

# Coprophilous contributions to the phylogeny of Lasiosphaeriaceae and allied taxa within Sordariales (Ascomycota, Fungi)

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**Abstract** The phylogenetic relationships of *Lasio-sphaeriaceae* are complicated in that the family is paraphyletic and includes *Sordariaceae* and *Chaetomiaceae*, as well as several polyphyletic genera. This study focuses on the phylogenetic relationships of the coprophilous genera, *Anopodium*, *Apodospora*, *Arnium*, *Fimetariella* and *Zygospermella*. They are traditionally circumscribed based on ascospore characters, which have proven homoplasious in other genera within the family. Our results based on LSU nrDNA and  $\beta$ -tubulin sequences distinguish four lineages of *Lasio-sphaeriaceae* taxa. *Anopodium* joins the clade of morphologically similar, yellow-pigmented species of *Cercophora* and *Lasio-sphaeria*. *Apodospora* is monophyletic and joins a larger group of taxa with unclear affinities to each other, while *Arnium* is polyphyletic being scattered throughout three of the four major clades of *Lasio-sphaeriaceae*. *Fimetariella* is represented by a single collection and joins the clade containing *Cercophora scortea* and *Podospora appendiculata*. *Zygospermella* shows affinities to the *Lasio-sphaeria* clade. Based on a combination of morphological and molecular data, *Echria stat. nov.* is recognized at the genus level for the former *Arnium* section and two new combinations are proposed: *E. gigantospora* and *E. macrotheca*.

**Keywords** Ascomal wall · Ascomycete ·  $\beta$ -tubulin · LSU nrDNA · Systematics, Taxonomy

## Introduction

*Sordariales* is a large group of microfungi that occur worldwide as degraders of dung, wood, plant material, and soil (Cannon and Kirk 2007). The order includes several well-known members including species of *Chaetomium* that are common indoor contaminants associated with high humidity, and the model organisms *Neurospora crassa*, *Podospora anserina*, *Sordaria fimicola*, and *S. macrospora* (Alexopoulos et al. 1996). Uniting morphological features of *Sordariales* species are the erumpent to superficial, perithecial ascomata with large-celled ascomal walls, as well as the predominantly one or two-celled ascospores formed in unitunicate asci which show a remarkable morphological diversity (Huhndorf et al. 2004). The ascospores have hyaline and/or dark brown cells that typically form an apical head and basal tail when two-celled, and commonly are surrounded by a gelatinous sheath or have appendages in various sizes and shapes (von Arx et al. 1986; Lundqvist 1972). Most species have smooth ascospores, but distinctive ornamentations occur in some taxa (Dettman et al. 2001; Garcia et al. 2004; Lundqvist 1967).

Huhndorf et al. (2004) reduced *Sordariales* to the present three families, *Chaetomiaceae*, *Lasio-sphaeriaceae* and *Sordariaceae*, and the order forms a monophyletic clade in phylogenies based on molecular data (Cai et al. 2006; Miller and Huhndorf 2005; Zhang et al. 2006). However, *Sordariales* relationships at the family and generic level are complicated. *Chaetomiaceae* and *Sordariaceae* are nested within the largest family *Lasio-sphaeriaceae* (Greif et al. 2009; Huhndorf et al. 2004; Réblová 2008), and several of the 31 genera that belong to *Lasio-sphaeriaceae* are polyphyletic, i.e. *Cercophora*,

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*Podospora*, *Triangularia*, and *Zopfiella* (Cai et al. 2006; Chang et al. 2010; Miller and Huhndorf 2005).

The circumscriptions of genera have traditionally been based on the morphology of the ascospores, but molecular results have shown that ascospore characters clearly are unreliable for interpreting relationships at the family and generic level (Miller and Huhndorf 2005). Attempts have been made to group taxa based on other characters, such as those pertaining to the ascomal wall morphology, which show better agreement with molecular data for some clades (Miller and Huhndorf 2005). For example, members of *Cercophora* and *Lasio-sphaeria* with an outer wall layer of hyphae forming a tomentum, group together with strong support. Unfortunately, a large number of the species have relatively simple ascomal walls without obvious characters with which to distinguish them. There are also several well-supported clades that include morphologically dissimilar species (Miller and Huhndorf 2005).

