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# Systematics of the genus *Chaetosphaeria* and its allied genera: morphological and phylogenetic diversity in north temperate and neotropical taxa

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***Abstract:*** *Chaetosphaeria* is a common saprobic pyrenomycete genus with simple, homogeneous teleomorphs and complex, diverse anamorphs. As currently circumscribed in the literature, the genus encompasses 30 species distributed in four 'natural groups', and includes morphological entities in 11 anamorphic genera. Species frequently have been defined primarily based on characters of the anamorphs resulting in species with almost indistinguishable teleomorphs. This study aimed to assess the value and significance of morphological characters in resolving phylogenetic relationships in *Chaetosphaeria* and its allied genera. Phylogenetic relationships of 42 taxa, representing 29 species distributed in *Chaetosphaeria* and five related genera, were estimated with partial sequences of the nuclear LSU rDNA and β-tubulin genes. Sequences were analyzed with maximum parsimony, maximum likelihood and Bayesian methods. Phylogenetic analyses of these two genes combined revealed two major lineages. The *Chaetosphaeria* lineage includes 21 species possessing both typical and new sexual and asexual morphologies. The lineage contains a strongly supported monophyletic clade of 13 species and eight paraphyletic taxa; the latter includes *C. innumera*, the type species of the genus. The second major lineage includes groupings concordant with the morphological circumscriptions of the genera *Melanochaeta*, *Melanopsammella*, *Striatosphaeria*, *Zignoëlla* and the new genus *Tainosphaeria*.

***Key words:*** anamorph, β-tubulin, phylogeny, rDNA, taxonomy, teleomorph

## INTRODUCTION

*Chaetosphaeria* Tul. & Tul. and its allied genera are commonly found worldwide occurring as saprobic pyrenomycetous ascomycetes which reproduce both sexually and asexually on extensively decomposed plant substrates. Since its original description (Tulasne and Tulasne 1863), the genus has been repeatedly redefined (Saccardo 1883; Booth 1957, 1958; Müller and von Arx 1962; Gams and Holubova-Jechova 1976; Réblová 1999, 2000). The circumscription and phylogeny of the genus recently has been reviewed (Réblová 2000, Réblová and Winka 2000). It currently encompasses 30 species distributed in four natural groups, which also includes morphological entities in 11 anamorphic genera (Réblová 2000). Taxonomic opinions at the family level have placed the genus in the Trichosphaeriaceae (Dennis 1978), Lasiosphaeriaceae (Barr 1990), and Chaetosphaeriaceae (Réblová et al 1999).

In general *Chaetosphaeria* teleomorphs are simple and relatively homogeneous, while their anamorphs are complex and diverse. Therefore, species identification is based primarily on characters of the anamorphs (Gams and Holubova-Jechova 1976, i.e. in genera such as Chloridium, Codinaea, Dictyochaeta), resulting in species with almost indistinguishable teleomorphs in many instances. Species identification can become even more challenging when morphological information on anamorphs is not available. When anamorph data are available from culture, there is the possibility of encountering altered or aberrant morphologies. In addition, some anamorphic taxa connected to *Chaetosphaeria* are monophyletic.

Closely allied genera have been distinguished and sometimes segregated from *Chaetosphaeria* based on teleomorph characters alone or in combination with anamorph characters. The preferred teleomorph character used in generic delimitation has been ascospore morphology: ascospore pigmentation in *Melanochaeta* E. Müll., Harr & Sulmont, ascospore disarticulation in *Melanopsammella* Höhn. and ascospore surface ornamentation in *Striatosphaeria* Sam-

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TABLE I. List of taxa used in the molecular analyses. All sequences are from the large subunit nrDNA and β-tubulin genes unless indicated

