 cm petiole on the ground, 29-IX-1995, *S. M. Huhndorf*, SMH1643, GenBank AY346295 (F).

Lentomitella pallibrunea Huhndorf, M. Greif, Mugambi & A.N. Mill., sp. nov. FIGS. 51–60

Ascomata obpyriformia vel lageniformia, brevibus vel longirostris, 275–325 μm diam, 700–800 μm alta, superficialia, obliquata. Pariet pseudoparenchymaticus, e cellulis multangularibus vel elongatis compositus, 25–35 μm crassus. Asci cylindrici, cum annulo apicali, 70–85 \times 8–10 μm . Ascosporeae ellipsoideae, brunneae pallide, 11.5–14 \times 4–5 μm .

Etymology. referring to the pale brown ascospores.

Ascomata obpyriform to lageniform, short to long-beaked, not collapsing when dried, surface roughened, dark brown, 275–325 μm diam, 700–800 μm high, numerous, gregarious, erumpent to superficial, oblique to horizontal on the substrate, at the base connected by sparse subicular hyphae to the substrate. *Ascomal wall* of *textura angularis-globosa* in surface view, in longitudinal section 25–35 μm thick, composed of large, pale brown, polygonal to globose, pseudoparenchymatic cells, outer layer a very thin, darker brown crust. *Ascomatal apex* conical to elongate beak-like, often with several deep sulcations, 375–425 μm high, 100–125 μm wide, ostiole circular, with periphyses. *Paraphyses* 5–6 μm wide, sparse, disintegrating. *Asci* cylindrical, short stipitate, 70–85 \times 8–10 μm , unitunicate, apex rounded, with large, refractive ring, 2–3 μm high, 2–3 μm wide, with 8 uniseriate ascospores. *Ascospores* ellipsoid, straight to slightly curved, pale brown, smooth, one-celled, 11.5–14 \times 4–5 μm .

Habitat. On bark or wood.

Known distribution. USA (Illinois, Michigan).

Specimens examined: USA. Illinois. Cook County, Swallow Cliffs Forest Preserve, on 38 cm log, 3-VII-1996, *S.M. Huhndorf*, F.A. Fernández, M. Huhndorf, SMH2533; on 50 cm log, SMH2534, GenBank EU527993; on wood fragment on the ground, SMH2539. Michigan. Berrien County, Warren Woods, south end of trail, through picnic area, up to creek, on 12 cm branch on the ground, 8-IX-1998, F.A. Fernández, A.N. Miller, SMH3877, GenBank EU527994 (HOLOTYPE F).

Lentomitella tropica Huhndorf, M. Greif, Mugambi & A.N. Mill., sp. nov. FIGS. 61–69

Ascomata obpyriformia vel lageniformia, brevibus vel longirostris, 375–475 μm diam, 700–900 μm alta, superficialia. Pariet pseudoparenchymaticus, e cellulis multangularibus vel elongatis compositus, 40–50 μm crassus. Asci cylindrici, cum annulo apicali, 90–100 \times 9–11 μm . Ascosporeae ellipsoideae, brunneae pallide, 14.5–16 \times 5–6 μm .

Etymology. referring to the tropical distribution.

Ascomata obpyriform to lageniform, short to long-beaked, not collapsing when dried, surface glabrous,

dark brown, 375–475 μm diam, 700–900 μm high, numerous, gregarious, superficial, at the base connected by sparse subicular hyphae to the substrate. *Ascomal wall* of *textura angularis-globosa* in surface view, in longitudinal section 40–50 μm thick, composed of large, pale brown, polygonal to globose, pseudoparenchymatic cells, outer layer a very thin, darker brown crust. *Ascomatal apex* conical to elongate beak-like, often with several deep sulcations, 150–200 μm wide, ostiole circular, with periphyses. *Paraphyses* 6–7 μm wide, abundant, persistent, with gelatinous coating. *Asci* cylindrical, short stipitate, 90–100 \times 9–11 μm , unitunicate, apex rounded, with large, refractive ring, 2–3.5 μm high, 3.5–4.5 μm wide, with 8 uniseriate ascospores. *Ascospores* ellipsoid, straight to slightly curved, pale brown, smooth, one-celled, 14.5–16 \times 5–6 μm .

Habitat. On bark or wood.

Known distribution. Costa Rica, Puerto Rico.