Delimiting genera within *Lasio-sphaeriaceae* is obviously very difficult and developing a stable taxonomic outline of the group is a continuously evolving project. A number of genera currently placed in *Lasio-sphaeriaceae* have not yet been studied with molecular methods and their addition to the *Sordariales* phylogeny would strengthen the understanding of intra- and interfamilial relationships. The primary purpose of this study is to further resolve the phylogenetic relationships of *Lasio-sphaeriaceae* and its allied taxa, with a focus on five coprophilous genera: *Anopodium*, *Apodospora*, *Arnium*, *Fimetariella*, and *Zygospermella*.

## Methods

**Taxon sampling** We assembled 62 newly generated sequences from 23 species and eight genera currently classified in the *Lasio-sphaeriaceae* plus 126 GenBank (<http://www.ncbi.nlm.nih.gov>) sequences representing various members of Sordariomycetes. A representative of Xylariomycetidae (*Eutypa* sp.) was used as an outgroup based on results from previous phylogenetic analyses (Huhndorf and Miller 2011; Zhang et al. 2006). We compiled a combined data set with 117 LSU nrDNA sequences and 71  $\beta$ -tubulin sequences. Collection data and GenBank accession numbers for the sequenced specimens are listed in Table 1, Electronic Supplementary Material.

**DNA extraction, PCR amplification and sequencing** DNA was extracted from ascomata or cultures using the EZNA Fungal DNA kit (Omega bio-tek, Doraville, Georgia, USA). The protocol was used according to the manufacturer's instructions, except that the material was homogenized for 3 min using a Mini-Beadbeater (BioSpec Products, Bartlesville, Oklahoma, USA) with Buffer FG1 and 2.5 mm

Zirconia/Silica beads (BioSpec Products, Bartlesville, Oklahoma, USA). Double-stranded copies of the LSU and  $\beta$ -tubulin were obtained by polymerase chain reaction (PCR) amplifications using AccuPower™ PCR premix (BioNeer, Alameda, California, USA) to which DNA extract, primers (10  $\mu$ M) and water were added. The DNA was amplified with primers LR0R/LR7 (R. Vilgalys website), and BT1819R/BT2916 (Miller and Huhndorf 2005), while thermocycler conditions followed Miller and Huhndorf (2005). PCR-products were cleaned with MultiScreen HTS™ PCR plates (Millipore, Cork, Ireland). Macrogen Ltd (Seoul, Korea) performed sequencing reactions using the following primers: LR0R, LR5, LR3R, LRAM1, LR3, LR7; BT1819R, BT1283R, BTAM1R, BT2916 (Huhndorf et al. 2004; Miller & Huhndorf 2005; R. Vilgalys website). Sequence fragments were assembled and edited in Sequencher 4.8 (Gene Codes Corp., Ann Arbor, Michigan, USA).

**Sequence alignment** Alignment of each set of sequences were done in Geneious Pro 5.6.5 (Biomatters Ltd, Auckland, New Zealand), using the MAFFT application (Katoh et al. 2002). We used algorithm E-INS-i, scoring matrix 100PAM/k=2, gap open penalty 1.53 and offset value 0 for the  $\beta$ -tubulin data set, and algorithm G-INS-i, scoring matrix 100PAM/k=2, gap open penalty 1.53 and offset value 0.123 for the LSU data set. We excluded parts of the 5' and 3' ends, plus one intron from the analyses of the  $\beta$ -tubulin data set (96 characters), and two ambiguous indel sections of the LSU data set (259 characters). The alignment is deposited in TreeBASE (accession number S14831).

