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A re-evaluation of genus *Chaetomidium* based on molecular and morphological characters

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Abstract: *Chaetomidium*, a genus in the Chaetomiaceae, comprises 12 species that produce similar cleistothecial ascomata with a membranous, mostly pilose, peridium. Approximately six species of this genus produce some type of modified peridium composed of cephalothecoid plates that previous authors have hypothesized to be a homologous character within the genus. To better understand the phylogenetic affiliations of *Chaetomidium* and distribution of the cephalothecoid peridium within this genus we performed phylogenetic analyses with LSU, β-tubulin and *rpb2* sequence data. The results of these analyses showed that *Chaetomidium* is polyphyletic and should be restricted to its type, *C. fimeti*, and *C. subfimeti*. The remaining cephalothecoid and non-cephalothecoid species were scattered throughout the Chaetomiaceae and Lasiosphaeriaceae. The cephalothecoid species of *Chaetomidium* were distributed in three unrelated clades, suggesting that the morphological similarity among these particular species resulted from convergence instead of ancestry.

Key words: cephalothecoid, Chaetomiaceae, phylogeny, Sordariales, systematics

INTRODUCTION

Within the Sordariales, family Chaetomiaceae currently accommodates 13 genera (Lumbsch and Huhndorf 2007), most notably the perithecial genus *Chaetomium* Kunze, as well as its nonostiolate counterpart genus *Chaetomidium* (Zopf) Sacc., which itself currently includes 12 species, all of which produce membranous, pigmented cleistothecia bearing (with the exception of *C. triangulare* Stchigel & Guarro) long, flexuous hairs and ellipsoidal to limoniform, single-celled ascospores bearing a single apical germ pore (von Arx 1975, Stchigel et al 2004, Greif and Currah 2007). Of the currently recognized *Chaetomidium* species, half possess a membranous cleistothecium composed of a pseudoparenchymous peridium while the other six have a peridium composed of cephalothecoid plates (FIGS. 1–2). Fungi with cephalothecoid cleistothecia have peridia composed of large or small plates of tightly packed cells radiating outward from a central point and often are bounded by lines of dehiscence (Hawksworth and Booth 1974, Greif et al 2004, Greif and Currah 2007). This particular peridial type is an adaptation that lets the cleistothecium fragment through exposure to a variety of abiotic and/or biotic triggers and release ascospores into the surrounding environment (Benny et al 1980, Hawksworth 1986, Samuels and Rodrigues 1989, Greif et al 2004, Greif and Currah 2007). While appearing to be morphologically similar, cephalothecoid fungi have evolved independently in a diverse number of unrelated fungal families currently placed in the Sordariales, Ophiostomatales and Dothideales (Suh and Blackwell 1999, Lumbsch and Huhndorf 2007). In addition to *Chaetomidium* several genera outside the Chaetomiaceae are characterized by a particular cephalothecoid peridium composed of multiple small plates (Malloch and Cain 1970, Hawksworth and Booth 1974, Suh and Blackwell 1999, Lumbsch and Huhndorf 2007). While DNA sequence analyses have shown that the cephalothecoid peridium is a convergent feature across families (Suh and Blackwell 1999), the evolution of this structure within genera, at least in the Chaetomiaceae, has not been investigated in great detail.

The distribution of different peridial forms in *Chaetomidium* along with the variation in ascospore and peridium appendage morphology (Meyer 1983, Stchigel et al 2004, Greif and Currah 2007) implies that this genus as currently accepted might include unrelated lineages. However the similarity among cephalothecoid *Chaetomidium* in morphology and development of the peridium (Benny et al 1980, Greif and Currah 2007) suggests that these species could form a monophyletic clade within this taxon (Silva and Hanlin 1996). Given the morphological variation among species in *Chaetomidium*, the objec-

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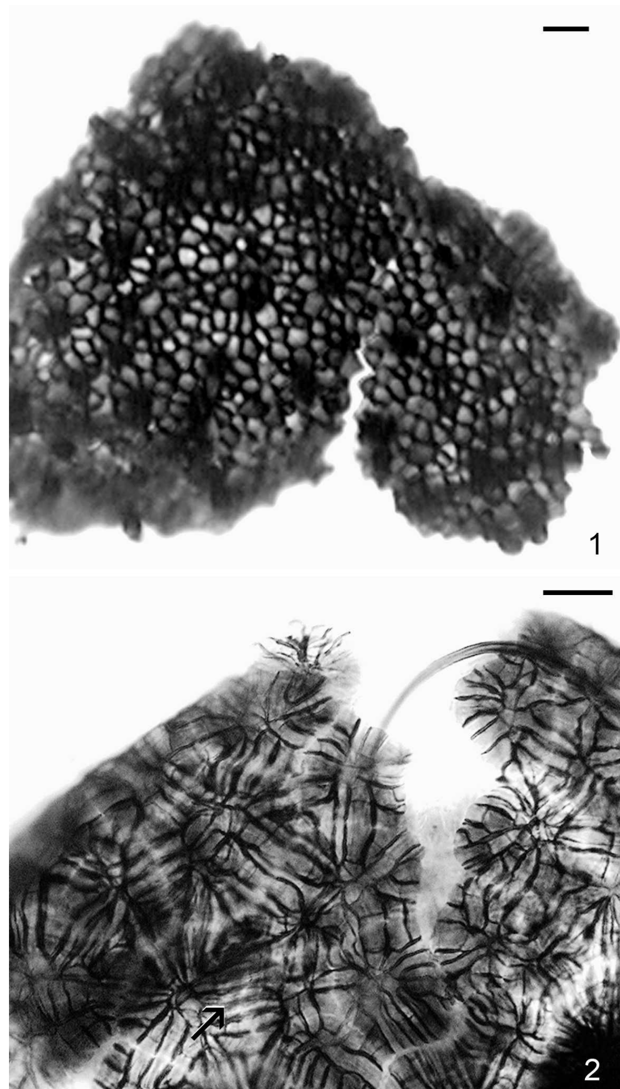