| Taxa | Culture designation[a] | Geopolitical origin | nr-lsu-DNA | β-tubulin |
|---|---|---|---|---|
| Cercophora newfieldiana (Ellis & Everh.) R. Hilber | SMH 2622 | USA (Michigan) | AF064642 | AF466019 |
| Chaetosphaeria caesariata (Cooke & Peck) F. A. Fernández & Huhndorf | SMH 2794 | USA (South Carolina) | AF466060 | AF466020 |
| Chaetosphaeria capitata Sivan. & H. S. Chang | SMH 3239 | Costa Rica | AF466061 | AF466021 |
| Chaetosphaeria callimorpha (Mont.) Sacc. | SMH 2791 | USA (South Carolina) | AF466062 | AF466022 |
| Chaetosphaeria chalaroides Hol.-Jech. | SMH 2018 | Puerto Rico | AY017372 | AF466023 |
| Chaetosphaeria chalaroides | SMH 2223 | Costa Rica | AF466063 | AF466024 |
| Chaetosphaeria chlorotunicata F. A. Fernández & Huhndorf | SMH 1565 | Puerto Rico | AF466064 | AF466025 |
| Chaetosphaeria chlorotunicata | SMH 3074 | Puerto Rico | AF466065 | AF466026 |
| Chaetosphaeria conirostris F. A. Fernández & Huhndorf | SMH 2183 | Costa Rica | AF466066 | AF466027 |
| Chaetosphaeria cubensis Hol.-Jech. | SMH 3258 | Costa Rica | AF466067 | AF466028 |
| Chaetosphaeria decastyla (Cooke) Réblová & W. Gams | SMH 2629 | USA (Indiana) | AF466068 | AF466029 |
| Chaetosphaeria hebetiseta Réblová & W. Gams | SMH 2729 | USA (North Carolina) | AF466069 | AF466030 |
| Chaetosphaeria innumera Berk. & Broome ex Tul. & C. Tul. | SMH 2748 | USA (North Carolina) | AY017375 | AF466018 |
| Chaetosphaeria lateriphiala F. A. Fernández & Huhndorf | SMH 2629-1 | USA (Indiana) | AF466070 | AF466031 |
| Chaetosphaeria lateriphiala | SMH 3294 | USA (North Carolina) | AF466071 | AF466032 |
| Chaetosphaeria lateriphiala | SMH 3320 | USA (South Carolina) | AF466072 | AF466033 |
| Chaetosphaeria lignomollis F. A. Fernández & Huhndorf | SMH 3015 | Puerto Rico | AF466073 | AF466034 |
| Chaetosphaeria longiseta F. A. Fernández & Huhndorf | SMH 1725 | Puerto Rico | AF279416 | AF466035 |
| Chaetosphaeria longiseta | SMH 3854 | USA (South Carolina) | AF279417 | AF466036 |
| Chaetosphaeria luquillensis F. A. Fernández & Huhndorf | SMH 2973 | Puerto Rico | AF466074 | AF466037 |
| Chaetosphaeria minuta F. A. Fernández & Huhndorf | SMH 3396 | Panama | AF466075 | AF466038 |
| Chaetosphaeria myriocarpa C. Booth nom. cons. | MUCL 34784 | Belgium | AF466076 | AF466039 |
| Chaetosphaeria pygmaea (P. Karst.) Constant., K. Holm & L. Holm | UPSC 2523 | Sweden | AF466077 | AF466040 |
| Chaetosphaeria raciborskii F. A. Fernández & Huhndorf | SMH 2017 | Puerto Rico | AF466078 | AF466041 |
| Chaetosphaeria spinosa F. A. Fernández & Huhndorf | SMH 2754 | North Carolina | AF466079 | AF466042 |
| Chaetosphaeria sylvatica F. A. Fernández & Huhndorf | SMH 2893 | Puerto Rico | AF279419 | AF466043 |
| Chaetosphaeria tropicalis F. A. Fernández & Huhndorf | SMH 1267 | Puerto Rico | AF279418 | AF466044 |
| Chaetosphaeria tropicalis | SMH 2250 | Costa Rica | AF466080 | AF466045 |
| Lasiosphaeria ovina (Pers.) Ces. & De Not. | SMH 1538 | USA (Wisconsin) | AF064643 | AF466046 |
| Melanochaeta aotearoae (S. Hughes) E. Müll., Harr & Sulmont | SMH 1655 | Puerto Rico | AF466081 | AF466047 |
| Melanochaeta aotearoae | SMH 3551 | Panama | AF466082 | AF466048 |
| Melanochaeta hemipsila (Berk. & Broome) E. Müll., Harr & Sulmont | SMH 2125 | Puerto Rico | AY346292 | AF466049 |

TABLE I. Continued

| Taxa | Culture designation[a] | Geopolitical origin | nr-lsu-DNA | β-tubulin |
|---|---|---|---|---|
| Melanochaeta hemipsila | SMH 3251 | Costa Rica | AF466084 | AF466050 |
| Melanopsammella gonytrichii F. A. Fernández & Huhndorf | SMH 3785 | Puerto Rico | AF466085 | AF466051 |
| Melanopsammella vermicularioides (Sacc. & Roum.) Réblová, M. E. Barr & Samuels | SMH 1985 | Puerto Rico | AF064644 | AF466053 |
| Melanopsammella vermicularioides | SMH 3883 | USA (Michigan) | AF466086 | AF466054 |
| Melanopsammella vermicularioides | FC404 | France | AF466087 | AF466052 |
| Striatosphaeria codinaeaphora Samuels & E. Müll. | SMH 1524 | Puerto Rico | AF466088 | AF466055 |
| Tainosphaeria crassiparies F. A. Fernández & Huhndorf | SMH 1934 | Puerto Rico | AF466089 | AF466056 |
| Zignoëlla ovoidea (Fr.) Sacc. | SMH 2605 | USA (Michigan) | AF064641 | AF466057 |
| Zignoëlla pulviscula (Curr.) Sacc. | MUCL 15710 | Germany | AF466090 | AF466059 |
| Zignoëlla pulviscula | SMH 3289 | USA (North Carolina) | AF466091 | AF466058 |

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uels & E. Müll. Preliminary molecular phylogenetic studies of *Chaetosphaeria* and allied taxa determined that ascospore pigmentation is a homoplasious morphological character in the group (Fernández et al 1998, 1999a, b). Relevant data have revealed ascospore morphology to be phylogenetically informative only at the species level.

Previous analyses of the nuclear large subunit (LSU) rDNA and β-tubulin genes have provided concordant phylogenies for *Chaetosphaeria* and some of its allied genera (Fernández et al 1999b). The studies presented here expand on the previous unpublished data and attempt to improve the phylogenetic framework for the genus *Chaetosphaeria* and its allied genera by analyzing partial sequence data from the LSU and β-tubulin genes from a diverse group of temperate and tropical taxa. The main goal of this study was to assess the value and significance of morphological characters in resolving phylogenetic relationships in these selected taxa, which is necessary for a taxonomic revision of *Chaetosphaeria* and related genera. Questions we considered were: (i) Does the anamorph have predictive value in these taxa? and (ii) Does the ascospore morphology reflect relatedness?