Specimens examined: COSTA RICA. Guanacaste, Parque Nacional Guanacaste (ACG), Sector Cacao, trail to Estacion Biologica Cacao, elev. 1100 m, [10.9264, -85.4686], on wood fragment on the ground, 24-VI-1997, *S.M. Huhndorf*, SMH3225, GenBank EU527999. PUERTO RICO. Caribbean National Forest, El Verde Research Area, 16 ha Grid, Luquillo Mts., on 50 cm log, 10-VI-1995, *S. M. Huhndorf*, SMH1458; on 25 cm log, 8-X-1995, SMH1797, GenBank EU527992 (HOLOTYPE F).

Muraeriata Huhndorf, M. Greif, Mugambi & A.N. Mill., gen. nov.

Ascomata lageniformia vel globosa longirostris, omnino collabentia, pagina aspera vel glabrata. Pariet pseudoparenchymaticus, e cellulis hyalinis, multangularibus vel elongatis, evacuatis, compositus. Asci cylindrici vel ventricosi, cum annulo apicali, elati, angusti. Ascosporeae angustae fusiformes, septatae.

Ascomata lageniform to globose, long-beaked, collapsing when dry, surface roughened or smooth. *Ascomal wall* with a middle layer of large, polygonal to irregular, hyaline cells that collapse or rupture creating large empty pockets, with an external brown crust. *Asci* cylindrical to ventricose, with a tall, narrow, apical ring. *Ascospores* narrowly fusiform, septate.

Typus generic. *Muraeriata collapsa* Huhndorf, M. Greif, G. Mugambi et A.N. Mill.

Etymology. “mur” meaning wall and “aer” meaning air, referring to the large vacuolate cells present in the central layer of the ascomal wall.

Muraeriata africana Huhndorf & Mugambi, sp. nov.

FIGS. 70–79

Ascomata lageniformia vel globosa longirostris, omnino collabentia, 325–525 μm diam, 800–1200 μm alta rostratis, pagina aspera. Pariet pseudoparenchymaticus, e cellulis hyalinis, multangularibus vel elongatis, evacuatis, compositus, 65–75 μm crassus. Asci ventricosi, 90–110 \times 13–16 μm ,

cum annulo apicali elati, angusti. Ascospores angustae fusiformes, $35\text{--}48 \times 2.7\text{--}3.7 \mu\text{m}$, triseptatae, hyalinae.

Etymology. referring to the locality.

Ascomata globose, long-beaked, venter and neck collapsing when dry, dark brown, surface roughened, $325\text{--}525 \mu\text{m}$ diam, neck $500\text{--}650 \mu\text{m}$ long, superficial, gregarious occurring in small to large groups. *Ascomal wall* of *textura angularis-globosa* in surface view; in longitudinal section $65\text{--}75 \mu\text{m}$ thick, composed of an inner layer of small brown cells, a middle layer of small to large, polygonal to irregular, hyaline cells that collapse or rupture creating large empty pockets, an outer layer of small, brown, cells with an external brown crust. *Ascomatal apex* elongate beak-like, $500\text{--}650 \mu\text{m}$ high, $130\text{--}170 \mu\text{m}$ wide, composed of cells similar to the ascomal wall cells, ostiole circular, with periphyses. *Paraphyses* abundant, $3.5\text{--}6 \mu\text{m}$ wide just above the asci, long tapering above. *Asci* cylindrical to ventricose, $90\text{--}110 \times 13\text{--}16 \mu\text{m}$; with a tall, narrow, apical ring, $1.5\text{--}2.5 \mu\text{m}$ wide, $1.5\text{--}2.5 \mu\text{m}$ high; with 8, tri-tetraseriate ascospores. *Ascospores* narrowly fusiform, ends slightly curved, hyaline, 3-septate, $35\text{--}48 \times 2.7\text{--}3.7 \mu\text{m}$.

Habitat. On bark or wood.

Known distribution. Kenya

Specimens examined: KENYA. Nairobi Province: Nairobi Arboretum, $1^{\circ}16'S$ $36^{\circ}48'E$, on wood, 7-VI-2005, G.K. Mugambi & D. Odhiambo GKM1084, GenBank EU527995 (HOLOTYPE EA, ISOTYPE F).

Muraeriata collapsa Huhndorf, M. Greif, Mugambi & A.N. Mill., sp. nov. FIGS. 80–88

Ascomata lageniformia vel globosa longirostris, omnino collabentia, $500\text{--}950 \mu\text{m}$ diam, $2\text{--}5 \text{mm}$ alta rostratis, pagina glabrata. Paries pseudoparenchymaticus, e cellulis hyalinis, multangularibus vel elongatis, evacuatis, compositus, $65\text{--}95 \mu\text{m}$ crassus. *Asci* cylindrici, $100\text{--}125 \times 8\text{--}12 \mu\text{m}$, cum annulo apicali, elati, angusti. *Ascospores* angustae fusiformes, $45\text{--}52 \times 3.7\text{--}4.3 \mu\text{m}$, triseptatae, hyalinae.