FIGS. 1–2. Examples of membranous and cephalothecoid peridia in *Chaetomidium*. Light microscopy (LM) image of the peridial surface of *Chaetomidium fimeti* (CBS 114382). The peridium is composed of a membranous sheet of tightly packed pseudoparenchymous cells. 2. LM image of the peridial surface of *Chaetomidium leptoderma* (CBS 538.74). The peridium consists of cephalothecoid plates composed of radially elongated cells clustered around a central undifferentiated cell and surrounded by lines of dehiscence (arrow). Bar = 10 μm.

tives of this study therefore were to verify the generic placement of recognized species by means of DNA sequence analysis and to examine the phylogeny of this genus within the Sordariales. Secondarily, this study will test the hypothesis that among cephalothecoid members of *Chaetomidium* the peridium is homologous (Silva and Hanlin 1996) and provide insight into the distribution of this character at the generic level.

MATERIALS AND METHODS

Isolates of *Chaetomidium* species were obtained from the Centraalbureau voor Schimmelcultures, Utrecht (CBS), Commonwealth Agricultural Bureaux International, Genetic Resource Collection, Oxfordshire (CABI), and the Faculty of Medicine, Reus (FMR) (TABLE I) and grown on cornmeal agar (CMA, 17 g/L sterile distilled water Acumedia™ cornmeal medium, Neogen Corp., Lansing, Michigan) to generate material for study. For all species, except *Chaetomidium fimeti* (Fuckel) Zopf, at least one isolate representing type material was used for sequencing (TABLE I). Type material in culture is not available for *Chaetomidium fimeti*, however isolate CBS 168.71, used by Malloch and Cain (1973) and von Arx (1975) in their revisions of the genus, was used for sequencing. CMA plates were incubated at room temperature under ambient light. Material for DNA extraction was obtained from cultures grown on CMA overlain with sterilized cellophane sheets (Hoefer Inc., Holliston, Massachusetts). After approximately 3 wk 100 mg of hyphal tissue and developing fruiting bodies were scraped off the cellophane, placed in sterile 1.5 mL plastic vials and dried 24 h. Dried tissue was disrupted with the aid of a sterile steel bead in a QIAGEN Tissuelyser (QIAGEN Inc., Hilden, Germany) set at 30 Hz for 45 s.

Genomic DNA was extracted and purified with a QIAGEN DNeasy Plant Mini Kit (QIAGEN Inc., Hilden, Germany) according to the manufacturer's specifications with some modification (tissues were incubated 24 h at 65 C to lyse tissues after disruption). Primer pairs 5.8SR-LR7 were used to amplify the LSU region, primers BT2a-BT1b for the β-tubulin region, and RPB2AM-3bF-RPB2AM-6R and fRPB2-5F-RPB2AM-7R for *rpb2* (Lane et al 1985, http://www.biology.duke.edu/fungi/mycolab/primers.htm; Miller and Huhndorf 2004, 2005). PCR was run 37 cycles in a Dyad DNA engine (Bio-Rad Laboratories Inc., Hercules, California) set to these parameters: initial denaturation at 94 C for 2 min, denaturation at 94 C for 1 min, annealing at 55 C for 1 min, extension at 72 C for 1 min, final extension at 74 C for 7 min followed by a cool-down at 4 C. The amplicon was purified with a Nucleofast 96-well PCR plate (Macherey-Nagel, Evanton, Pennsylvania). Cycle sequencing was done with primers 5.8sr, LROR, LR3r, LR16, LR5, and LR7 for LSU, BT1a, BT1b, BT2a, BT2b, T12 and T22 for β-tubulin, and primers fRPB2-5F, RPB2AM-6R, RPB2AM-1f, RPB2AM-1R, RPB2AM-1bF, RBP2AM-1bR, and RPB2AM-7R for *rpb2* (Lane et al 1985, White et al 1990, http://www.biology.duke.edu/fungi/mycolab/primers.htm; Liu et al 1999, Miller and Huhndorf 2004, 2005). Sequencing reactions were done with the BigDye terminator cycle sequencing kit (Applied Biosystems, Foster City, California) and amplicons were run on an ABI 3730 (Amersham Pharmacia Biotech Inc., Piscataway, New Jersey). A consensus sequence was constructed and edited with Sequencher 4.5 (Gene Codes Corp., Ann Arbor, Michigan). The newly determined sequences were subjected to a BLAST analysis (Altschul et al 1997) comparing our species with published sequences submitted to GenBank (www.ncbi.nlm.nih.gov).

Chaetomidium sequences initially were aligned with

TABLE I. Culture collection accession data and locality information for isolates sequenced in this study

Species	Accession number	Substrate	Type locality	GenBank accession numbers		
				LSU	β-tubulin	*rpb2*
Chaetomium longicolleum	FMR 9050	Soil	Estado do Paraná, Brazil	FJ666365		FJ666395
Chaetomidium arxii	CBS 104.79 (type culture)	Kangaroo rat dung	California, USA	FJ666359	FJ666375	FJ666390
Chaetomidium arxii	CBS 100576	Desert soil	Basrah, Iraq	FJ666363	FJ666379	
Chaetomidium cephalothecoides	CABI 180791 (type culture)	Mouse dung	California, USA		FJ666367	FJ666382
Chaetomidium fimeti	CBS 168.71	Decaying hay	Ontario, Canada	FJ666358	FJ666374	FJ666389
Chaetomidium fimeti	CBS 114382	Barely leaf and stem	Tabriz, Iran	FJ666351	FJ666366	FJ666381
Chaetomidium fimeti (Originally deposited as *C. pilosum*)	CBS 343.73	Old rug	California, USA	FJ666355	FJ666371	FJ666386
Chaetomidium galaicum	CBS 113678 (type culture)	Black spot on granite	Galicia, Spain	FJ666361	FJ666377	FJ666392
Chaetomidium galaicum	FMR 9124	Soil	Zamora, Spain	FJ666360	FJ666376	FJ666391
Chaetomidium leptoderma	CBS 538.74 (type culture)	Pine wood soil	Surrey, UK	FJ666353	FJ666369	FJ666384
Chaetomidium pilosum	CBS 335.67 (type culture)	Surface sterilized wheat grains	Perth, Australia	FJ666356	FJ666372	FJ666387
Chaetomidium subfimeti	CBS 370.66 (type culture)	Paper and vegetable matter	Wales, UK	FJ666354	FJ666370	FJ666385
Chaetomidium subfimeti	CBS 169.71	Soil	California, USA	FJ666357	FJ666373	FJ666388
Chaetomidium triangulare	FMR 7545 (type culture)	Soil	Tucumán, Argentina	FJ666362	FJ666378	FJ666393
Chaetomidium trichorobustum	CBS 563.67 (type culture)	Rabbit dung	Hamburg, Germany	FJ666352	FJ666368	FJ666383
Corynascus sepedonium	FMR 9123	Soil	Tarragona, Spain	FJ666364	FJ666380	FJ666394