## MATERIALS AND METHODS

*Taxon sampling.*—Taxa in this study are listed (TABLE I), along with their geographical localities, collection or culture numbers and GenBank accession numbers. Two members of the Sordariales, *Cercophora newfieldiana*, and *Lasiosphaeria ovina* were used as outgroups because morphologically both genera have been traditionally considered as closely allied to *Chaetosphaeria* (Barr 1990) and they have been shown previously to have phylogenetic affinities to *Chaetosphaeria* based on molecular data (Fernández et al 1999a, Huhndorf et al 2004, Miller and Huhndorf 2004). All voucher specimens are deposited in the Field Museum Mycology Herbarium (F). Cultures of multispore isolates were obtained following the techniques of Huhndorf et al (2004) and are stored at the Field Museum. Cultures with an origin of USA or Puerto Rico are available through ATCC or CBS. Images were captured and photographic plates produced following the methods of Huhndorf and Fernández (1998).

*DNA isolation, PCR amplification, sequencing and sequence alignment.*—DNA isolation was performed as outlined in Fernández et al (1999a). Portions of the 5′ end of LSU rDNA and the 3′ end of β-tubulin genes (ca. 1.4 and 1.0 kb, respectively) were amplified with reagents in a Repli-pack Reagent set (Boehringer Mannheim Corp., Indianapolis, Indiana) in this manner: 2.5 μL of 10× reaction buffer with 25 mM $MgCl_2$(100 mM Tris, 500 mM KCl, pH 8.3), 5 μL of 8 mM dNTPs, 2.5 μL each of 10 μM primers LR0R and LR7 for the LSU rDNA (Moncalvo et al 1993) and primers BT1819R and BT2916 for the β-tubulin gene (Miller and Huhndorf 2005), 0.25 μL (1.25 units) of Taq DNA polymerase, 2 μL of the undiluted DNA extract and 32.75 μL of double distilled sterile water for a 50 μL total reaction volume. PCR was performed with these thermal cycling parameters: initial denaturation at 95 C for 2 min, followed by 35 cycles of denaturation at 95 C for 1 min, annealing at 50 C for 1 min and extension at 72 C for 1 min. A final extension step of 10 min at 72 C was added. Amplified products were separated from unincorporated nucleotides and primers with a Geneclean III kit (Bio 101 Inc., Vista, California).

Sequencing was performed on both strands with primers

LROR, LR3, LR3R, LR5, LR6 (Rehner and Samuels 1994, Vilgalys and Hester 1990, Vilgalys and Sun 1994) and LRFF1 (Huhndorf et al 2004) for the LSU rDNA and T22 (O'Donnell and Cigelnik 1997) Bt1a (Glass and Donaldson 1995), BT1819R, BT2916 and Bt1283 (Miller and Huhndorf 2005) for the β-tubulin gene. Sequencing reactions were performed with the ABI Prism Dye Terminator Cycle Sequencing kit (Perkin-Elmer Corp.). Sequenced products were precipitated with a 70% ethanol/5 mM $MgCl_2$ precipitation solution. Sequences were generated with an ABI Prism 377 DNA Sequencer (Applied Biosystems). Sequences were assembled and aligned with Sequencher version 3.0 (Gene Codes Corp.). Alignment was checked by eye and corrected manually when necessary. The alignment of the combined LSU and β-tubulin data sets is deposited in TreeBase (accession SN2098).

*Phylogenetic analyses.*—All phylogenetic analyses were performed with PAUP* 4.0b8 and 4.0b10 (Swofford 1998, 2001) compiled for the Apple/Macintosh platform. A total of nine ambiguously aligned regions were detected and delimited in the LSU rDNA with the method outlined in Lutzoni et al (2000). Five sequences (SMH 1267, 1725, 2250, 2893, 3854) representing three species contained a single splicesomal intron in the LSU rDNA (Bhattacharya et al 2000). Both datasets initially were analyzed separately. Two maximum parsimony analyses were performed on the LSU rDNA. In one analysis, ambiguously aligned regions were excluded, nucleotide substitutions within the remaining unambiguously aligned sites were equally weighted, and gaps were treated as missing data. In a second analysis, gaps were treated as a fifth character. Also, the nine ambiguously aligned regions along with the single spliceosomal intron splice site were included as 10 unequivocally coded characters, each subjected to a specific step-matrix derived from pairwise comparisons of sequences using the program INAASE 2.2b (Lutzoni et al 2000). The unambiguously aligned portion of the alignment was subjected to a specific symmetric stepmatrix as previously described (Fernández et al 1999a). Two maximum parsimony analyses, one with equally weighted and another with unequally weighted nucleotide substitutions, also were performed on the β-tubulin data. Intron sequence data were excluded. Exons in the β-tubulin data set were partitioned into first, second, and third codon positions, which were subjected to a specific stepmatrix following the same weighing scheme as previously described by Fernandez et al (1999a).

A heuristic search with 1000 random-addition sequence replicates was implemented for each analysis, with the branch-swapping algorithm set to TBR, the MULPARS option was in effect, and zero-length branches were collapsed. Support for internodes was estimated by performing 1000 bootstrap replicates with a heuristic search consisting of two random-addition sequence replicates for each bootstrap replicate. Conflicts between the two data sets were assumed to be significant when conflicting clades both had ≥70% bootstrap support. The combined dataset included 29 species plus two outgroup species and a total of 435 parsimony informative characters. A total of 1000 random-addition sequence replicates were implemented as previously described. The internode support was estimated by 1000 bootstrap replicates with heuristic searches consisting of 50 random-addition sequence replicates for each bootstrap replicate.