Etymology. referring to the ascomal shape.

Ascomata globose, long beaked, venter and neck collapsing when dry, dark brown, surface smooth, $500\text{--}950 \mu\text{m}$ diam, neck $1.7\text{--}3.5 \text{mm}$ long, superficial, gregarious occurring separately. *Ascomal wall* opaque in surface view; in longitudinal section $65\text{--}95 \mu\text{m}$ thick, composed of an inner layer of small brown cells, a middle layer of small to large, polygonal to irregular, hyaline cells that collapse or rupture creating large empty pockets, an outer layer of small, brown cells with an external brown crust. *Ascomatal apex* extremely elongate beak-like, $1.7\text{--}3.5 \text{mm}$ high, $150\text{--}250 \mu\text{m}$ wide, composed of cells similar to the ascomal wall cells, ostiole circular, with periphyses. *Paraphyses* abundant, ca. $4\text{--}6.5 \mu\text{m}$ wide just above the asci, long tapering above. *Asci* cylindrical, $100\text{--}125 \times 8\text{--}12 \mu\text{m}$; with a tall, narrow, apical ring, $1.5\text{--}1.9 \mu\text{m}$

wide, $2\text{--}2.3 \mu\text{m}$ high; with 8, bi-triseriate ascospores. *Ascospores* narrowly fusiform, ends slightly curved, hyaline, 3-septate, $45\text{--}52 \times 3.7\text{--}4.3 \mu\text{m}$.

Habitat. On bark or wood.

Known distribution. Costa Rica, Ecuador.

Specimens examined: COSTA RICA. Alajuela, Parque Nacional Volcan Arenal, La Fortuna de San Carlos, Pilón trail, [10.4419, -84.7167], 15-VII-2001, on wood fragment on the ground, S.M. Huhndorf, F.A. Fernández, A.N. Miller, M.P. DaRin, SMH4553, GenBank EU527996 (HOLOTYPE USJ, ISOTYPE F); San Jose, San Gerardo de Dota, Albergue de Montana, Savegre, Sendero la Quebrada, 2300 m, [9.55, -83.8], on 8 cm branch on the ground, 12-V-1996, S.M. Huhndorf, F.A. Fernández, SMH2386, SMH2411 (F).

Ophioceras dolichostomum (Berk. & M.A. Curtis) Sacc., Syll. Fung. 2:358. 1883. FIGS. 89–97

Ascomata globose, long-beaked, not collapsing when dry, dark brown, surface smooth, $500\text{--}800 \mu\text{m}$ diam, neck up to $3\text{--}5 \text{mm}$ long, occurring in large clusters or separate. *Ascomal wall* of *textura angularis-globosa* in surface view; in longitudinal section composed of an inner layer of flattened hyaline cells, a middle layer of small, polygonal to irregular, dark brown cells, an outer layer of large, pale brown, polygonal to globose, pseudoparenchymatic cells. *Ascomatal apex* elongate beak-like, up to $3\text{--}5 \text{mm}$ long, $125\text{--}175 \mu\text{m}$ wide, composed of cells similar to the ascomal wall cells, ostiole circular, with periphyses. *Paraphyses* abundant, $6\text{--}9 \mu\text{m}$ wide just above the asci, long tapering above. *Asci* cylindrical, $130\text{--}140 \times 9\text{--}10.5 \mu\text{m}$; with a tall, narrow, apical ring, $2.5\text{--}3 \mu\text{m}$ wide, $1.5\text{--}2 \mu\text{m}$ high. *Ascospores* filiform, hyaline, 3–5 septate, $120\text{--}130 \times 2\text{--}3 \mu\text{m}$.

