A molecular re-appraisal of taxa in the Sordariomycetidae and a new species of Rimaconus from New Zealand

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Abstract: Several taxa that share similar ascomatal and ascospore characters occur in monotypic or small genera throughout the Sordariomycetidae with uncertain relationships based on their morphology. Taxa in the genera Duradens, Leptosporella, Linocarpon, and Rimaconus share similar morphologies of conical ascomata, carbonised peridia and elongate ascospores, while taxa in the genera Caudatispora, Erythromada and Lasiosphaeriella possess clusters of superficial, obovoid ascomata with variable ascospores. Phylogenetic analyses of 28S large-subunit nrDNA sequences were used to test the monophyly of these genera and provide estimates of their relationships within the Sordariomycetidae. Rimaconus coronatus is described as a new species from New Zealand; it clusters with the type species, R. jamaicensis. Leptosporella gregaria is illustrated and a description is provided for this previously published taxon that is the type species and only sequenced representative of the genus. Both of these genera occur in separate, well-supported clades among taxa that form unsupported groups near the Chaetosphaeriales and Helminthosphaeriaceae, Lasiosphaeriella and Linocarpon appear to be polyphyletic with species occurring in several clades throughout the subclass. Caudatispora and Erythromada represented by single specimens and two putative Duradens spp. have unclear affinities in the Sordariomycetidae.

Key words: Ascomycota, Caudatispora, Duradens, Erythromada, Lasiosphaeriella, Leptosporella, Linocarpon, LSU, systematics.

Taxonomic novelties: Rimaconus coronatus Huhndorf & A.N. Mill., sp. nov

INTRODUCTION

In recent years molecular data have helped to clarify relationships among the many taxa in the Sordariomycetidae. A number of taxonomic novelties have been described with sequence data useful in the placement of these new taxa. In our own phylogenetic studies of wood-inhabiting ascomycetes we have found species that consistently cluster around the Chaetosphaeriales but without the benefit of strongly supported branches. Some of these taxa share similar morphologies in possessing conical ascomata, carbonised peridia and elongate ascospores, while others possess clusters of superficial, obovoid ascomata with variable ascospores. Caudatispora biapiculatis, Duradens sp., Erythromada lanciospora, Lasiosphaeriella nitida, Leptosporella gregaria, Linocarpon appendiculatum, and Rimaconus jamaicensis were included in analyses of the 28S large-subunit (LSU) nrDNA and were consistently found to occur in the Sordariomycetidae on unsupported branches outside of the Chaetosphaeriales and Helminthosphaeriaceae (Huhndorf et al. 2004, Miller & Huhndorf 2004, Huhndorf et al. 2005, Miller & Huhndorf 2005). Ongoing surveys of wood-inhabiting ascomycetes have uncovered additional taxa with morphologies that suggest affinities to Duradens, Leptosporella, and Rimaconus. Sequence data from these taxa and Lasiosphaeriella and Linocarpon were assembled to further assess the phylogenetic relationships in this group of Sordariomycetidae. A new species of Rimaconus is described and illustrated from New Zealand.

Table 1. Taxa	sequenced	for	this	study.	All	specimens	are
deposited in F.							

Taxon	Source	Origin	LSU GenBank Accession No.
Duradens sp. 2	SMH4427	Ecuador	HM171282
Lasiosphaeriella nitida	SMH1290	Puerto Rico	HM171283
Lasiosphaeriella noonae- daniae	SMH2818	Thailand	HM171284
Lasiosphaeriella pseudobombarda l	SMH4365	Ecuador	HM171285
Lasiosphaeriella pseudobombarda II	SMH4370	Ecuador	HM171286
Leptosporella gregaria II	SMH4673	Ecuador	HM171287
Leptosporella gregaria III	SMH4867	Costa Rica	HM171288
Leptosporella gregaria IV	SMH4700	Ecuador	HM171289
Linocarpon-like sp. 1	SMH3782	Puerto Rico	HM171290
Linocarpon-like sp. 2	SMH1600	Puerto Rico	HM171291
Rimaconus coronatus	SMH5212	New Zealand	HM171292
Rimaconus jamaicensis	SMH4782	Ecuador	HM171293

MATERIALS AND METHODS

Taxon sampling

Taxa sequenced in this study are listed in Table 1 with additional collection data provided under the examined specimens for selected taxa. Representatives from families and orders within