A heuristic maximum likelihood tree search was performed on the combined dataset with PAUP* 4.0b8 (Swofford 1998). Constant characters were excluded. Likelihood model parameters for the combined data set were estimated with Modeltest 3.5 (Posada and Crandall 1998). A general-time-reversible likelihood model (Tavaré 1986) with among site rate variation following a gamma shape distribution (GTR+G) was selected. In a first step, 100 random-addition sequence replicates were performed without branch swapping, saving the best tree of each replicate. The topologies of the resulting 100 trees were compared and none were found to be identical. Therefore all 100 trees from this first analysis were used as starting points for a second round of heuristic searches, with TBR branch swapping and a limit of 15 000 rearrangements per random addition replicate.

Bayesian posterior probabilities for each internode were calculated with a Metropolis-coupled Markov chain Monte Carlo ($MCMCMC^3$) sampling method as implemented in the program MrBayes, version 3.0b4 (Huelsenbeck and Ronquist 2001). The combined data set was assumed to have four distinct partitions (LSU, β-tubulin 1st, 2nd, 3rd codon positions). To determine which model of nucleotide substitution with the least number of parameters best fit each of these partitions, hierarchical likelihood ratio tests (LRTs) were performed with Modeltest 3.5 (Posada and Crandall 1998). For the LSU data set, a Tamura-Nei-93 likelihood model (Tamura and Nei 1993) including a proportion of invariable sites and among site rate variation following a gamma shape distribution (TrNef+I+G) was selected. This model also was selected for first position codons for the β-tubulin dataset. Second position codons were subjected to a Jukes and Cantor model (Jukes and Cantor 1969) with a proportion of invariable sites and equal among site rate variation (JC+I), while a transversional model (Modeltest 3.5) with among site rate variation following a gamma shape distribution (TVM+G) was selected for third position codons.

Five independent MrBayes analyses were run for 5 000 000 generations each for the combined dataset. Four Markov chains were run with trees sampled every 100th generation, resulting in 50 000 total trees per run (250 000 total trees). Log-likelihood scores were plotted against generation time with Excel 2004 (Microsoft Corp.) to determine the number of generations before the chains reached stationarity (i.e. burn-in phase). The first 10 000 generations represented the burn-in phase in all runs so the first 10 000 trees were excluded from further analysis. A majority rule consensus tree with the remaining 40 000 trees from one of these runs was computed with the SUMT command in MrBayes. The resulting posterior probability support values for bipartitions were considered significant at ≥95%.

## RESULTS

Maximum parsimony (MP) analyses of each dataset resulted in trees with similar topologies and overall higher bootstrap support values in the unequally weighted analyses (not shown). Also, the number of equally most parsimonious trees was reduced from 36 in the equally weighted to six in the unequally weighted analyses for the LSU rDNA dataset. Both the equally and the unequally weighted analyses of the β-tubulin dataset yielded a single most parsimonious tree. Comparisons of bootstrap support for clades in both trees show no conflicts. The LSU dataset had 19 branches with more than 70% bootstrap support, the β-tubulin dataset had 14 branches and the combined dataset had 20 branches. The LSU dataset alone provided the support for the putative sister lineage, the *Melanopsammella* clade and clade A. Maximum parsimony and maximum likelihood (ML) analyses based on the combined dataset resulted respectively in a single most parsimonious tree of 3033 steps (data not shown) and one most likely tree (-ln = 9172) (data not shown). Both trees have similar topologies. MP and ML analyses concur on two major lineages: the *Chaetosphaeria* lineage, represented by *Chaetosphaeria innumera* (the type species of the genus) along with 20 additional species; and a putative sister lineage which contains five genera, *Melanochaeta*, *Melanopsammella*, *Striatosphaeria*, *Tainosphaeria* F. A. Fernández & Huhndorf 2005 and *Zignoëlla* Sacc. 1878. The *Chaetosphaeria* lineage received higher support from the Bayesian MCMCMC analysis (posterior probability = 100) than from the MP bootstrap analysis (57%) (FIG. 1). The lineage contains a strongly supported (BS = 84, PP = 100) monophyletic clade of 13 species, which is further subdivided into two well supported sister groups: a large clade (A) composed of 10 different species and a small clade (B) composed of three species (FIG. 1).

In clade A there are two well supported internal clades. One consists of *C. capitata* and *C. chlorotunicata*, which consistently were found to be well supported sister species by MP bootstrap and Bayesian analyses (FIG. 1). The second clade contains *C. raciborskii*, *C. spinosa* and *C. conirostris* and had high Bayesian support and bootstrap support of 69% (FIG. 1). Phylogenetic affinities among *C. caesariata* (in Réblová 1999 as *Umbrinosphaeria caesariata* (Cooke & Peck) Réblová), *C. lignomollis*, *C. decastyla* and *C. chalaroides* were not well supported by either analysis within clade A. *C. cubensis* similarly does not exhibit clear phylogenetic relationships to the other species in clade A (FIG. 1). *Chaetosphaeria tropicalis*, *C. sylvatica* and *C. lateriphiala* group together in a clade (clade B) with strong bootstrap and Bayesian support.

Relationships among the remaining eight *Chaetosphaeria* species outside the lineage containing clades A and B are uncertain (FIG. 1). *C. longiseta* is the most divergent species in this group. *C. luquillensis* and *C. minuta* come together but this clade is unsupported (FIG. 1). Similarly, *C. callimorpha* and *C. hebetiseta* come together but also without significant support (FIG. 1). *Chaetosphaeria innumera*, *C. myriocarpa* and *C. pygmaea* were revealed as a monophyletic group with high posterior probability (99%, FIG. 1), but without bootstrap support ≥50%.