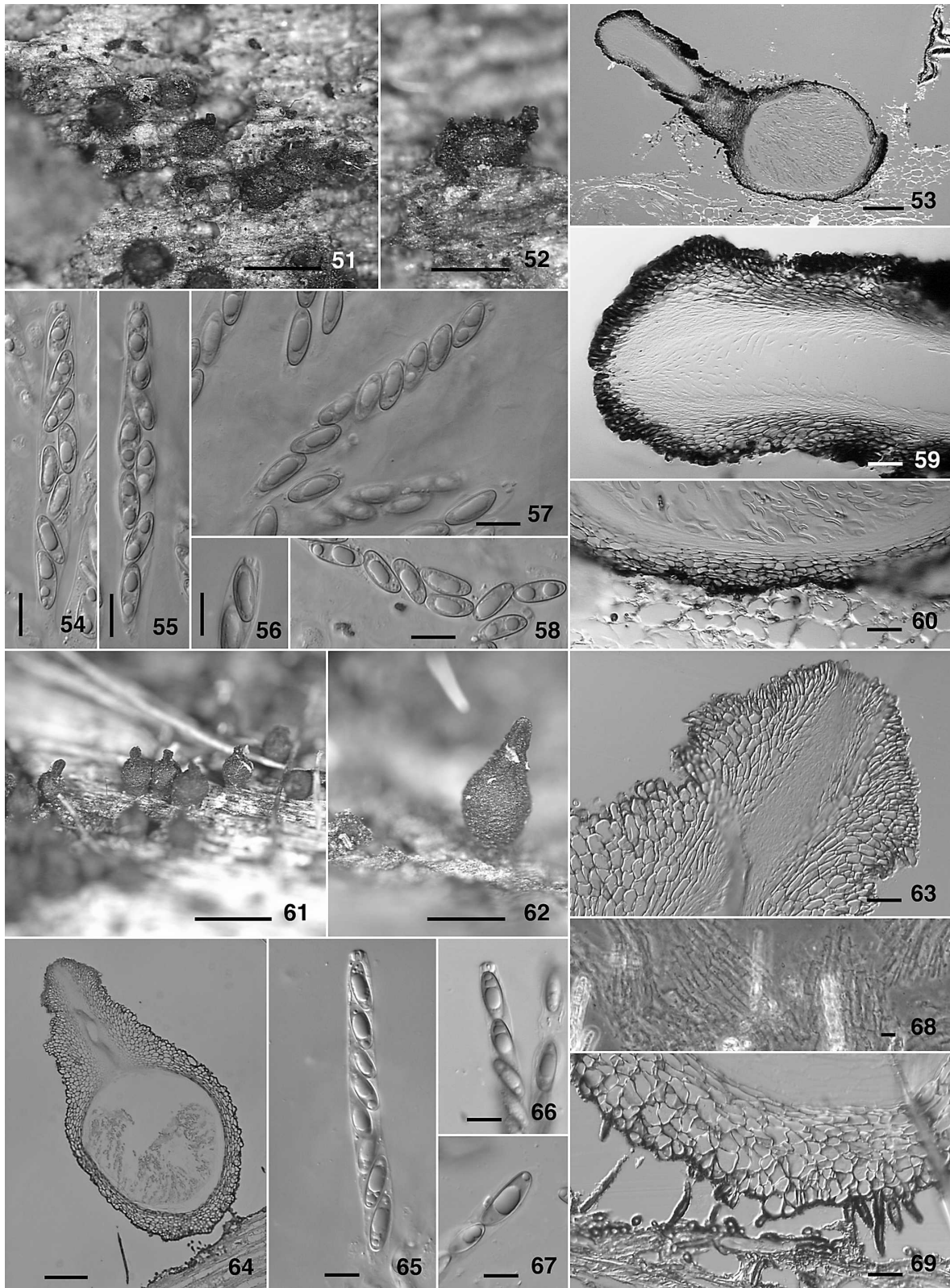
Habitat. On bark or wood.

Known distribution. Cuba, Puerto Rico, USA (Florida, however specimens cited in Conway and Barr [1977] at NY were not found).

Specimens examined: CUBA. Fungi Cubenses Wrightiani 856, C. Wright (ISOTYPE NY). PUERTO RICO. Caribbean National Forest, El Verde Research Area, 16 ha Grid, Luquillo Mts., on 3 cm branch, 25-IX-1995, S.M. Huhndorf, SMH1579, GenBank EU528000, EU528006; on 15 cm log, 15-I-1996, S.M. Huhndorf, SMH1888, GenBank EU528002, EU528005 (F).

DISCUSSION

When the Magnaporthaceae was established it included mostly necrotrophic parasites such as *Magnaporthe* R.A. Krause & R.K. Webster, *Gaeumannomyces* Arx & D.L. Olivier and *Buergenerula* Syd. among others (Cannon 1994). Molecular data has shown that these three genera indeed are related but the family has been expanded to include saprophytic taxa such as *Ophioceras*, *Pseudohalonectria* and *Ceratosphaeria* (Shearer et al 1999, Chen et al 1999, Réblová 2004).