**Phylogenetic analyses** We used PartitionFinder 1.0.1 (Lanfear et al. 2012) to select the best partitioning scheme and best-fit models of nucleotide evolution. The partitioning scheme with the best likelihood, according to the Bayesian information criterion, separated the data sets among genes and among nucleotide positions in the codons for the  $\beta$ -tubulin gene. The best-fit evolutionary models were the SYM+I+ $\Gamma$  model for  $\beta$ -tubulin codon position 1, the JC+I model for  $\beta$ -tubulin codon position 2, and the GTR+I+ $\Gamma$  model for  $\beta$ -tubulin codon position 3 and LSU. Bayesian Markov Chain Monte Carlo (MCMC) analyses were conducted in MrBayes 3.2 (Ronquist and Huelsenbeck 2003) and performed using resources at SNIC through Uppsala Multidisciplinary Center for Advanced Computational Science (UPPMAX). MCMC sampling was performed with four parallel runs, six chains, sampling every 1000th tree, and the temperature set to 0.25. Sampling was halted when the critical value for the topological convergence diagnostic had reached 0.01. Log files from the Bayesian analyses were analyzed with Tracer v 1.5 (Rambaut and Drummond 2009) to determine the burn-in value, and a 50 % majority rule consensus tree was

**Table 1** Information on the species sequenced in this study

Order: Family	Species	Source	GenBank accession no.	
			LSU	betatubulin
Xylariales	<i>Eutypa</i> sp.	GenBank	AY346280	AY780117
Diaporthales	<i>Valsa ceratosperma</i>	GenBank	AF408387	AY780144
Boliales	<i>Camarops amorphia</i>	GenBank	AY780054	AY780093
	<i>Camarops tubulina</i>	GenBank	AY346266	AY780095
Coniochaetales	<i>Coniochaetidium savoryi</i>	GenBank	AY346276	AY780114
	<i>Coniochaeta discoidea</i>	GenBank	AY346297	AY780134
Sordariales Chaetomiaceae	<i>Chaetomidium fimeti</i>	GenBank	FJ666358	FJ666374
	<i>Chaetomidium subfimeti</i>	GenBank	FJ666357	FJ666373
	<i>Chaetomidium triangulare</i>	GenBank	FJ666362	FJ666378
	<i>Chaetomium globosum</i>	GenBank	AY346272	AY780110
	<i>Corynascus sepedonium</i>	GenBank	FJ666364	FJ666380
	<i>Zopfiella ebriosa</i>	GenBank	AY346305	AY780146
	Lasiosphaeriaceae	<i>Anopodium ampullaceum</i>	1. UK, Scotland, on rabbit dung, Richardson, MJR 40/07 ( <sup>a</sup> UPS)	<b>KF557662</b>
<i>Anopodium ampullaceum</i>		2. UK, Scotland, on rabbit dung, Richardson MJ, E00218015 (E)	<b>KF557663</b>	<b>KF557702</b>
<i>Apiosordaria backusii</i>		GenBank	AY780051	AY780085
<i>Apiosordaria verruculosa</i>		GenBank	AY346258	AY780086