CABI: Commonwealth Agricultural Bureaux International, Genetic Resource Collection; CBS: Centraalbureau voor Schimmelcultures; FMR: Faculty of Medicine, Reus.

representative Sordariomycetes sequences obtained from Genbank. The data matrix was assembled with Se-Al 2.0a11 Carbon (Rambaut 2002) then exported to Mesquite 2.01 (Madison and Madison 2007) where it was aligned automatically with MUSCLE 3.6 (Edgar 2004). The aligned matrix was imported back into Se-Al and edited manually. For the LSU *Xylaria acuta* Peck was used as an outgroup taxon while *Xylaria bambusicola* Y.M. Ju & J.D. Rogers and *Xylaria hypoxylon* (L.) Grev. were used for the β-tubulin and *rpb2* analyses respectively. For each gene sequence data matrix a maximum parsimony analysis was performed with PAUP 4.0 b10 (Swofford 2002). A heuristic search was done with parsimony as the optimality criterion. Gaps were treated as missing data. Starting trees were obtained at random via stepwise addition with tree-bisection-reconnection as the branch-swapping algorithm and the MULTREES option in effect, with the exception of the LSU matrix where this option was turned off. Upon completing 200 stepwise addition sequences, confidence in the branches of the resulting trees was evaluated by bootstrap analysis (Felsenstein 1985) using 2000 replicates with each replicate consisting of 200 stepwise addition sequences. The resultant trees were viewed with PAUP 4.0 b10 (Swofford 2002). A Bayesian analysis was conducted with MrBayes 3.1.2 (Huelsenbeck et al 2001), which uses a Markov chain Monte Carlo (MCMC) method to estimate posterior probabilities of clades (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 temperature set at 0.1 for the LSU matrix, 0.15 for the β-tubulin and 0.2 for the *rpb2* matrix (the lower chain temperatures were necessary for the LSU and β-tubulin analyses to achieve stationarity due to large size and/or lack of variation among sequences in the respective matrices). The nucleotide substitution model was chosen by analyzing the data matrix with Modeltest 3.7 (Posada and Crandall 1998). Every 100th tree was sampled out of a total of 8 000 000, 3 000 000 and 2 000 000 generations for the LSU, β-tubulin and *rpb2* analyses respectively, with the first 30% of trees being deleted as burn-in for the LSU matrix and 25% for the remaining two matrixes. The SUMP command summarized the parameter values of the analyses and indicated if stationarity had been achieved. The SUMT command then