FIGS. 51–60. *Lentomitella pallibrunnea*. 61–69. *Lentomitella tropica*. 51, 52, 61, 62. Ascomata on the substrate. 53, 64. Longitudinal section through ascomata. 54, 55, 57, 65. Asci. 56, 66. Ascus apex with ring. 58, 67. Ascospores. 59, 63. Longitudinal section through ascomatal neck. 60, 69. Longitudinal section through ascomatal wall. 68. Paraphyses. 51, 52, 61, 62 by photomicrography; 53–60, 63–67, 69 by DIC; 68 by PH. Bars: 51, 61 = 1 mm; 52, 62 = 500 μ m; 53, 64 = 100 μ m; 59, 60,

In our analyses two additional genera are found to have relationships within the family sensu lato. The Magnaporthaceae containing all of these taxa is supported by LSU and SSU data and contains several well supported clades (FIGS. 1, 2).

In both the LSU and SSU analyses representatives of *Magnaporthe*, *Gaeumannomyces* and *Buergenerula* show affinities to each other but close relationships are not highly supported. Studies using ITS have shown that *M. grisea* and anamorphs are closely related to *Gaeumannomyces* species and anamorphs (Bussaban et al 2005). Close relatives of *Magnaporthe* and *Gaeumannomyces* at the species level appear to remain unsampled for LSU. The new genus *Muraeriata* forms a strong sister clade of these taxa. *Muraeriata* species are distinct from these taxa by having an ascomal wall structure that includes a central wall layer where cells rupture and form empty areas. This characteristic is not seen in the walls described in the literature for the other taxa (Walker 1980, Kohlmeyer and Gessner 1976, Hebert 1971). The multilayered wall of *Muraeriata* bears some resemblance to that seen in *Ceratosphaeria lampadophora*. Differences between the genera also are seen in the asci and ascospore morphologies. *Gaeumannomyces* has scolecosporous ascospores while *Magnaporthe* and *Muraeriata* have narrowly fusiform ascospores and *Buergenerula* has clavate ascospores.

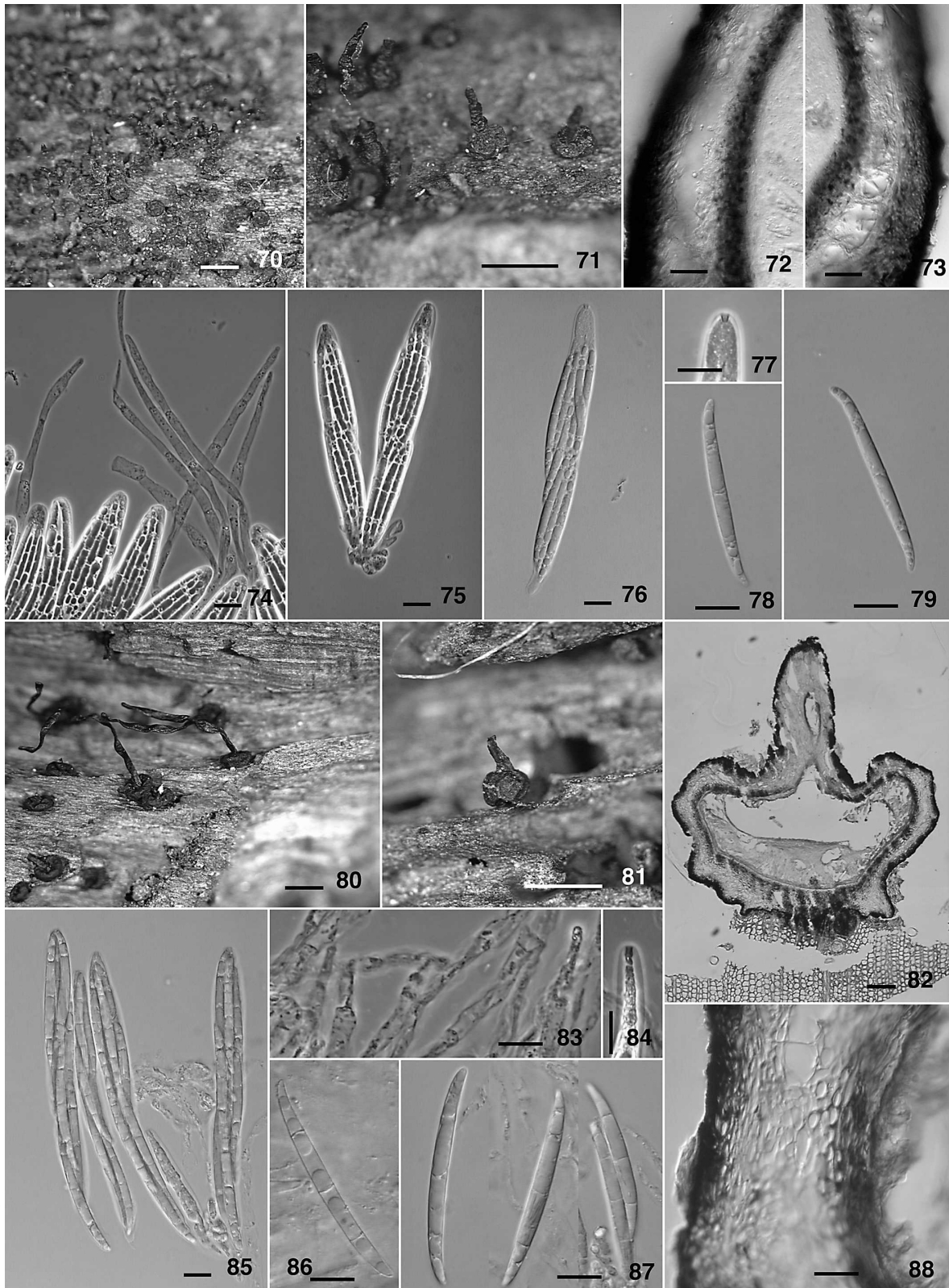
Our two collections of *Ophioceras dolichostomum* (from Puerto Rico) were compared to the type specimen (from Cuba) to verify identification, and the SSU was sequenced to verify their placement among other species of *Ophioceras* for which SSU was available. Morphologically our collections are identical to the type and geographically within proximity to the type locality to be confidently used to represent the species. The SSU does not resolve differences among our collections and *O. dolichostomum* representatives from southeastern Asia, *O. hongkongense* K.M. Tsui, H.Y.M. Leung, K.D. Hyde & Hodgkiss and *O. venezuelense* Shearer, J.L. Crane & W. Chen. Sequences of ITS might better resolve species-level distinctions within *Ophioceras*.