In contrast the putative sister lineage to *Chaetosphaeria*, is strongly supported (BS = 98 %, PP = 100%). Within this lineage three monophyletic groups were found that correspond to the morphological circumscriptions of three existing genera: *Melanopsammella*, *Melanochaeta* and *Zignoëlla*. *Tainosphaeria crassiparies* and *Striatosphaeria codinaeaphora* come together but with poor support. This clade is sister to the *Zignoëlla* clade showing high posterior probability. *Melanopsammella* is a sister group to *Melanochaeta*, but without support (FIG. 1). In the MP tree (not shown) *Melanochaeta* joins *Zignoëlla*, *Tainosphaeria* and *Striatosphaeria* in a clade that received bootstrap support (74%).

Spliceosomal introns were found in five taxa representing three species of *Chaetosphaeria*: *C. longiseta*, *C. tropicalis* and *C. sylvatica*. Details on their occurrence and molecular significance have been discussed previously (Bhattacharya et al 2000). Specimens of *C. tropicalis* (SMH 1267, SMH2250; TABLE I) are from disparate geographical areas but have identical intron sequences. Intron sequences for both specimens of *C. longiseta* show a total of eight base pair changes: seven transitions and one transversion, plus one insertion/deletion polymorphism. Intron sequences for *C. tropicalis* and *C. longiseta* are the same length. Comparisons among intron sequences revealed different levels of sequence similarity, reflecting of their level of phylogenetic relatedness (Bhattacharya et al 2000). No group 1 introns were found in nuclear rDNA in the specimens sequenced for this study.

## DISCUSSION

### *Relationships within* Chaetosphaeria

A phylogenetic scheme for *Chaetosphaeria* had been proposed previously based on analyses of morphology and nuclear ribosomal sequence data from northern European species (Réblová and Winka 2000). Our analyses of sequence data from 17 known species and 12 new species of temperate and neotropical *Chaeto-*