<i>Apodospora gotlandica</i>		France, on cattle dung, Richardson MJ, E00204952 (E)	<b>KF557664</b>	<b>KF557703</b>
<i>Apodospora peruviana</i>		Australia, on wombat dung, <sup>b</sup> CBS 118,394	<b>KF557665</b>	-
<i>Apodospora simulans</i>		1. Sweden, on moose dung, Kruys 701 (UPS)	<b>KF557666</b>	<b>KF557704</b>
<i>Apodospora simulans</i>		2. Sweden, on moose dung, Lantz H., 2007, Storvreta, Trollberget.	<b>KF557667</b>	<b>KF557705</b>
<i>Apodus deciduus</i>		GenBank	AY681165	-
<i>Apodus oryzae</i>		GenBank	AY681166	-
<i>Arnium arizonense</i>		1. USA, on sheep dung, Santesson 18,211-c (S)	<b>KF557668</b>	<b>KF557706</b>
<i>Arnium arizonense</i>		2. Sweden, on sheep dung, Kruys 724 (UPS)	<b>KF557669</b>	<b>KF557707</b>
<i>Arnium arizonense</i>		3. France, on sheep dung, Richardson MJ, E00204509 (E)	<b>KF557670</b>	<b>KF557708</b>
<i>Arnium arizonense</i>		4. Australia, on camel dung, CBS 120,289.	<b>KF557671</b>	-
<i>Arnium caballinum</i>		Iceland, on sheep dung, Lundqvist 7,098-e (S)	<b>KF557672</b>	-
<i>Arnium cirriferum</i>		Australia, on cow dung, CBS 120,041	<sup>d</sup> <b>KF557673</b>	<b>KF557709</b>
<i>Arnium hirtum</i>		1. UK, Scotland, on sheep dung, Richardson MJ, E00204950 (E)	<b>KF557675</b>	<b>KF557711</b>
<i>Arnium hirtum</i>		2. UK, Scotland, on cattle dung, Richardson MJ, E00204487 (E)	<b>KF557676</b>	<b>KF557712</b>
<i>Arnium hirtum</i>		3. USA, on bison dung, Sanchez LyRS8108,1 (F)	<b>KF557677</b>	-
<i>Arnium hirtum</i>		4. USA, on bison dung, Sanchez LyRS9246,2 (F)	<b>KF557678</b>	-
<i>Arnium inaequilaterale</i>	Sweden, on hare dung, Lundqvist 3,345-b (UPS)	<b>KF557679</b>	-	
<i>Arnium japonense</i>	Japan, on mouse dung <sup>c</sup> SANK 10,273	<b>KF557680</b>	<b>KF557713</b>	
<i>Arnium leporinum</i>	1. Netherlands, on rabbit dung, CBS 365.69	<b>KF557682</b>	-	
<i>Arnium leporinum</i>	2. Estonia, on hare dung, Lundqvist 18,080-c (UPS)	<b>KF557681</b>	-	
<i>Arnium leporinum</i>	3. USA, on rabbit dung, Huhndorf SMH3188	<b>KF557683</b>	-	
<i>Arnium mendax</i>	1. France, on rabbit dung, Lundqvist 20,874-c (S)	<b>KF557687</b>	<b>KF557716</b>	
<i>Arnium mendax</i>	2. UK, Scotland, on roe deer dung, Richardson MJ, E00122117 (E)	<b>KF557688</b>	<b>KF557717</b>	
<i>Arnium olerum</i>	1. Costa Rica, on horse dung, Huhndorf SMH3253 3 (F)	<b>KF557690</b>	-	
<i>Arnium olerum</i>	2. Australia, on wombat dung, CBS 120,012	<sup>d</sup> <b>KF557689</b>	<b>KF557718</b>	
<i>Arnium sudermanniae</i>	Sweden, on moose dung, Åke Strid 26. III.1989 (S)	-	<b>KF557719</b>	