In both analyses *Ophioceras* species occur in a strongly supported clade with the newly described *Ceratosphaerella*. In the SSU analyses *O. leptosporum* (S.H. Iqbal) J. Walker falls basal to the rest of the *Ophioceras* species, causing the genus to become

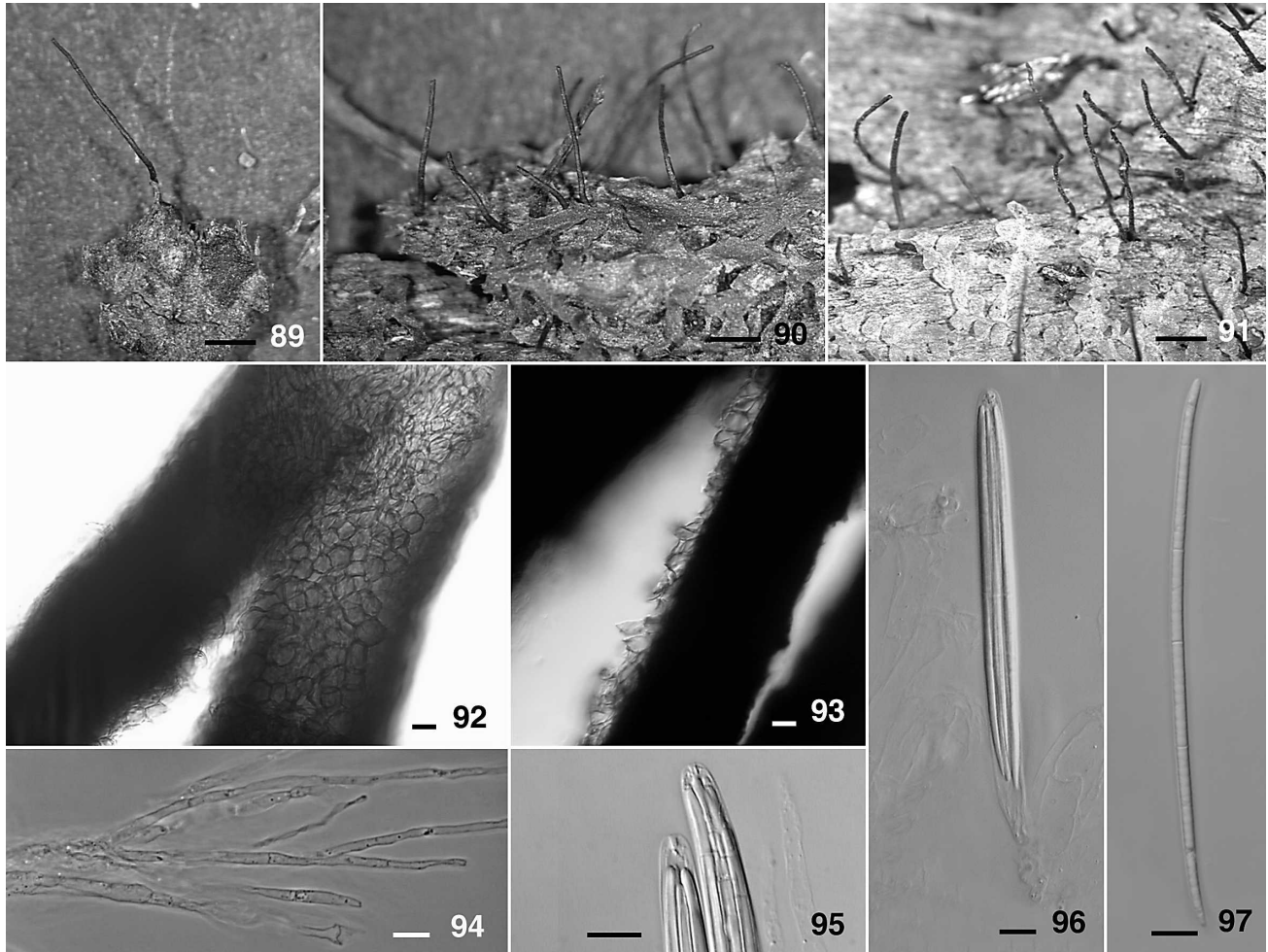
paraphyletic and *Ceratosphaerella* to become nested within. Morphologically however *Ceratosphaerella* differs from *Ophioceras* by its fusiform ascospores and ascomata with a large, sterile base. The two genera are similar in having ascomata with an outer layer of globose, pale brown cells, a feature that we consider phylogenetically important. In *Ceratosphaerella* the outer layer is much larger than that found in *Ophioceras*. In the SSU analyses, *O. arcuatissporum* Shearer, J.L. Crane & W. Chen remains outside the clade of *Ophioceras* species and indeed it does not show the outer ascomal layer of globose cells (Shearer et al 1999, FIGS. 1, 4). How *O. arcuatissporum* relates to the plant pathogens in the clade where it resides is unclear.

Ceratosphaeria lampadophora occurs in a third well supported clade in the Magnaporthaceae based on LSU data. The two known LSU sequences of this species are identical because the specimens were collected in the same locality, just over a year apart by different collectors and the same collaborator and probably represent the same population. Sequence data from specimens from additional localities would be useful. The species is distinguished by its large, erumpent, long-beaked ascomata with distinct yellow wall pigments (FIG. 35). The ascomal wall has been described as having four layers, the inner two layers of which contain the yellow