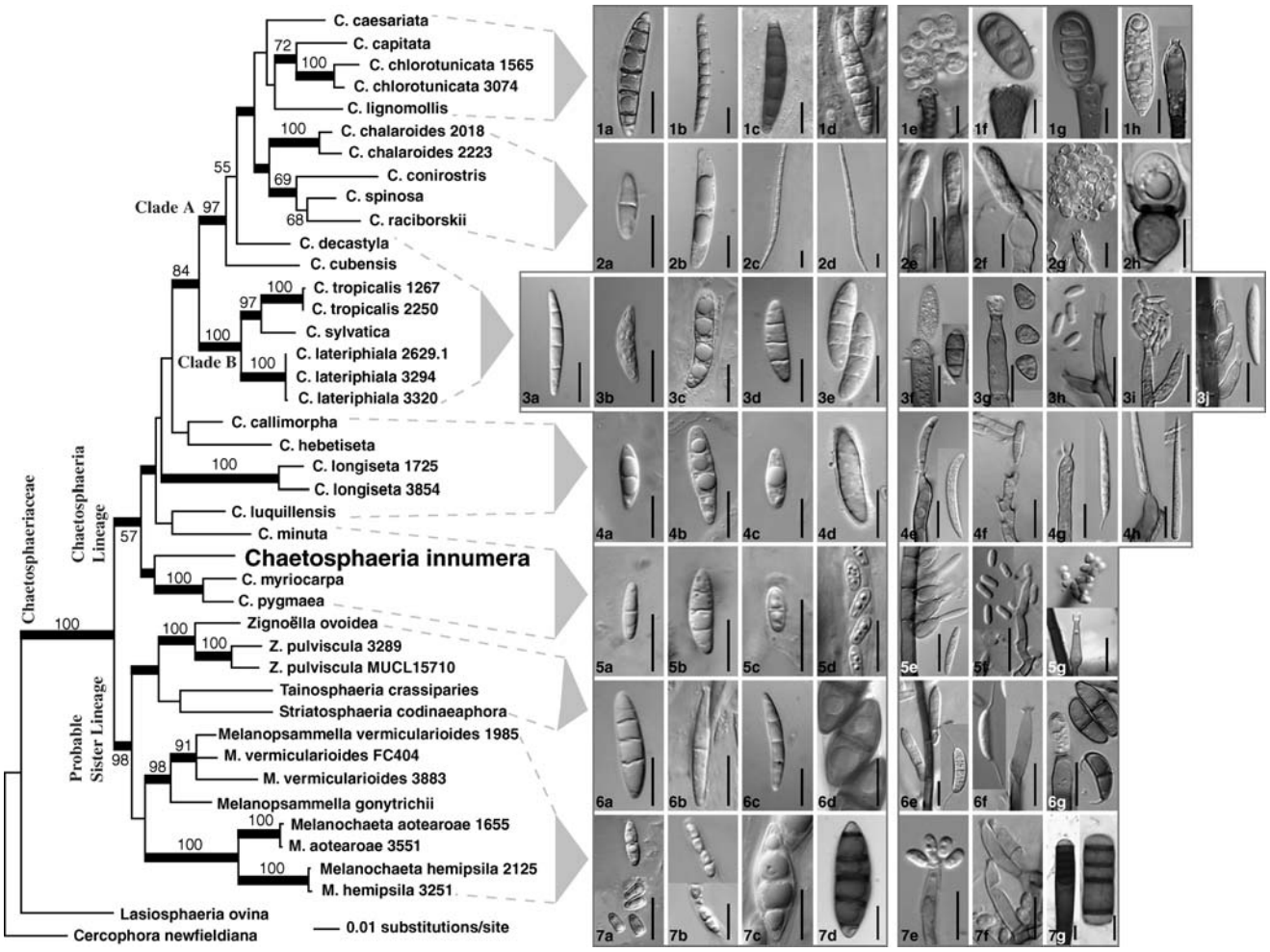


FIG. 1. Phylogram based on a Bayesian MCMCMC analysis of the combined LSU and β-tubulin dataset. The phylogram represents the majority rule consensus tree of 40 000 trees and has an arithmetic mean likelihood value of (−12648.37). Branch lengths are averaged over all trees. Numbers above the branches indicate bootstrap support based on 1000 replicates. Thickened branches represent significant posterior probabilities (≥95%) generated from Bayesian analyses. Images of ascospores. 1a. *C. caesariata,* 1b. *C. capitata,* 1c. *C. chlorotunicata,* 1d. *C. lignomollis*; 2a. *C. chalaroides,* 2b. *C. conirostris,* 2c. *C. spinosa,* 2d. *C. raciborskii*; 3a. *C. decastyla,* 3b. *C. cubensis,* 3c. *C. tropicalis,* 3d. *C. sylvatica,* 3e. *C. lateriphiala*; 4a. *C. callimorpha,* 4b. *C. hebetiseta,* 4c. *C. longiseta,* 4d. *C. luquillensis*; 5a. *C. minuta,* 5b. *C. innumera,* 5c. *C. myriocarpa,* 5d. *C. pygmaea*; 6a. *Z. ovoidea,* 6b. *Z. pulviscula,* 6c. *T. crassiparies,* 6d. *S. codinaeophora*; 7a. *M. vermicularioides,* 7b. *M. gonytrichii,* 7c. *M. aotearoae,* 7d. *M. hemipsila.* Images of anamorphs. 1e. *C. caesariata* (*Chloridium*-like), 1f. *C. capitata* (*Exserticlava*), 1g. *C. chlorotunicata* (*Exserticlava*), 1h. *C. lignomollis* (*Kylindria* DiCosmo, S.M. Berch & W.B. Kendr.); 2e. *C. chalaroides* (*Chalara*), 2f. *C. conirostris* (*Craspedodidymum* Hol.-Jech.), 2g. *C. spinosa* (*Phialophora*-like), 2h. *C. raciborskii* (*Craspedodidymum*-like); 3f. *C. decastyla* (*Cacumisporium* Preuss), 3g. *C. cubensis* (*Catenularia* Grove), 3h. *C. tropicalis* (*Phaeostalagmus* W. Gams), 3i. *C. sylvatica* (*Phaeostalagmus*), 3j. *C. lateriphiala* (*Zanclospora* S. Hughes & W.B. Kendr.); 4e. *C. callimorpha* (*Codinaea* Maire), 4f. *C. hebetiseta* (*Chloridium*), 4g. *C. longiseta* (*Dictyochaeta*), 4h. *C. luquillensis* (dematiaceous phialidic); 5e. *C. minuta* (*Zanclospora*), 5f. *C. innumera* (*Chloridium*), 5g. *C. myriocarpa* (*Chloridium*-like); 6e. *Z. ovoidea* (*Menispora* Pers.), 6f. *T. crassiparies* (*Codinaea*), 6g. *S. codinaeaphora* (*Dictyochaeta*); 7e. *M. vermicularioides* (*Chloridium*), 7f. *M. gonytrichii* (*Gonytrichum*), 7g. *M. hemipsila* (*Sporoschisma*). Bars = 10 μm.

*sphaeria* and allied genera provide new perspectives on phylogenetic relationships within the group. Morphological interpretation of the two-gene molecular phylogeny presents *Chaetosphaeria* as an assemblage of species groups with varying degrees of overlap in their teleomorph and anamorph characters. Analyses presented here show *Chaetosphaeria* as a morphologically complex genus with a distinct monophyletic group of species with diverse morphologies (clade A + B), and as a highly divergent group of paraphyletic, mostly poorly supported species (that includes the type species) with relatively uniform teleomorphs. Given the diverse phylogenetic spectrum of the genus, predominance of particular

teleomorph and anamorph characters can be observed in the different parts of the trees (FIG. 1). One-septate, short-fusiform ascospore morphology predominates in the paraphyletic or typical species that originated early in the diversification of *Chaetosphaeria* (e.g. *C. myriocarpa, C. pygmaea, C. minuta*). Species with 3-septate ascospores originated early and late in the evolution of this genus (e.g. *C. innumera, C. hebetiseta, C. tropicalis*). In the well supported monophyletic clade A, ascospores that are multiseptate and hyaline or pigmented are present in *C. caesariata, C. capitata, C. chlorotunicata, C. lignomollis, C. raciborskii, C. spinosa* and *C. decastyla*. One outlier is *C. chalaroides,* which has 1-septate short-fusiform ascospores typical of the basal species (FIG. 1). A similar morphological trend of reduced ascospore septation in basal species was observed in a phylogenetic study of temperate species (Réblová and Winka 2000).