**Table 1** (continued)

Order: Family	Species	Source	GenBank accession no.	
			LSU	betatubulin
	<i>Arnimium tomentosum</i>	Spain, on stalks of <i>Brassica oleracea</i> , Francoise Candoussau (S)	<b>KF557691</b>	<b>KF557720</b>
	<i>Belloisia rhynchostoma</i>	GenBank	EU999217	-
	<i>Bombardia bombardia</i>	1. GenBank	AY346263	AY780090
	<i>Bombardia bombardia</i>	2. GenBank	AY780052	AY780089
	<i>Bombardia bombardia</i>	3. GenBank	AY780053	AY780091
	<i>Bombardioidea anartia</i>	GenBank	AY346264	AY780092
	<i>Cercophora aff mirabilis</i>	GenBank	AY780061	AY780104
	<i>Cercophora ambigua</i>	GenBank	AY999114	-
	<i>Cercophora appalachianensis</i>	GenBank	AF132328	-
	<i>Cercophora areolata</i>	GenBank	AY587936	AY600252
	<i>Cercophora atropurpurea</i>	1. GenBank	AY780056	AY780099
	<i>Cercophora atropurpurea</i>	2. GenBank	AY780057	AY780100
	<i>Cercophora caudata</i>	GenBank	AY999113	-
	<i>Cercophora coprophila</i>	1. GenBank	AY780058	AY780102
	<i>Cercophora coprophila</i>	2. Jamaica, on cow dung, Fernandez SMH4089 (F)	<b>KF557692</b>	-
	<i>Cercophora costaricensis</i>	GenBank	AY780059	AY780103
	<i>Cercophora grandiuscula</i>	<sup>o</sup> Australia, on wombat dung, CBS 120,013	<sup>d</sup> <b>KF557693</b>	-
	<i>Cercophora macrocarpa</i>	GenBank	AY780060	-
	<i>Cercophora mirabilis</i>	GenBank	AY346271	-
	<i>Cercophora newfieldiana</i>	GenBank	AY780062	AY780106
	<i>Cercophora samala</i>	GenBank	AY999111	-
	<i>Cercophora scortea</i>	GenBank	AY780063	AY780107
	<i>Cercophora septentrionalis</i>	GenBank	U47823	-
	<i>Cercophora sordarioides</i>	GenBank	AY780064	-
	<i>Cercophora</i> sp.	GenBank	AY780055	AY780098
	<i>Cercophora sparsa</i>	GenBank	AY587937	AY600253
	<i>Cercophora striata</i>	1. GenBank	AY780065	AY780108
	<i>Cercophora striata</i>	2. GenBank	AY780066	-
	<i>Cercophora sulphurella</i>	GenBank	AY587938	AY600254
	<i>Cercophora terricola</i>	GenBank	AY780067	AY780109
	<i>Corylomyces selenosporus</i>	GenBank	DQ327607	-
	<i>Cuspidatispora xiphiago</i>	GenBank	DQ376251	DQ376252
	<i>Echria gigantospora</i>	<sup>o</sup> USA, on submerged wood, culture made from the holotype: Raja, Miller & Crane, F77-1	<sup>d</sup> <b>KF557674</b>	<b>KF557710</b>
	<i>Echria macrotheca</i>	1. Sweden, on horse dung, Lundqvist 2,674 (UPS)	-	<b>KF557714</b>
	<i>Echria macrotheca</i>	2. Sweden, on horse dung, Lundqvist 2,311 (UPS)	<b>KF557684</b>	<b>KF557715</b>
	<i>Echria macrotheca</i>	3. Costa Rica, on horse dung, Huhndorf SMH3253,1 (F)	<b>KF557686</b>	-
	<i>Echria macrotheca</i>	4. New Zealand, on cow dung, Huhndorf SMH5141 (F)	<b>KF557685</b>	-
	<i>Fimetariella rabenhorstii</i>	Sweden, on moose dung, Lundqvist 20,410-c (S)	<b>KF557694</b>	<b>KF557721</b>
	<i>Immersiella caudata</i>	GenBank	AY436407	-
	<i>Immersiella immersa</i>	GenBank	AY436408	-
	<i>Jugulospora rotula</i>	GenBank	AY346287	AY780120
	<i>Lasiosphaeria glabrata</i>	GenBank	AY436410	AY600255
	<i>Lasiosphaeria hirsuta</i>	GenBank	AY436417	AY780121
	<i>Lasiosphaeria hispida</i>	GenBank	AY436419	AY780122
	<i>Lasiosphaeria lanuginosa</i>	GenBank	AY587940	AY600258