pigments (Hyde et al 1997). The yellow cast is also present in the beak and often stains the woody substrate (FIGS. 33, 34). A sister taxon of *C. lampadophora* is *C. phialidica* (FIG. 1), which was identified erroneously by us (Huhndorf et al 2004) as *Ophioceras tenuisporum* Shearer, J.L. Crane & W. Chen (AY346295). The true *O. tenuisporum* groups with the other species of *Ophioceras* in the SSU analyses (FIG. 2). Our collection differs from the type of *O. tenuisporum* by having pale, yellow brown coloration of the ascomata and the substrate and in such it matches the description of *Pseudohalonectria phialidica*, which has scolecosporous ascospores and immersed, yellow-hued ascomata with long beaks (Shearer 1989). It shares the characteristic of yellow pigmentation seen in *C. lampadophora* however to a lesser extent. The yellow pigment is present around the outer wall cells and in the substrate at the base of the venter (FIG. 47). *Ceratosphaeria phialidica* has material deposited on the outside wall of the beaks, described as being "orange-brown amorphous" (Shearer 1989: 1952) and in this collection the

←



FIGS. 70–79. *Muraeriata africana*. 80–88. *Muraeriata collapsa*. 70, 71, 80, 81. Ascomata on the substrate. 72, 73, 88. Longitudinal section through ascomatal wall. 74, 83, Paraphyses. 75, 76, 85. Asci. 77, 84. Ascus apex with ring. 78, 79, 86, 87. Ascospores. 82. Longitudinal section through ascoma. 70, 71, 80, 81 by photomicrography; 72, 73, 76, 78, 79, 82, 85–88 by DIC; 74, 75, 77, 83, 84 by PH. Bars: 70, 71, 80, 81 = 1 mm; 82 = 100 μ m; 72, 73, 88 = 20 μ m; 74–79, 83–87 = 10 μ m. 70–79 from Mugambi 1084; 80, 81, 83–85, 87 from SMH4553; 82, 86, 88 from SMH2411.