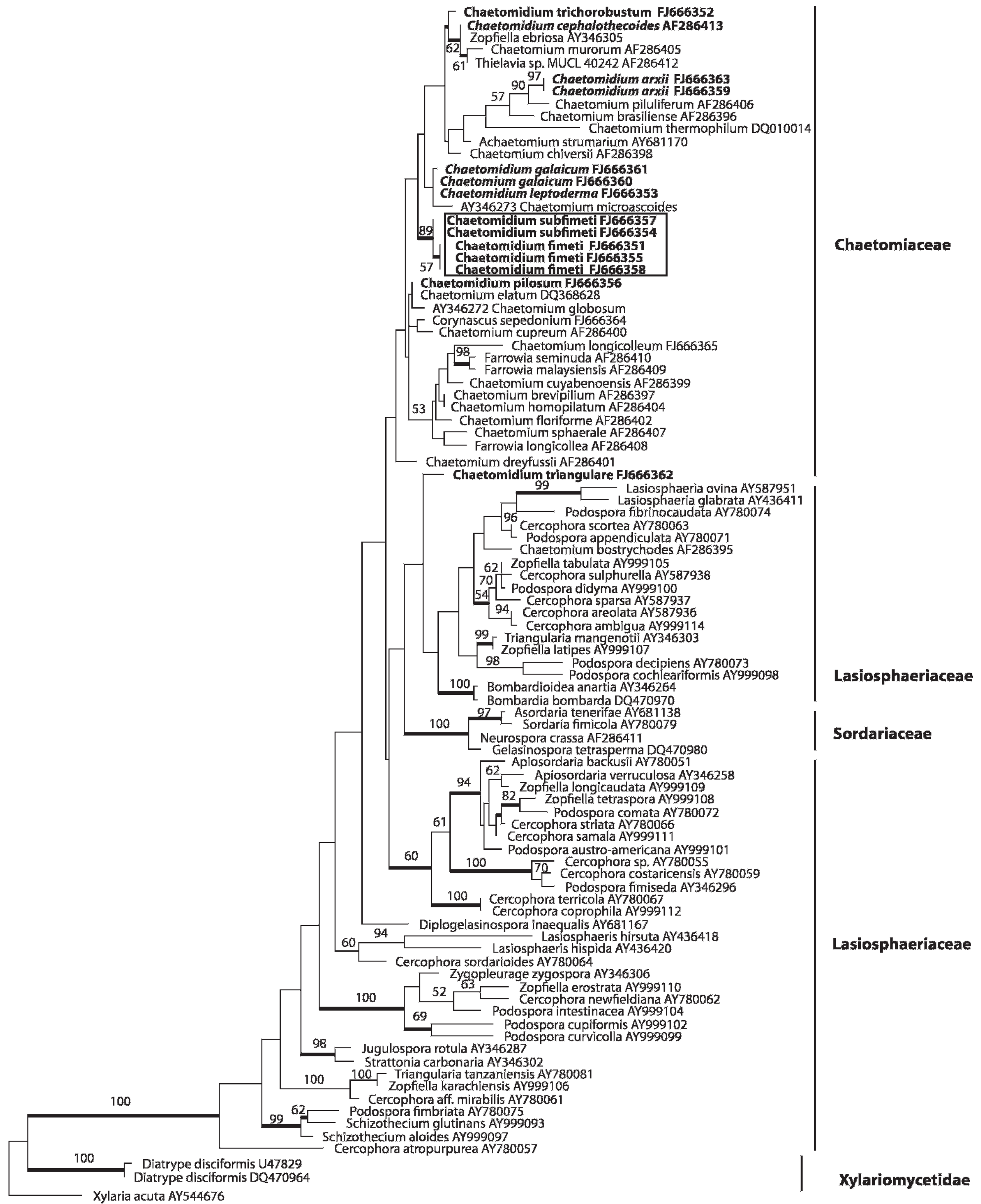


FIG. 3. Phylogenetic tree obtained from maximum parsimony showing the relationship among species of *Chaetomidium* based on the LSU rDNA sequence data. *Chaetomidium fimeti* and *C. subfimeti* form a strongly supported clade while most other *Chaetomidium* species are scattered among the Chaetomiaceae. *Chaetomidium triangulare* is located outside the Chaetomiaceae without branch support. Bootstrap support values > 50% are shown above branches and Bayesian posterior probabilities ≥ 95% are indicated by thickened branches. *Chaetomidium* species are in boldface and those with a cephalothecoid peridium are also italicized.

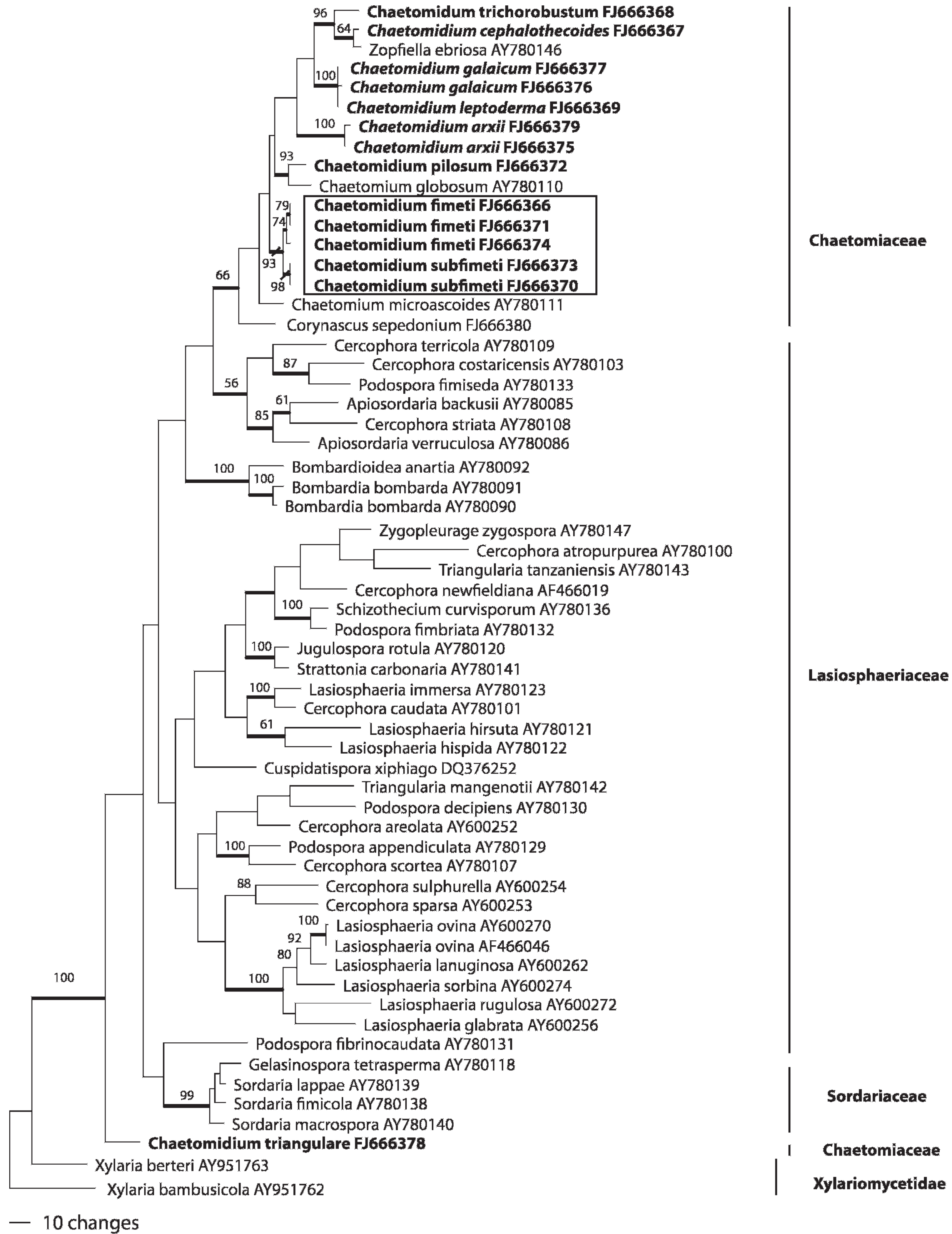


FIG. 4. Phylogenetic tree obtained from maximum parsimony showing the relationship among species of *Chaetomidium* based on the β-tubulin sequence data. *Chaetomidium fimeti* and *C. subfimeti* form a strongly supported clade while most other *Chaetomidium* species are scattered among the Chaetomiaceae. Isolates of *Chaetomidium leptoderma* and *C. galaicum* form a strongly supported clade with little sequence differentiation. *Chaetomidium triangulare* is located outside the Chaetomiaceae

summarized the posterior probability of each clade and branch length. Only clades with posterior probability values ≥ 95% were considered to have significant support. Consensus trees were viewed with PAUP 4.0 b10 (Swofford 2002).