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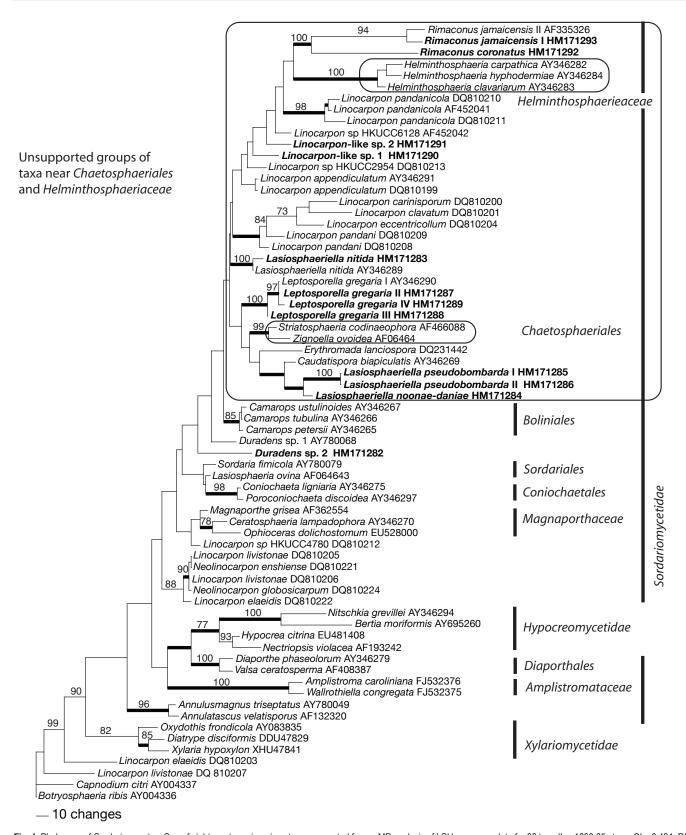


Fig. 1. Phylogeny of Sordariomycetes. One of eight most-parsimonious trees generated from a MP analysis of LSU sequence data for 68 taxa (L = 1690.65 steps, CI = 0.431, RI = 0.691, RC = 0.298). Taxa sequenced for this study are in bold. Thickened branches indicate Bayesian posterior probabilities \geq 95 % while numbers above or below branches refer to MP bootstrap values \geq 70 %. Two species in the *Dothideomycetes* are outgroups.

the *Sordariomycetes* were included to determine the phylogenetic position of the target taxa. Two members of the *Dothideomycetes* were used as outgroups. 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 using photomacrography, bright field (BF),

phase contrast (PH), and differential interference microscopy (DIC). Photographic plates were produced following the methods of Huhndorf & Fernández (1998). Format of the individual figures for the species follow those produced for the pyrenomycetes website (*Pyrenomycetes of the World*: www-s.life.illinois.edu/pyrenos/). The scale bars for the figures are as follows: ascomata bars = 500 μ m; ascus bars = 10 μ m; ascospore bars = 10 μ m.

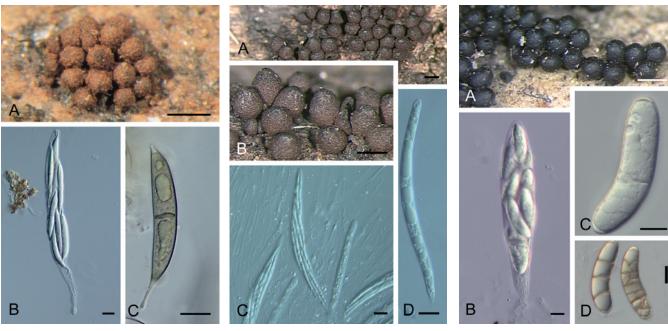


Fig. 2. *Caudatispora biapiculatis* (AY346269; *SMH1873*). A. Ascomata. B. Ascus. C. Ascospore.

Fig. 3. *Erythromada lanciospora* (DQ231442; SMH1526). A, B. Ascomata. C. Ascus. D. Ascospore.

Fig. 4. Lasiosphaeriella nitida (HM171283; *SMH1290*). A. Ascomata. B. Ascus. C, D. Ascospores.

DNA extraction, PCR amplification and sequencing

Detailed protocols for the extraction, amplification and sequencing of partial LSU are described in Huhndorf *et al.* (2004).