In the anamorph, two general developmental patterns are observed in *Chaetosphaeria*. One pattern shows aseptate to multiseptate conidia that are broadly attached to an integrated conidiogenous cell and are produced at endogenous conidiogenous loci (e.g. *Exserticlava* S. Hughes and *Chalara* [Corda] Rabenh. anamorphs of *C. chlorotunicata* and *C. chalaroides,* respectively). The second pattern shows aseptate to 1-septate, ''enteroblastic conidia'' produced from a narrow conidiogenous locus in a discrete or integrated conidiogenous cell (e.g. *Dictyochaeta* Speg. and some *Chloridium* Link anamorphs). While previous data suggested that anamorph developmental patterns reflect phylogenetic relatedness in *Chaetosphaeria* (Réblová 2000) the data presented here only partially support this observation. The data (FIG. 1) do show some patterns regarding the location, arrangement, shape, and proliferation mode of conidiogenous cells, but there are exceptions. For example the first pattern of conidial development is observed in some of the species that are part of clade A: *C. capitata, C. chlorotunicata, C. decastyla* and *C. chalaroides*. Others (e.g. *C. lignomollis, C. caesariata* and *C. spinosa*) produce anamorphs fitting the typical patterns of enteroblastic conidiogenesis (Fernández and Huhndorf 2005). This pattern of conidiogenesis is present predominantly in species that make up the basal grade (i.e. *C. innumera, C. hebetiseta, C. longiseta* and *C. myriocarpa*). It should be noted that endogenous conidial formation does not occur in any of the species that make up the basal grade (FIG. 1) but does occur outside the genus (i.e. *Melanochaeta*).

The relative simplicity of *Chaetosphaeria* teleomorphs and the intra- and inter-specific morphological character plasticity in the anamorphs suggest a versatile holomorph design within a constrained general morphological framework in these species. Simple and plastic morphologies might let these species reproduce in a wider range of environmental conditions and perhaps exploit a wider range of substrates. It also would allow asexual reproduction to proceed under varying degrees of nutritional and/or environmental constraints, with noticeable effects on morphological features of reproductive structures. Evidence of intra-specific morphological versatility is evident in the *Dictyochaeta* anamorphs of *C. callimorpha* (Booth 1957), *C. longiseta* and others (Réblová 2000) with morphologically similar conidia produced in a single apical conidiogenous locus on long conidiophores and/or in a sympodially proliferating conidiogenous cell bearing several conidiogenous loci on short conidiophores. The large number of existing morphospecies described under the anamorphic genus *Dictyochaeta* (Kuthubutheen and Nawawi 1991) suggests a high success level of the polyphialidic morphological design. These potential attributes pose enormous challenges to attempts to delimit morpho-species based on anamorph characters in the presence of relatively uniform teleomorph characters across species. These conditions appear to be predominant among species forming the basal grade of *Chaetosphaeria*. Inter-specific overlap of morphological features among anamorphic genera can be far-reaching and would explain the morphological continua suggested for anamorphs of *Chaetosphaeria*: *Catenularia-Chloridium* (Kendrick 1980), *Chloridium-Dictyochaeta-Cylindrotrichum* pro parte-*Cacumisporium* (Réblová 2000). Although discrete morphological units are distinguished in those continua, phylogenetic relationships among most of them are unclear.

*Relationships within the Chaetosphaeriaceae*

*Chaetosphaeria* occurs as a poorly bootstrap-supported clade but with high Bayesian posterior probability. The analyses also show several strongly supported clades corresponding to existing named genera: *Zignoëlla, Melanochaeta* and *Melanopsammella*. These taxa, together with single representatives of *Striatosphaeria* and *Tainosphaeria* form a strongly supported clade that appears to be a sister group to *Chaetosphaeria*.

Teleomorphs in *Zignoëlla* strongly resemble those of *Chaetosphaeria*. The segregation of *Zignoëlla* from *Chaetosphaeria* was proposed based on position of ascomata on the substrate and production of *Menispora* anamorphs (Cannon 1997). Our analyses confirm the close phylogenetic relationships between *Z. ovoidea* and *Z. pulviscula* based on their teleomorphs and anamorphs. The diagnostic characters are found in the general morphology of the macro-

conidial synanamorph: discrete cylindrical phialides, with recurved or straight apices, borne along the conidiophore axis and hyaline, non-septate or 3-septate conidia, with non-cellular, often subterminal setula (when present) at both ends. It can be speculated that anamorphs with morphologies similar to those of *Menispora* and possibly other closely related anamorphic taxa would have phylogenetic placement in *Zignoëlla*. For example, *Z. ovoidea* (as *Chaetosphaeria ovoidea*) and the anamorph *Menispora tortuosa* form a strongly supported monophyletic group (Réblová and Winka 2000). *M. tortuosa* might be the anamorph of a putative, third species of *Zignoëlla* if its distinguishing characters (e.g. densely clustered phialides on branches along the mid-conidiophore axis) prove to be autapomorphic. Other *Menispora* morphospecies could represent new *Zignoëlla* species as well.

Some groupings in this lineage agree with morphological circumscriptions of genera based on putative synapomorphies in the teleomorph such as distinctive ascomal setae and ascospore pigmentation in *Melanochaeta* (Müller et al 1969), and ascospore cell fragmentation tendency in *Melanopsammella* (Höhnel 1919). In *Melanochaeta*, analyses corroborate the close phylogenetic relationships between *M. aotearoae* and *M. hemipsila* based on morphology. These two taxa share some distinctive morphological characters such as capitate setae on the ascoma, incompletely brown-pigmented ascospores (versicolorous), and a distinctive endophialidic (*Sporoschisma* Berk. & Broome) anamorph (Hughes 1966, Müller et al 1969, Müller and Samuels 1982). A third species, *M. garethjonesii* Sivichai & Hywel-Jones, produces uniformly pigmented ascospores and conidiophores distinct from those in *M. aotearoae* and *M. hemipsila* (Sivichai et al 2000).