RESULTS

The LSU alignment consisted of 94 taxa and 821 characters, 584 of which were constant and 193 were parsimony informative. The heuristic search produced eight equally parsimonious trees (one of which is shown in FIG. 3) with a consistency index of 0.375 and a retention index of 0.708. The β-tubulin alignment consisted of 60 taxa and 875 characters, 612 of which were constant and 226 were parsimony informative. The heuristic search produced five equally parsimonious trees (one of which is shown in FIG. 4) with a consistency index of 0.254 and a retention index of 0.561. Finally, the *rpb2* alignment consisted of 64 taxa and 993 characters, 407 of which were constant and 543 were parsimony informative. The heuristic search produced one most parsimonious tree (FIG. 5) with a consistency index of 0.218 and a retention index of 0.566.

Chaetomidium formed a polyphyletic group in all three analyses (FIGS. 3–5). The majority of *Chaetomidium* species were among the Chaetomiaceae with the exception of *C. triangulare*, which occurred in an uncertain position within the Lasiosphaeriaceae. *Chaetomidium fimeti* and *C. subfimeti* Seth formed a strongly supported clade with both high bootstrap (> 80) and posterior probability (> 95) in all three analyses (FIGS. 3–5). *Chaetomidium pilosum* (C. Booth & Shipton) Arx formed a clade with *Chaetomium globosum* Kunze and *C. elatum* Kunze (with the exception of the β-tubulin matrix, FIG. 4, where the DNA sequence for *C. elatum* was unavailable). *Chaetomidium cephalothecoides* (Malloch & Benny) Arx and *C. trichorobustum* Seth consistently formed a strongly supported clade with *Zopfiella ebriosa* Guarro, P.F. Cannon & Aa in both the β-tubulin (FIG. 4) and *rpb2* analyses (FIG. 5) and a poorly supported clade with *Z. ebriosa*, *Chaetomium murorum* Corda and a strain of *Thielavia* Zopf in the LSU analysis (FIG. 3). *Chaetomidium leptoderma* (C. Booth) Greif & Currah formed a clade with isolates of *C. galaicum* Stchigel & Guarro with strong support in the β-tubulin (FIG. 4) and *rpb2* (FIG. 5) analyses but formed an unsupported clade with *Chaetomium murorum* in the LSU analysis (FIG. 3). A subsequent examination of the sequence data for *Chaetomidium leptoderma* and *C. galaicum* revealed them to be almost identical in all three genes, and therefore they can be regarded as conspecific. *Chaetomidium arxii* Benny was only weakly supported in a clade containing *Chaetomium piluliferum* J. Daniels and *Chaetomium brasiliense* Bat. & Pontual in the LSU analyses (FIG. 3). While unsupported in the LSU tree, fungi comprising the Chaetomiaceae formed a moderately supported (β-tubulin) or strongly supported (*rpb2*) clade separate from fungi representing the Lasiosphaeriaceae or Sordariaceae (FIGS. 4, 5). A combined analysis of all three genes also was performed, but the resultant distribution of *Chaetomidium* was similar to that seen in the LSU, β-tubulin and *rpb2* analyses, and therefore these results were not considered further (data not shown).

DISCUSSION

Chaetomidium was first erected as a subgenus by Zopf to accommodate a nonostiolate *Chaetomium* described by Fuckel and later was elevated to generic status by Saccardo (1882). The acceptance of *Chaetomidium* has fluctuated over time. Bainier (1910), Chivers (1915), Skolko and Groves (1953), Cain (1961), Whiteside (1962) and Lodha (1974) maintained it as a separate taxon although Lodha maintained it for species with two-pored ascospores. Others doubted the validity of various morphological features as indicators of generic affiliation and synonymized the genus with either *Chaetomium* (Gwynne-Vaughan 1922, Clements and Shear 1931) or *Thielavia* (Malloch and Cain 1973). Von Arx (1975) reinstated *Chaetomidium*, separating it from *Thielavia* and *Chaetomium* on the basis of peridial tissue type and thickness and lack of an ascomal ostiole respectively. He restricted *Chaetomidium* to species with clavate asci and ascospores bearing a single germ pore and transferred species with variant ascus and ascospore morphologies into genera such as *Corynascus* Arx (i.e. globose asci and spores with two germ pores). *Chaetomidium* has since remained an accepted genus in the Chaetomiaceae (Mukerji and Saxena 1974, von Arx et al 1984, Stchigel et al 2000, Lumbsch and Huhndorf 2007).

Results from the analyses of the molecular data

←

with branch support. Bootstrap support values > 50% are shown above branches and Bayesian posterior probabilities ≥ 95% are indicated by thickened branches. *Chaetomidium* species are in boldface and those with a cephalothecoid peridium are also italicized.