Sequence alignment and phylogenetic analyses

Sequences were assembled and aligned by eye using Sequencher v. 4.7 (Gene Codes Corp., Ann Arbor, Michigan). Maximum parsimony (MP) and maximum likelihood (ML) analyses were performed using PAUP v. 4.0b10 (Swofford 2002). Fifty-nine and 210 bp of the 5' and 3' ends respectively were excluded from all analyses due to missing data in most taxa. Twelve ambiguously aligned regions totaling 340 bp were delimited and excluded from analyses along with two spliceosomal introns (Bhattacharya et al. 2000) with lengths of 67 bp and 75 bp. A portion of the phylogenetic signal was recovered from three of the ambiguously aligned regions by recoding them using the program INAASE (Lutzoni et al. 2000). The remaining nine ambiguously aligned regions could not be recoded due to their size so they were excluded from all analyses. The remaining unambiguously aligned characters were subjected to a symmetrical stepmatrix to differentially weight nucleotide transformations using STMatrix v. 2.2 (François Lutzoni & Stefan Zoller, Biology Dept., Duke University, Durham, North Carolina), which calculates the costs for changes among character states based on the negative natural logarithm of the percentages of reciprocal changes between any two character states. Unequally weighted MP analyses were performed with 1 000 stepwise random addition heuristic searches, TBR branch-swapping, MULTREES option in effect, zero-length branches collapsed, constant characters excluded and gaps treated as missing. Branch support was estimated by performing 100 bootstrap replicates (Felsenstein 1985) each consisting of 10 stepwise random addition heuristic searches as above. MODELTEST v. 3.7 (Posada & Crandall 1998) determined the best-fit model of evolution for LSU to be the GTR model (Rodríguez et al. 1990) with a proportion of invariable sites while the remaining sites were subjected to a gamma distribution shape parameter. ML analyses

were performed using the above model with 100 stepwise random addition replicates and TBR branch-swapping with a reconnection limit of twelve. Constant characters were included and ambiguously aligned characters were excluded from the ML analyses. Bayesian analyses were performed using MrBayes v. 3.1 (Huelsenbeck & Ronquist 2001, Ronquist & Huelsenbeck 2003) as an additional means of assessing branch support. Constant characters were included, the above model of evolution was implemented, and 100 M generations were sampled every 1000th generation resulting in 100 000 total trees. The Markov chain always achieved stationarity after the first 100 000 generations, so the first 10 000 trees, which extended well beyond the burn-in phase of each analysis, were discarded. Posterior probabilities were determined from a 95 % consensus tree generated using the remaining 90 000 trees. This analysis was repeated twice starting from different random trees to ensure trees from the same tree space were ultimately being sampled during each analysis.

RESULTS

Sequence alignment and phylogenetic analyses

The LSU alignment contained 68 taxa and 1 338 characters of which 1 134 were excluded. Three ambiguously aligned regions were delimited and recoded resulting in 204 parsimony-informative characters. The MP analysis generated eight most-parsimonious trees, which did not differ significantly in topology. One of these most-parsimonious trees is shown in Fig. 1. The ML analysis generated two most likely trees, which did not differ significantly from one another or from the most-parsimonious trees (data not shown).

Species relationships

The LSU phylogeny contains a clade representing the proposed new species of *Rimaconus* supported by both bootstrap support

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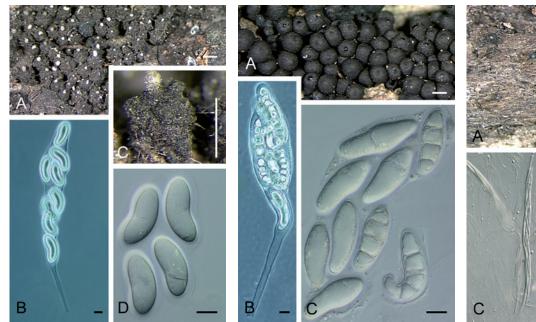


Fig. 5. Lasiosphaeriella noonae-daniae (HM171284; SMH2818). A, C. Ascomata. B. Ascus. D. Ascospores.

Fig. 6. Lasiosphaeriella pseudobombarda (HM171286; SMH4370). A. Ascomata. B. Ascus. C. Ascospores.