FIGS. 89–97. *Ophioceras dolichostomum*. 89–91. Ascomata on the substrate. 92, 93. Ascomatal neck surface. 94. Paraphyses. 95. Ascus apices with rings. 96. Ascus. 97. Ascospores. 89–91 by photomicrography; 92, 93, 95–97 by DIC; 94 by PH. Bars: 89–91 = 1 mm; 92–97 = 10 μ m. 89, 90 from NY isotype; 91–97 from SMH1888.

material appears to be yellow crystalline (FIG. 43). The phialidic anamorph that further distinguishes *C. phialidica* also was found in this collection. The conidia in this collection are longer than those reported by Shearer (1989: 1952), but the illustrations (Shearer 1989: 1953) show sizes of conidia in relation to bar lines that are similar to those reported here. The anamorph of *C. phialidica* is similar to the *Harpophora*-like anamorph reported for *C. lampadophora* (Réblová 2006).

Another member of this LSU *Ceratospaeria* clade, although without support, is *Pseudohalonectria lignicola* Minoura & T. Muroi. Yellow pigments occur in cultures of this species (Shearer 1989), although in the collection from which our sequence was generated this feature was not observed. In the SSU analyses *Pseudohalonectria falcata* Shearer and a different collection of *P. lignicola* do not cluster with *C. lampadophora*.

Genus *Lentomitella* is enlarged with the addition of two new species that occur as a sister clade of two known species in the LSU analyses. The size range of ascospores in *L. tropica* is similar to that of *L. tomentosa* Réblová & J. Fourn., while that of *L. pallibrunnea* is similar to *L. crinigera*. However *Lentomitella pallibrunnea* and *L. tropica* differ from the other species in the genus by having ascospores that are pigmented before discharge. This is a characteristic attributed to *Xylomelasma* Réblová (Réblová 2006), but the new species are not genetically close to this genus nor do they have the other generic characteristics of terminal ascospore pores, free-floating asci and ramifying ascogenous hyphae. The ascomal morphologies in all the species of *Lentomitella* appear to be similar but *L. pallibrunnea* differs from *L. tropica* by smaller ascomata that consistently lie oblique to horizontal on the substrate with the neck at one end. Several

attempts were made to obtain these two species of *Lentomitella* in culture, but germination was not observed.

CONCLUSION

The Magnaporthaceae s.l. is expanded to include two new genera, *Ceratosphaerella* and *Muraeriata*. Morphological characteristics vary along the following lines. Robust aggregations of hyphae that form into rhizomorph-like structures are found in *Ceratosphaerella rhizomorpha* and are not known from other taxa in the group. Ascomata occur superficially and in large clusters on the substrate in both *Ceratosphaerella* and *Muraeriata*. In both these genera and in *Ceratosphaeria* and *Ophioceras* the ascomata have very long necks. Ascomatal walls are multilayered assemblages in *Ceratosphaeria lampadophora* and *Muraeriata*; the outer wall layer of *Ceratosphaerella* and *Ophioceras* is composed of globose cells; simpler two-layered structures are seen in the other taxa. Ascus rings are tall wedge-shaped in *Ceratosphaeria*, *Ceratosphaerella*, *Muraeriata*, *Pseudohalonectria* and *Ophioceras*; shorter, donut-like in *Buergenerula*, *Magnaporthe* and *Gaeumannomyces*. Fairly wide, simple paraphyses or paraphyses-like structures are reported for all taxa. Ascospore morphology in the family can vary from clavate in *Buergenerula*, fusiform in *Magnaporthe*, *Ceratosphaeria*, *Ceratosphaerella* and *Muraeriata*, to scolecosporous in *Ophioceras*, *Gaeumannomyces*, *Pseudohalonectria* and *Ceratosphaeria*, even to a mixture of different kinds within the different genera. The previously hyaline-spored *Lentomitella* is expanded to include two species with pale brown ascospores, but the genus remains of uncertain placement in Sordariomycetidae.

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