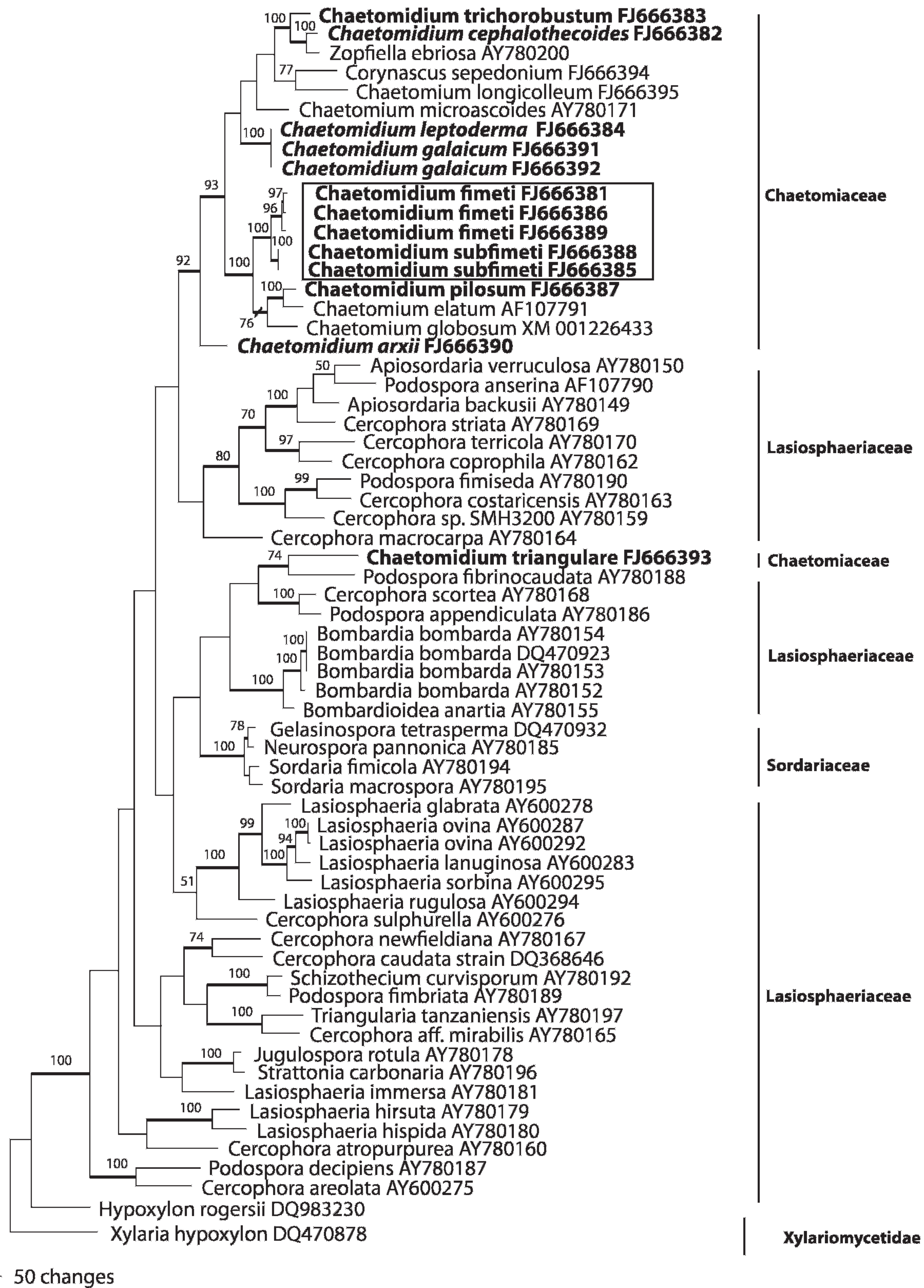
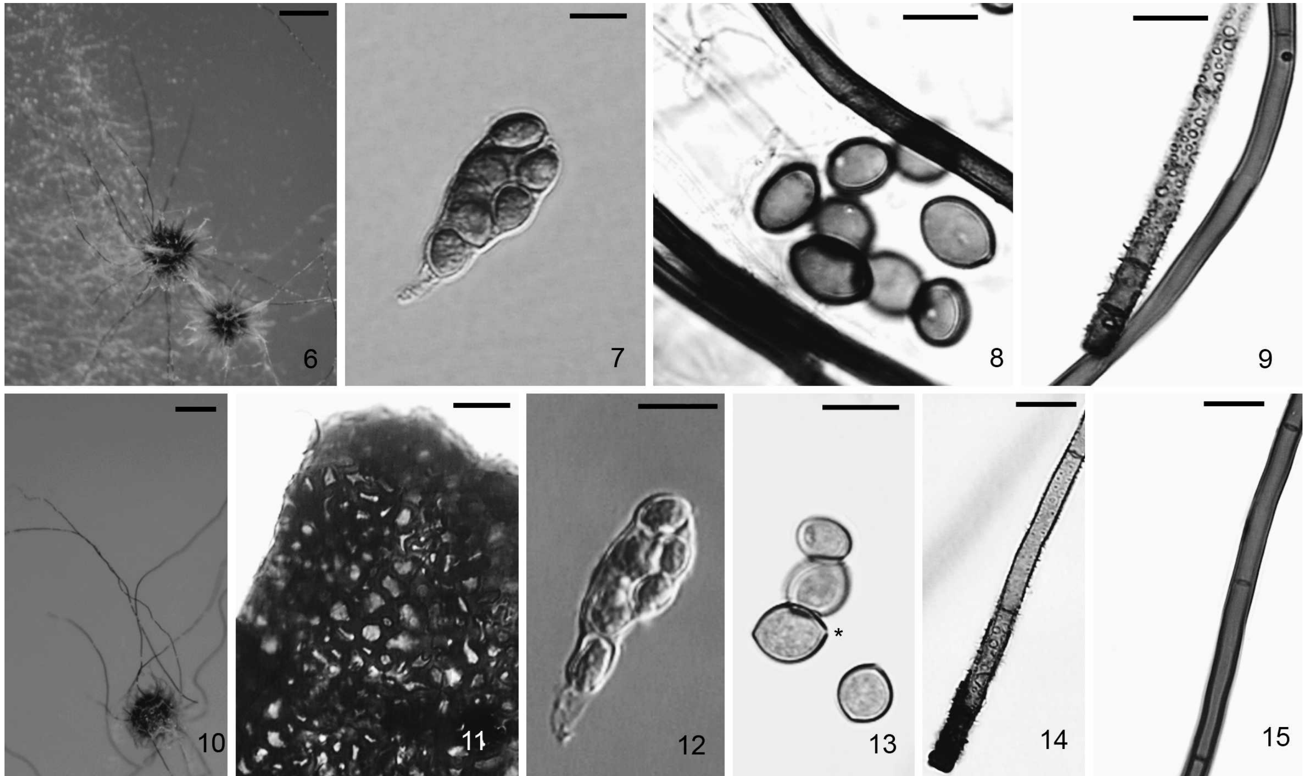