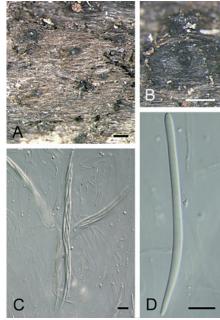


Fig. 7. Duradens sp. 1 (AY780068; SMH1708). A, B. Ascomata. C. Ascus. D. Ascospore.

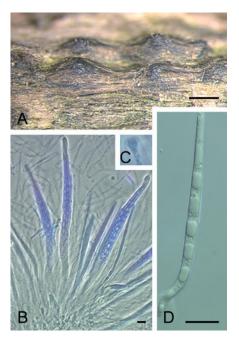


Fig. 8. Duradens sp. 2 (HM171282; SMH4427). A. Ascomata. B. Asci. C. Ascus ring. D. Ascospore.

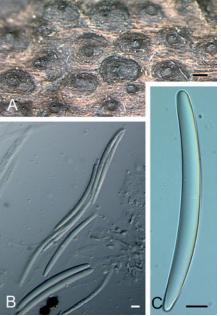


Fig. 9. Linocarpon-like sp. 2 (HM171291; SMH1600). A. Ascomata. B. Ascus. C. Ascospore.

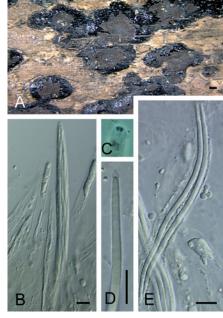


Fig. 10. Linocarpon-like sp. 1 (HM171290; SMH3782). A. Ascomata. B. Ascus. C. Ascus ring. D. Ascospore appendage. E. Ascospores.

(BS) and significant Bayesian posterior probability (PP). These data reveal a strongly supported clade containing all the collections of Leptosporella gregaria. The genus Lasiosphaeriella appears to be polyphyletic with the species clustering in two separate clades. The two collections of Lasiosphaeriella nitida group together with 100 % BS as do the two collections of Lasiosphaeriella pseudobombarda. In these analyses L. pseudobombarda groups with L. noonaedaniae, Duradens sp. 1, Duradens sp. 2, Linocarpon-like sp. 1, and Linocarpon-like sp. 2 occur on single unsupported branches in the Sordariomycetidae. The genus Linocarpon appears to be polyphyletic with species clustering in multiple separate clades scattered throughout the tree.

TAXONOMY

Images of sequenced taxa are included for comparison of morphological characteristics: Caudatispora biapiculatis (Fig. 2), Erythromada lanciospora (Fig. 3), Lasiosphaeriella nitida (Fig. 4), L. noonae-daniae (Fig. 5), L. pseudobombarda (Fig. 6), Duradens sp. 1 (Fig. 7), Duradens sp. 2 (Fig. 8), Linocarpon-like sp. 2 (Fig. 9), Linocarpon-like sp. 1 (Fig. 10), Leptosporella gregaria (Figs 11-15) and Rimaconus jamaicensis (Fig. 16). A description of Leptosporella gregaria is included here because it was not provided previously (Huhndorf & Fernández 2005).

Leptosporella gregaria Penz. & Sacc., Malpighia 11: 407. 1897. Figs 11-15.

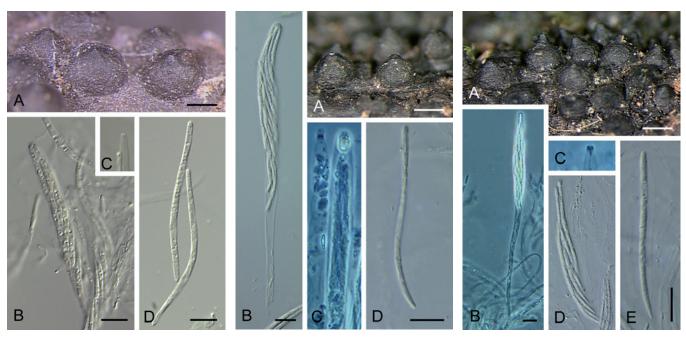
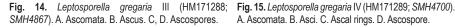


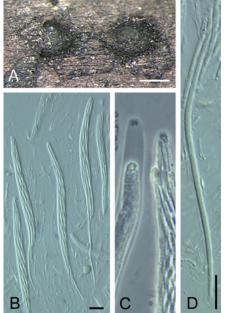
Fig. 11. Leptosporella gregaria (holotype; PAD). A. Ascomata. B. Ascus. C. Ascus ring. D. Ascospores.