**Table 1** (continued)

Order: Family	Species	Source	GenBank accession no.	
			LSU	betatubulin
	<i>Lasiosphaeria rugulosa</i>	GenBank	AY436414	AY600272
	<i>Lasiosphaeria sorbina</i>	GenBank	AY436415	AY600273
	<i>Podospora appendiculata</i>	1. GenBank	AY780071	AY780129.
	<i>Podospora appendiculata</i>	2. GenBank	AY999103	-
	<i>Podospora australis</i>	1. USA. on bison dung, Sanchez LyRS9247 1 (F)	<b>KF557695</b>	-
	<i>Podospora australis</i>	2. USA. on bison dung, Sanchez LyRS9341 5 (F)	<b>KF557696</b>	-
	<i>Podospora austroamericana</i>	GenBank	AY999101	-
	<i>Podospora cochleariformis</i>	GenBank	AY999098	-
	<i>Podospora comata</i>	GenBank	AY780072	-
	<i>Podospora cupiformis</i>	GenBank	AY999102	-
	<i>Podospora curvicolla</i>	GenBank	AY999099	-
	<i>Podospora decipiens</i>	GenBank	AY780073	AY780130
	<i>Podospora didyma</i>	GenBank	AY999100	-
	<i>Podospora fimiseda</i>	GenBank	AY346296	AY780133
	<i>Podospora intestinacea</i>	GenBank	AY999104	-
	<i>Podospora pauciseta</i>	GenBank	EU999216	-
	<i>Schizothecium curvisporum</i>	GenBank	AY346300	-
	<i>Schizothecium fimbriatum</i>	GenBank	AY780075	-
	<i>Schizothecium glutinans</i>	GenBank	AY999093	-
	<i>Schizothecium vesticola</i>	GenBank	AY780076	-
	<i>Strattonia carbonaria</i>	GenBank	AY346302	AY780141
	<i>Triangularia mangenotii</i>	GenBank	AY346303	AY780142
	<i>Triangularia tanzaniensis</i>	GenBank	AY780081	AY780143
	<i>Zopfiella leucotricha</i>	Japan, on field soil, CBS 389.84	<b>KF557697</b>	-
	<i>Zygopleurage zygospora</i>	GenBank	AY346306	AY780147
	<i>Zygospermella insignis</i>	1. USA, on muskox dung, Burdsall 1308AK (F)	<b>KF557700</b>	-
	<i>Zygospermella insignis</i>	2. Sweden, on cow dung, Lundqvist 2,444-h (UPS)	<b>KF557698</b>	<b>KF557722</b>
	<i>Zygospermella insignis</i>	3. France, on cattle dung, Richardson MJ, E00204312 (E)	<b>KF557699</b>	<b>KF557723</b>
Sordariaceae	<i>Asordaria tenerifae</i>	GenBank	AY681138	-
	<i>Gelasinospora tetrasperma</i>	GenBank	AY346281	AY780118
	<i>Neurospora crassa</i>	GenBank	AF286411	-
	<i>Sordaria fimicola</i>	GenBank	AY780079	AY780138

Sequences produced in this study are in bold

<sup>a</sup> Herbarium acronyms follow Holmgren et al. 1990

<sup>b</sup> CBS, Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands

<sup>c</sup> SANK, Research laboratories of the Daiichi Sanko Pharmaceutical Co., Ltd., Tokyo, Japan

<sup>d</sup> We have sequenced duplicate cultures of *Arniium cirriferum*, *A. olerum*, *E. gigantosporum* and *Cercophora grandiuscula*. Their nLSU rDNA sequences were identical and only one set was included in the analyses

<sup>e</sup> Included ex-typesY

generated in mrBayes using the remaining trees. We evaluated the contribution of individual genes and the amount of incongruence among them by comparing significantly supported clades from separate Bayesian analyses with two parallel runs and eight chains (Mason-Gamer and Kellogg

1996; de Queiroz 1993). Significant support in this study refers to Bayesian posterior probability values (BPP)  $\geq 95$  % and bootstrap values (BS)  $\geq 70$  % (Alfaro et al. 2003; Hillis and Bull 1993). We also conducted a Maximum Likelihood search with rapid bootstrap analysis

for the best tree with the GTR+ $\Gamma$  model for all partitions combined with 5,000 bootstrap replicates in RAxML 7.2.6 (Stamatakis 2006; Stamatakis et al. 2008).

## Results

**Sequence alignment and phylogenetic analyses** Our combined  $\beta$ -tubulin and LSU alignment included 2,408 (984+1,424) characters. The number of parsimony informative sites compared to variable sites was 257/316 (81 %) for the  $\beta$ -tubulin partition and 215/335 (64 %) for the LSU.

The MCMC analysis of the two combined genes proceeded for  $17.45 \times 10^6$  generations, and resulted in 69 824 trees. The first 2 500 trees per run were discarded as burn-in, and the consensus tree was generated using the remaining 59 824 trees (Fig. 1). We found no significant incongruence in the tree topologies of the separate gene analyses. The MCMC analysis of LSU resulted in 34 051 trees per run and 48 102 were included in a consensus tree. The  $\beta$ -tubulin analysis produced 104 401 trees per run and 158 802 were used to build the consensus tree. The significantly supported topology of the best ML tree coincides with the Bayesian tree (data not shown).