FIG. 5. Phylogenetic tree obtained from maximum parsimony showing the relationship among species of *Chaetomidium* based on the *rpb2* sequence data. The topology of *Chaetomidium* among the tree is similar to that of the previous two analyses with the exception that *Chaetomidium triangulare* forms a clade with good branch support (74% bootstrap and significant posterior probability) with *Podospora fibrinocaudata*. Bootstrap support values > 50% are shown above branches and Bayesian posterior probabilities ≥ 95% are indicated by thickened branches. *Chaetomidium* species are in boldface and those with a cephalothecoid peridium are also italicized.

(FIGS. 3–5) provide evidence that the current morphological concept of *Chaetomidium* is polyphyletic. The type species, *Chaetomidium fimeti*, and *Chaetomidium subfimeti* formed a strongly supported clade in all three analyses (FIGS. 3–5), but most *Chaetomidium* species are interspersed among species of *Chaetomium*, *Farrowia* D. Hawksw. and *Thielavia*, taxa representative of the Chaetomiaceae. Our results are consistent with studies that supported the placement of *Chaetomidium* within the Chaetomiaceae, while at the same time indicating the polyphyletic nature of related taxa such as *Chaetomium* and *Farrowia* (Untereiner et al 2001) as well as other genera in the Sordariales such as *Cercophora* Fuckel, *Podospora* Ces. and *Zopfiella* G. Winter (Miller and Huhndorf 2005, Cai et al 2006a). Traditional morphological classification frequently has not held up against modern phylogenetic evidence, which has revealed many instances where morphologically similar species are scattered among different families or where morphologically dissimilar species form well supported clades (Cai et al 2006a, b). However applying morphological concepts to monophyletic clades identified in molecular analyses in some instance has provided ancillary support in classifying taxa. Ascomal wall morphology has been useful in clarifying familial and generic taxonomy in the Sordariales (Huhndorf et al 2004, Miller and Huhndorf 2004), while ascospore morphology has been useful in providing morphological support for clades containing species of *Zopfiella*, *Podospora* and *Schizothecium* Corda (Cai et al 2006a).

Past efforts to expand the concept of *Chaetomidium* to include variant peridial and spore characteristics are not supported by the results of these molecular analyses. However a comparison of morphological characters among *Chaetomidium fimeti*, *C. subfimeti* and other *Chaetomidium* taxa reveals that the *C. fimeti-subfimeti* clade (= *Chaetomidium s.s.* clade) can be defined in part by the combination of a membranous, non-cephalothecoid peridium, the production of broadly ellipsoidal ascospores with strictly umbonate ends and a solitary pore on one end, two specific types of peridial hairs (one smooth and one rough-walled) and a lack of an anamorphic state in culture (FIGS. 6–15) (Seth 1967, von Arx 1975). This unique combination of characters is lacking from all other described species of *Chaetomidium* and should be strictly applied to prevent additional ambiguous species from being placed in the genus. While *C. fimeti* and *C. subfimeti* are most similar phylogenetically and morphologically, the remaining *Chaetomidium* species are scattered inside and outside the Chaetomiaceae. This suggests that many taxonomic characters used to describe the genus have been incorrectly applied when formally describing these other species. *Chaetomidium trichorobustum*, which consistently formed a clade with *Chaetomidium cephalothecoides* and *Zopfiella ebriosa* (FIGS. 3–5), originally was placed in the genus on the basis of appendage morphology. However, according to Seth (1968), this species is reported to bear three different kinds of hairs and produces three distinct ascospore types, which differ from that of *C. fimeti*. *Chaetomidium triangulare* that fell outside the Chaetomiaceae (FIGS. 3–5), was placed in the genus based on the membranous nature of the peridium and the terminal pore on the ascospore, but in this species the peridium lacks hairs and the ascospores are distinctly triangular instead of limoniform (Stchigel et al 2004). Generic placement of *Chaetomidium pilosum*, which formed a clade with *Chaetomium globosum* and *C. elatum* (FIGS. 3, 5), was based on the presence of a membranous peridium bearing hairs and limoniform ascospores, but the ascospores in this species are unique in having conspicuous tapered ends that are absent from other species described here (Booth and Shipton 1966). *Chaetomidium cephalothecoides*, *C. arxii*, *C. leptoderma* and *C. galaicum* (the latter three forming monophyletic terminal clades separate from the *Chaetomidium fimeti* clade, FIGS. 3–5) were placed in the genus based on the presence of peridial hairs and single-celled ascospores bearing a single solitary germ pore. These four species all produce a cephalothecoid peridium (a feature absent from the C. *fimeti-subfimeti* clade), and *C. cephalothecoides* produces a *Botryotrichum* Sacc. & Marchal anamorph in culture (Booth 1961, Malloch and Benny 1973, Benny 1980, Greif and Currah 2007). In view of these observations we consider it unlikely that the *Chaetomidium* species that we were unable to obtain for culturing and analysis (*C. megasporum* Doveri, Guarro, Cacialli & Caroti, *C. khodense* Cano, Guarro & El Shafie and *C. heterotrichum* R.J. Mey.) would cluster with the true *Chaetomidium* clade. *Chaetomidium megasporum* and *C. khodense* were placed in the genus based on the similarity of their peridia to those of *C. arxii* and *C. cephalothecoides* (Cano et al 1993, Doveri et al 1998), suggesting they will ally with the cephalothecoid lineages in the Chaetomiaceae. *Chaetomidium heterotrichum* was placed in the genus due to its membranous peridium ornamented with hairs (Meyer 1983), but the combination of stout setae in addition to the peridial hairs, globose ascospores and an unnamed phialidic anamorph make its generic affiliation with *Chaetomidium* highly unlikely. At this time we are choosing to not introduce nomenclatural changes within the Chaetomiaceae based on these phylogenetic analyses. Additional isolates of named *Chaetomidium* species and a greater sampling of fungi in the Chaetomiaceae will be necessary to verify generic and familial placement.