Fig. 12. Leptosporella gregaria I (AY346290; SMH4290). A. Ascomata. B. Ascus. C. Ascal rings. D. Ascospore.

Fig. 13. Leptosporella gregaria II (HM171287; SMH4673). A. Ascomata. B, D. Asci. C. Ascus ring. E. Ascospore.







A. Ascomata. B. Asci. C. Ascal rings. D. Ascospore.



Fig. 16. Rimaconus jamaicensis (HM171293; SMH4782). A. Ascomata. B. Ascus. C. Ascal rings. D. Ascospore.

Anamorph: None known.

Ascomata conical, hemispherical to mammiform, papillate, ostiolate, 600-1100 µm diam, 500-800 µm high, separate, gregarious often in large groups, immersed, becoming erumpent with or without fragments of host cells adherent to ascomal wall, surface roughened, dark brown appearing black. Ascomal wall in longitudinal section 40-60 µm thick, composed of polygonal, strongly melanised, pseudoparenchymatic cells, often mixed with host cells, very thin at base, mostly composed of fungal hyphae growing in host cells, a wedge of elongate, thinner-walled cells ca. 95 µm thick at periphery. Ascomal apex acute or rounded, ostiole circular, with indistinct periphyses. Paraphyses abundant, persistent, narrow, tapering towards apex, with gelatinous coating, centrum with distinct yellow pigment. Asci cylindrical, 90-110 ×

8-10 µm, stalked, numerous, basal and lateral, partially lining the peripheral wall of centrum, unitunicate, apex tapered, with refractive ring, with 8 tri- to tetraseriate ascospores. Ascospores filiform, mostly 44-60 \times 2-3 μ m, long-spored collections 85-90 \times 2-3 to 107–137 × 2.8–4 μm, curved, hyaline, at times staining yellow from centrum pigments, one-celled, without sheath or appendages.

Habitat: On decorticated wood.

Distribution: Costa Rica, Ecuador, Indonesia.

Specimens examined: Costa Rica, Puntarenas, Area de Conservacion Osa, Parque Nacional Corcovado, Sirena Station, Espaveles trail, elev. 5 m, 8.4814 N, 83.595 W, on wood fragment, 17 July 2000, F.A. Fernández SMH4290, F; Alajuela Prov., Alberto Manuel Brenes Biological Reserve, near San Ramón, elev. 1000 m, on branch, 2-5 Dec. 2002, S. M. Huhndorf, F.A. Fernández SMH4867, F. Ecuador,

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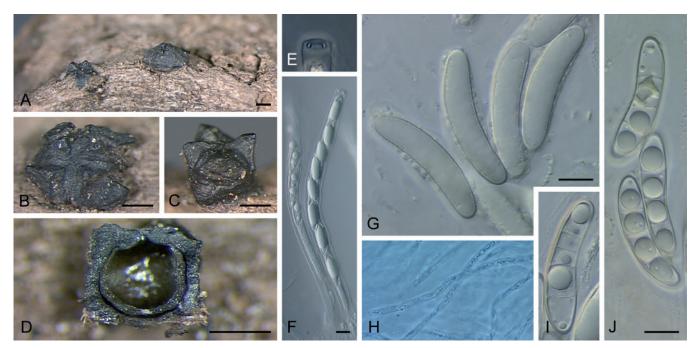


Fig. 17. Rimaconus coronatus (HM171292; SMH5212). A-D. Ascomata. E. Ascus ring. F. Ascus. G, I, J. Ascospores. H. Paraphyses.

Orellana Prov., Yasuni Biosphere Reserve, Tiputini Biological Station, Guacamayo trail, first 500 m, 0.6361 S, 76.1528 W, on log, 24 Mar. 2002, F. A. Fernández, A. N. Miller SMH4673, F; Matapalo trail, 0.6361 S, 76.1528 W, on palm petiole, 25 Mar. 2002, F. A. Fernández, A. N. Miller SMH4700, F. Indonesia, hab. in ligno putri, Tjibodas, 2 Feb. 1897, n. 135, Holotype PAD.