**Phylogenetic relationships** *Sordariales* is monophyletic with high posterior probability support, while the largest family *Lasiosphaeriaceae* is paraphyletic and includes *Sordariaceae* and *Chaetomiaceae* (Fig. 1). We distinguish four core lineages of *Lasiosphaeriaceae* taxa (clades I–IV) with only clade IV having both significant BPP and BS support. Clades I, II and III are unsupported sister taxa. Clade I includes representatives of eleven genera of *Lasiosphaeriaceae* (Fig. 1a). In clade II, the monophyletic *Sordariaceae* and one member of *Chaetomiaceae* are nested among members of eight genera of *Lasiosphaeriaceae*. Clade III includes members of seven genera of *Lasiosphaeriaceae* with significant BPP support (Fig. 1b). Clade IV is sister group to the basal *Chaetomiaceae* and includes members of five *Lasiosphaeriaceae* genera with significant BS and BPP support.

The main focus of this study, the genera *Anopodium*, *Apodospora*, *Arnium*, *Fimetariella* and *Zygospermella*, belong to different clades in the tree. The 26 representatives of *Arnium* are scattered in clades I, II and IV. The three *Apodospora* species form a well-supported monophyletic group in clade II where the single representative of *Fimetariella* is also placed. *Anopodium* belongs to clade III and *Zygospermella* to clade I.

**Taxonomy** Based on a combination of morphological and molecular data, *Arnium* section *Echria* is raised to genus level and two species are transferred as new combinations.

*Echria* (N. Lundq.) Krays, Huhndorf and A.N. Mill., stat. nov. MycoBank #: MB808571.

$\equiv$  *Arnium* section *Echria* N. Lundq. Symb. Bot. Upsal. 20(1): 237. 1972 (basionym).

*Echria gigantospora* (Raja and Shearer) Krays, Huhndorf and A.N. Mill., comb. nov. MycoBank #: MB808572.

$\equiv$  *Arnium gigantosporum* Raja and Shearer, *Fungal Diversity* 22: 220. 2006 (basionym).

*Echria macrotheca* (P. Crouan and H. Crouan) Krays, Huhndorf and A.N. Mill., comb. nov.

MycoBank #: MB808573.

$\equiv$  *Sphaeria macrotheca* P. Crouan and H. Crouan, *Florule Finistère* (Paris): 24. 1,867 (basionym).