The cephalothecoid *Chaetomidium* species did not



FIGS. 6–15. Morphological characters of *Chaetomidium fimeti* (CBS 114382) and *Chaetomidium subfimeti* (CBS 169.71). 6. *Chaetomidium fimeti* cleistothecia. Primary hairs visible as elongate black strands while secondary hairs are short and gray. 7. An immature ascus of *Chaetomidium fimeti*. 8. Ascospores of *Chaetomidium fimeti*. Ascospores broadly ellipsoidal with umbonate ends and a solitary pore on one end. 9. Peridial hairs types of *Chaetomidium fimeti*. Primary hairs thick-walled and smooth while the secondary hairs are thin-walled and covered by numerous protrusions. 10. *Chaetomidium subfimeti* cleistothecia. Primary hairs visible as elongate black strands while secondary hairs are short and gray on the surface of the peridium. 11. The peridium of *Chaetomidium subfimeti* composed of a membranous sheet of tightly packed cells. 12. An immature ascus of *Chaetomidium subfimeti*. 13. Ascospores of *Chaetomidium subfimeti*. They are broadly ellipsoidal with umbonate ends and a solitary pore on one end (star). Secondary hairs of *Chaetomidium subfimeti*. They are thin-walled and covered by numerous protrusions. 15. Primary hairs of *Chaetomidium subfimeti*. Primary hairs are thick-walled and smooth. FIGS. 6–10. Stereomicroscopy, all other images light microscopy. Bar: 6 = 400 μm; 7–9, 11–15 = 10 μm; 10 = 250 μm.

form a monophyletic clade, contrary to both our original working hypothesis and a cladistic analysis based on morphology carried out by Silva and Hanlin (1996). Our results indicate that the morphological similarity among these cephalothecoid taxa was the result of convergence instead of ancestry. The cephalothecoid peridium has been found to be an analogous character among unrelated families (Suh and Blackwell 1999). Cephalothecoid taxa are often interspersed among perithecial genera, reflecting a trend from an ostiolar form with active ascospore ejecting asci to an enclosed fruiting body bearing dehiscent asci (von Arx et al 1984, Suh and Blackwell 1999). Similarly the cephalothecoid *Chaetomidium* taxa are distributed among ostiolate taxa in the Chaetomiaceae. In ultrastructural studies of both *Chaetomidium arxii* and *C. leptoderma* the peridium is initially composed of a sheet of polygonal pseudoparenchymous cells that eventually develops into a series of small plates of simple elongated cells surrounding an unmodified central cell (Benny et al 1980, Greif and Currah 2007). The overall morphological similarity among immature cephalothecoid *Chaetomidium* species (presumably including *C. cephalothecoides* and *C. galaicum* although developmental studies for these two taxa are lacking) and non-cephalothecoid Chaetomiaceae suggest only minor modifications to the peridium, in terms of enclosure of the ascal tissue and the formation of the plates, occur during maturity. The current analyses of *Chaetomidium* add at least three unrelated cephalothecoid lineages to the Chaetomiaceae, and an expanded study of this family will help identify additional lineages as well as non-cephalothecoid sister taxa. Performing comparative studies examining gene expression on cephalothecoid and non-cephalothecoid sister taxa in

the Chaetomiaceae will address whether the adaptation from non-cephalothecoid ancestral taxa are due to minor or major genetic changes and might identify potential ''cephalothecoid'' genes in this family.

Along with the polyphyly of cephalothecoid taxa there is extensive morphological variation among cephalothecoid peridial types, ranging from large complex plates (e.g. *Cephalotheca* Fuckel and *Cryptendoxyla* Malloch & Cain) to small simple plates of elongated cells (e.g. *Chaetomidium* and *Rhytidospora* Jeng & Cain) to even partially cephalothecoid perithecial ascoma (e.g. *Cercophora*) (Chesters 1934, Malloch and Cain 1973, Jeng and Cain 1977, Benny et al 1980, Hanlin 1999, Greif et al 2004). The cephalothecoid peridium is an adaptation that allows for a weakening of the outer ascomal wall and liberation of the ascospores (Hawksworth and Booth 1974). Depending on the development and complexity of the cephalothecoid plates, the mature peridium on desiccation either unfolds to reveal the ascospores (e.g. *Cryptendoxyla*, *Batistia* Cif. and *Cephalotheca*) or becomes weakened due to the creation of small tears along thin-walled lines of dehiscence (e.g. *Chaetomidium*) (Chesters 1934, Malloch and Cain 1973, Samuels and Rodrigues 1989, Greif et al 2004). Studies on *Cryptendoxyla hypophloia* Malloch & Cain and *Chaetomidium leptoderma* revealed different developmental sequences and modes of peridial disruption and ascospore dispersal (Greif et al 2004, Greif and Currah 2007). However *Chaetomidium leptoderma* and *Chaetomidium arxii* displayed a high degree of morphological and developmental similarity in the production of relatively simple peridial plates, and this convergence presumably reflects an adaptation toward a common dispersal strategy, ostensibly by arthropods providing the force needed to successfully rupture the distressed peridium (Benny et al 1980, Greif and Currah 2007) that might extend to the other cephalothecoid *Chaetomidium* species. The cephalothecoid *Chaetomidium* have been isolated from bovine and rodent dung in addition to granite and soil samples (Booth 1961, Malloch and Benny 1973, Benny 1980, Cano et al 1993, Doveri et al 1998, Stchigel et al 2004). Sampling for cephalothecoid *Chaetomidium* from arthropods attracted to such substrates would test further the hypothesis that the convergent development of this peridial form in the Chaetomiaceae is driven in part by arthropod interactions.

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