Notes: Leptosporella was described for two species, L. gregaria and L. sparsa; L. gregaria was selected as the type of the genus in Clements & Shear (1931). Currently 13 species are listed in Index Fungorum (www.indexfungorum.org). The type specimen of L. gregaria has abundant perithecia in marginal condition. Ascomatal contents are mostly agglutinated and distinct ascospores and asci are not abundant. Recent collections of this species from Costa Rica and Ecuador show unitunicate asci and hyaline scolecosporous ascospores. Leptosporella sparsa is probably a species of Lasiosphaeria based on the original drawings on the type specimen packet. The type specimen of L. sparsa no longer contains any perithecia, therefore, the name should be disregarded.

Rimaconus coronatus Huhndorf & A.N. Mill., **sp. nov**. MycoBank MB518333. Fig. 17. *Anamorph*: None known.

Etymology: coronatus refers to the crown-shaped ascomatal apex.

Similis *R. jamaicensis* sed ascomata conica vel cylindrica, 900–1 500 μ m diametro, 700–1 000 μ m alta, apex planus ad depressus, coronatus. Asci cylindrici, pars sporiferi 190–250 × 13–15 μ m, stipitati 18.5–51 × 2–5 μ m. Ascosporae fusiformes vel cylindricae, 36–42 × 7.5–9 μ m, hyalinae, triseptatae usque ad hexaseptatae.

Ascomata conical to applanate when young, becoming hemispherical or conical to cylindrical with coronate projections around apical rim, non-papillate, ostiolate, 900–1 500 μm diam, 700–1 000 μm high, separate to gregarious in small groups, immersed becoming erumpent, with fragments of host cells adherent to ascomal wall when young, surface roughened, dark brown appearing black. Ascomal wall in longitudinal section ca. 100–130 μm thick, composed of strongly melanised cells, thicker, ca. 250–400 μm, with coronate projections around periphery of

apex, somewhat thinner at base. Ascomal apex flattened to sunken, crater-like, ostiole circular; periphyses not seen. Paraphyses 3–4 μm wide, abundant, persistent, narrow, tapering towards apex. Asci cylindrical, spore- bearing part 190–250 \times 13–15 μm , stalk 36–50 μm long, numerous, basal and lateral, partially lining peripheral wall of centrum, unitunicate, apex tapered, with refractive ring 5 μm wide, with 8 overlapping uniseriate ascospores. Ascospores broadly fusiform to short cylindrical, broadly rounded at apex and base, 36–42 \times 7.5–9 μm , curved symmetrical, hyaline, smooth, mostly 3-septate, a few up to 6-septate, without constrictions at septa, primary septum median, septa evenly distributed, without sheath or appendages.

Habitat: On decorticated wood.

Distribution: New Zealand.

Specimen examined: **New Zealand**, Auckland, Kawakawa Bay, Morehu Reserve, 36.9708 S, 175.1793 E, on large, decorticated log, 5 June 2008, S. M. Huhndorf, P. R. Johnston SMH5212, **holotype** PDD, **isotype** F.

DISCUSSION

A number of taxa in the *Sordariomycetidae* occur as unsupported, single lineages or appear to have uncertain relationships in their molecular phylogenies often grouping with other taxa in unsupported clades. This does not mean they have entirely unknown affinities since they often consistently cluster together or near certain well-supported taxa. The taxa that consistently cluster outside but near the well-supported clades of *Chaetosphaeriales* and *Helminthosphaeriaceae* are one such group that has a diverse mix of morphological characteristics.

Within this admixture, a few groups of taxa form well-supported clades. *Rimaconus coronatus* occurs in a clade with the type species, *R. jamaicensis*. Both taxa reside on long branches indicating that a significant amount of divergence has occurred between these species. The two species share morphological

similarities such as dark-coloured, strongly melanised ascomata that are erumpent through the woody substrate. *Rimaconus coronatus* differs by forming flaring, crown-shaped extensions of the ascomatal wall. Both species share a wide, flat, refractive ascus ring and hyaline, septate ascospores. However, the ascospores differ in their shape and septation. In *R. jamaicensis* the 7+ septate ascospores are long cylindrical with a distinct bend at the slightly submedian position. In *R. coronatus* the 3+ septate ascospores are shorter, wider, and more evenly curved. The highly supported clade containing these two species occurs as an unsupported sister group to the *Helminthosphaeriaceae*, but that relative placement is unstable.