*Melanopsammella* is resolved as part of the most basal divergence in the MP analysis (not shown). It currently encompasses four species: *M. chloroconia* (W. Gams & Hol.-Jech.) Réblová, M.E. Barr & Samuels 1999, *M. inaequalis* (Grove) Höhn. 1919, *M. vermicularioides* and *M. gonytrichii*. The genus exhibits the simplest morphologies in the lineage: relatively small setose ascomata, small, cylindrical asci and fusiform, 1-septate ascospores that often disarticulate at the septum. Ascospore disarticulation, which can be a variable character in *Melanopsammella*, is homoplastic. For example *M. vermicularioides* (No. SMH3883) has ascospores that do not readily disarticulate, although it produces the typical anamorph for that species. On the other hand, *C. preussii* W. Gams & Hol.-Jech., which possesses ellipsoid, 1-septate disarticulating ascospores, has phylogenetic affinities in *Chaetosphaeria*, closely related to *C. pygmaea* and *C. myriocarpa* (Réblová and Winka 2000).

In *Melanopsammella*, conidiophores are macronematous and mononematous, bearing a terminal, discrete, apical phialide and/or lateral phialides in whorls, with multiple conidiogenous loci in a single phialide, corresponding to morphologies in the *Chloridium virescens* (Pers.) W. Gams & Hol.-Jech. anamorph of *M. vermicularioides* and the *Gonytrichum* Nees & T. Nees anamorph of *M. gonytrichii*. At first glance, gross morphology in the *Gonytrichum* and *Chloridium virescens* anamorphs is different and could be interpreted logically as indicative of polyphyly. However, the complex lateral branching observed in the *Gonytrichum* morphology becomes simplified in culture, with conidiophores resembling the *C. virescens* morphology (Gams and Holubová-Jechová 1976, Réblová 2000). This is a good example of how a cultural anamorph (Seifert and Samuels 2000) can be phylogenetically informative when compared to a naturally occurring anamorph.

*Melanopsammella*, *Striatosphaeria* and *Tainosphaeria* share anamorphs presenting percurrent proliferation of conidiogenous cells. Close phylogenetic relationships among *Zignoëlla*, *Striatosphaeria* and *Tainosphaeria* (FIG. 1) may coincide with the occurrence of setulose conidia in their anamorphs. This observation agrees with the grouping of anamorphs with setulose conidia represented by *Codinaea sensu stricto* and *Menispora* (Réblová 2000). It also supports the distinction of *Dictyochaeta* anamorphs from *Codinaea* and representative anamorphs of some *Chaetosphaeria* species. Ontogeny of setulae in anamorphs of *Striatosphaeria*, *Tainosphaeria* and *Zignoëlla* could be a phylogenetically informative morphological character. For example setulae observed in conidia of the *Dictyochaeta* anamorphs of *C. montana* Réblová and *C. longiseta* are located terminally and appear to be extensions of end cells. Conversely setulae in conidia of *M. glauca*, and probably in other *Menispora* species, are located subterminally, unfold from the concave side of the conidium when placed in water (Hughes and Kendrick 1963) and appear as non-cellular, unilateral extensions of the conidium wall. These subtle differences in the conidiogenesis and development in the anamorphs may be phylogenetically informative.

The putative phylogenetic placement of *Tainosphaeria* in the lineage sister to *Chaetosphaeria* is reflected in some of its shared morphological characters. Its setulose, aseptate conidia are reminiscent of those described for the anamorphs *Dictyochaeta simplex* (S. Hughes & W. B. Kendr.) Hol.-Jech. (Holubová-Jechová 1984) and *Codinaeopsis gonytri-*

*choides* (Shearer & J. L. Crane) Morgan-Jones (Morgan-Jones 1976). It also resembles *Codinaea aristata* Maire in the terminal integrated conidiogenous cell, the conspicuous collaret and the terminally setulate conidia. *Tainosphaeria* also shares similar hyaline, 3-septate, ascospores with *Zignoëlla* and some species of *Chaetosphaeria* . The genus is distinguished by relatively thick-walled (22-33 µm) ascomata and conidiophores with single integrated terminal phialides exhibiting multiple percurrent proliferations. Similar morphologies of conidiogenous cells were observed in the anamorph of *Striatosphaeria codinaeaphora* on the natural substrate (F. A. Fernández personal observations) and in *Melanopsammella* (Réblová et al 1999).

## CONCLUSIONS

The inclusion of the phylogenetic framework presented here into the existing classification for *Chaetosphaeria* is problematic. The wide diversity of morphologically distinct anamorphs in *Chaetosphaeria* has not been viewed as problematic because characters that have conformed to the original taxonomic ranking have centered on the teleomorph. This has led to the generalized assumption that in *Chaetosphaeria* and allied taxa, teleomorph characters define genera and anamorph characters define species.

The lack of a well- supported clade that includes *C. innumera*, the type species of the genus, advises against establishing *Chaetosphaeria sensu stricto* at this time. Based on our diverse, but still biased taxon sampling, we accept a widely defined *Chaetosphaeria* with additional species that further expand the circumscription of the genus. Some of these *Chaetosphaeria* species might represent 'phylogenetic windows' providing glimpses of useful synapomorphies. However, some of these taxa hardly provide enough morphological variation to define a species, let alone define a genus. These problems are discussed in detail by Lumbsch (2002). Some interesting questions can be considered: Is the larger number of species in *Chaetosphaeria* relative to its allied taxa the result of biased taxon sampling? Or is *Chaetosphaeria* phylogenetically and morphologically more speciose than the other genera as inferred by the combined two-gene phylogenies?

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