Multiple specimens of Leptosporella gregaria form another wellsupported clade within the pectinate topology of taxa clustering with the Chaetosphaeriales and Helminthosphaeriaceae. The species is distinguished by conical, erumpent ascomata and scolecosporous ascospores. The type specimen provides adequate morphological information to allow identification of fresh specimens. With ascospores measuring 60-67 × 2-3 µm, the type specimen from Indonesia fits in the middle of the range of measurements from the sequenced collections. Among the four specimens with sequence data, the morphology is not entirely uniform. Leptosporella gregaria I (SMH4290) and L. gregaria II (SMH4673) have ascomata and ascospores that are somewhat smaller in size (ascospores $37-56 \times 2-3 \mu m$) than those of the type specimen; L. gregaria IV (SMH4700) has smaller ascomata and spores longer than the type specimen (85–90 × 2–3 µm). Leptosporella gregaria III (SMH4867) has ascomata that are of a size close to the type specimen but the ascospores are almost twice as long (107-137 × 2.8-4 µm). All of them share a distinctive yellow colouration of the centrum that in some collections is often pronounced enough to stain some of the ascospores and asci yellow (Fig. 14). Leptosporella gregaria III (SMH4867) may represent a distinct species but given the mixture of collections in this overall group, sequences of additional specimens are necessary before another new species is described. In this analysis the clade containing these specimens occurs as an unsupported sister group to the Chaetosphaeriales and several other taxa, but their relative placement is not stable.

Other taxa clustering near the Chaetosphaeriales and Helminthosphaeriaceae possess conical, immersed to erumpent ascomata and scolecosporous ascospores. The numerous species of *Linocarpon* included in this analysis do not form a monophyletic group. Representatives of the type species, L. pandani, form a supported group with three other species, while three collections of *L. pandanicola* form the only well-supported clade in the genus. Additionally, several named species of Linocarpon occur well outside this group of taxa scattered among other Sordariomycetidae as well as outside the subclass. Our own collections of *Linocarpon*like taxa do not provide any resolution to the question of what indicates relationships within the genus. Linocarpon-like sp. 1 (SMH1600) differs from the other described Linocarpon species in having erumpent ascomata, no clypeus and wide ascospores (Fig. 9). Linocarpon-like sp. 2 (SMH3782) appears to differ by having ascomata that are not separate but cluster together under a united clypeate covering. The ascomata have separate central ostioles thus precluding its placement in the genus Palmicola. Using molecular data their unsupported positions leave unclear the affinities of taxa within *Linocarpon*.

Near the Chaetosphaeriales and Helminthosphaeriaceae reside a number of taxa that have dense clusters of obovoid ascomata and occur superficially on the substrate. Species of Lasiosphaeriella have widely allantoid to ellipsoid ascospores that

suggest morphological relatedness (Figs 4-6). However, these species do not form a single clade, but instead separate into two clades with L. nitida appearing to be distant from the other two species. In this analysis two additional unsupported taxa basal to *L.* noonae-daniae and L. pseudobombarda have clusters of superficial ascomata. Caudatispora biapiculatis has roughened ascomata as does L. noonae-daniae, but the ascospores have unique apical and basal wall extensions (Figs 2, 5). Erythromada lanciospora differs from the other gregarious taxa in having thin, elongate, lanceolate ascospores (Fig. 3). The presence of this scolecosporous ascospore type resembles those found in other species that are prevalent in this unsupported group. Lastly, two collections designated as Duradens spp. with morphology suggestive of inclusion in the unsupported group nest outside the group on branches between the Boliniales and Sordariales. Duradens was described as a monotypic genus for a single collection from Guyana (Samuels & Rogerson 1990). Duradens lignicola occurs as heavily carbonised, conical, erumpent ascomata on decorticated wood and has long, relatively wide ascospores. The generic description could match either unnamed species as well as the unnamed Linocarpon-like sp. 2. Describing these species in *Duradens* would create another polyphyletic genus. Choosing which species in the tree best fits the genus based on *D. lignicola* is problematic.

Where then is the predictability from the morphology in this group? Same-named species occur widely spaced in the tree suggesting difficulty in correctly identifying species and applying names. Beyond the molecular work, we find the same difficulty among our own collections when faced with only morphological data for identification. For the taxa remaining unnamed in this tree, there is no enthusiasm for erecting additional monotypic genera of uncertain affinities based on single collections. We choose to supply the sequences and illustrations in hopes that sister taxa may yet be uncovered that will allow for some confidence in applying names. The stability in classification surrounding the *Chaetosphaeriales* and *Helminthosphaeriaceae* will probably require extensive future sequencing of multiple genes.

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