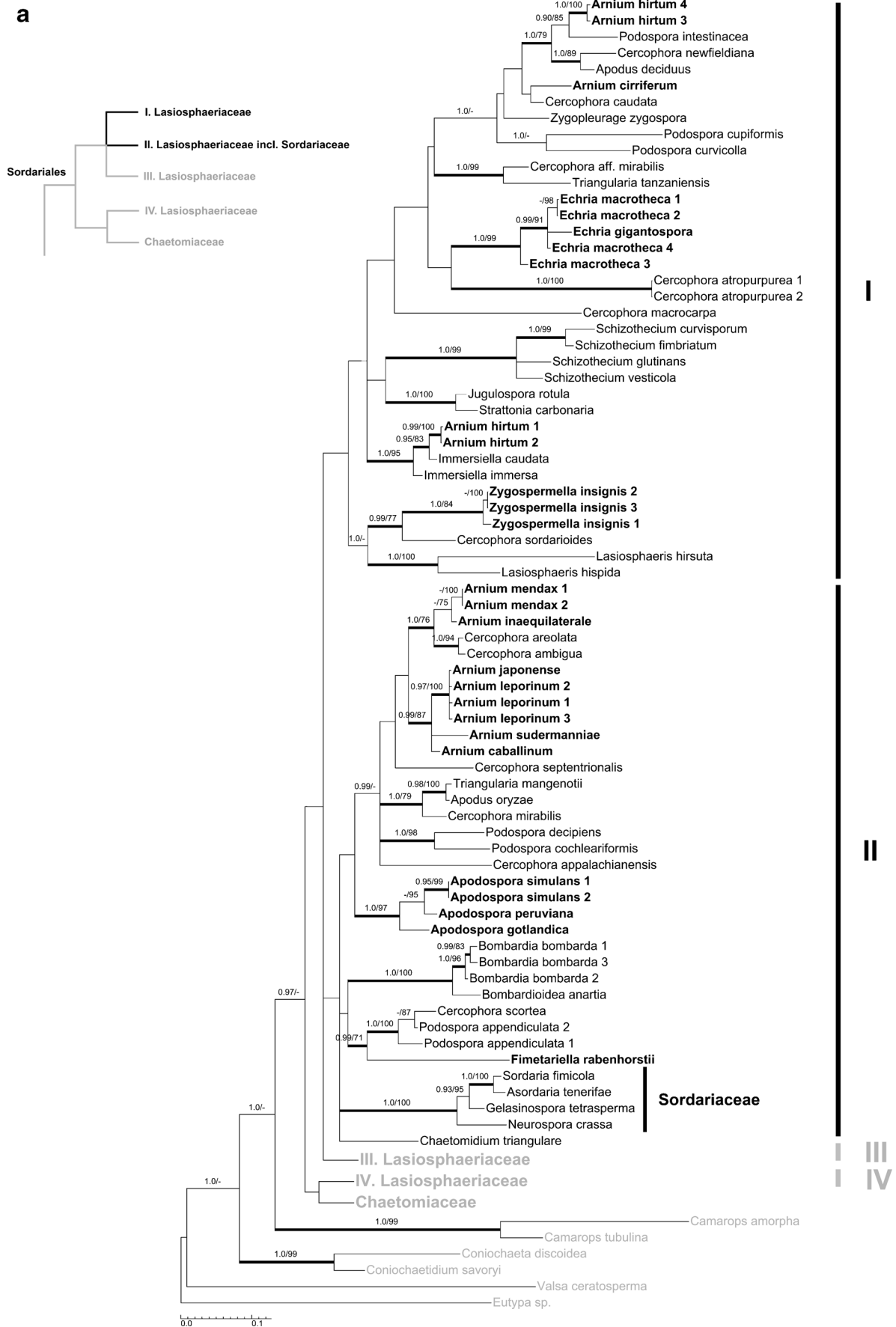
Note: *Echria gigantospora* and *E. macrotheca* are morphologically similar in that both have ascospores covered with long pointed tufts of agglutinated rigid hairs and striated gelatinous sheaths that swell in water and surround their ascospores. They differ in that *E. gigantospora* has larger ( $84\text{--}126 \times 20\text{--}34 \mu\text{m}$ ), fusiform spores with a roughened spore wall (Raja and Shearer 2006), while *E. macrotheca* (Fig. 3o–q) has smaller ( $43\text{--}54 \times 20\text{--}29 \mu\text{m}$ ), ellipsoidal spores (Lundqvist 1972). They also seem to prefer different substrates as *E. gigantospora* is described from submerged wood, while *E. macrotheca* is coprophilous.

## Discussion

The main topology of our *Sordariales* tree is consistent with previous phylogenies in which *Chaetomiaceae* and *Sordariaceae* are nested within the paraphyletic *Lasiosphaeriaceae* (Miller and Huhndorf 2005; Réblová 2008). Although our study includes additional taxa, the clades that Miller and Huhndorf (2005) distinguished based on the morphology of the ascomal walls remain unchanged. We reveal several new significantly supported clades, but the major challenge is still to find morphological features to characterize them when monophyletic genera are mostly the exception rather than the rule.

*Anopodium* Lundqvist (1964a) established the genus *Anopodium* for its unique uniseptate ascospores with one dark

**Fig. 1** The Bayesian tree is a 50 % majority rule consensus of 59 824 combined trees of the  $\beta$ -tubulin and LSU nrDNA for 119 taxa. Thickened branches have both significant BPP values ( $\geq 95$  %) and BS values ( $\geq 70$  %). Numbers above the branches indicate significant support values in the following order: BPP/BS, and support values below these thresholds are indicated by a dash. Outgroup is *Eutypa* sp. *Sordariales* include four clades of *Lasiosphaeriaceae* taxa (I–IV) **a.** *Lasiosphaeriaceae* clade I and II in focus. **b.** *Lasiosphaeriaceae* clade III and IV in focus



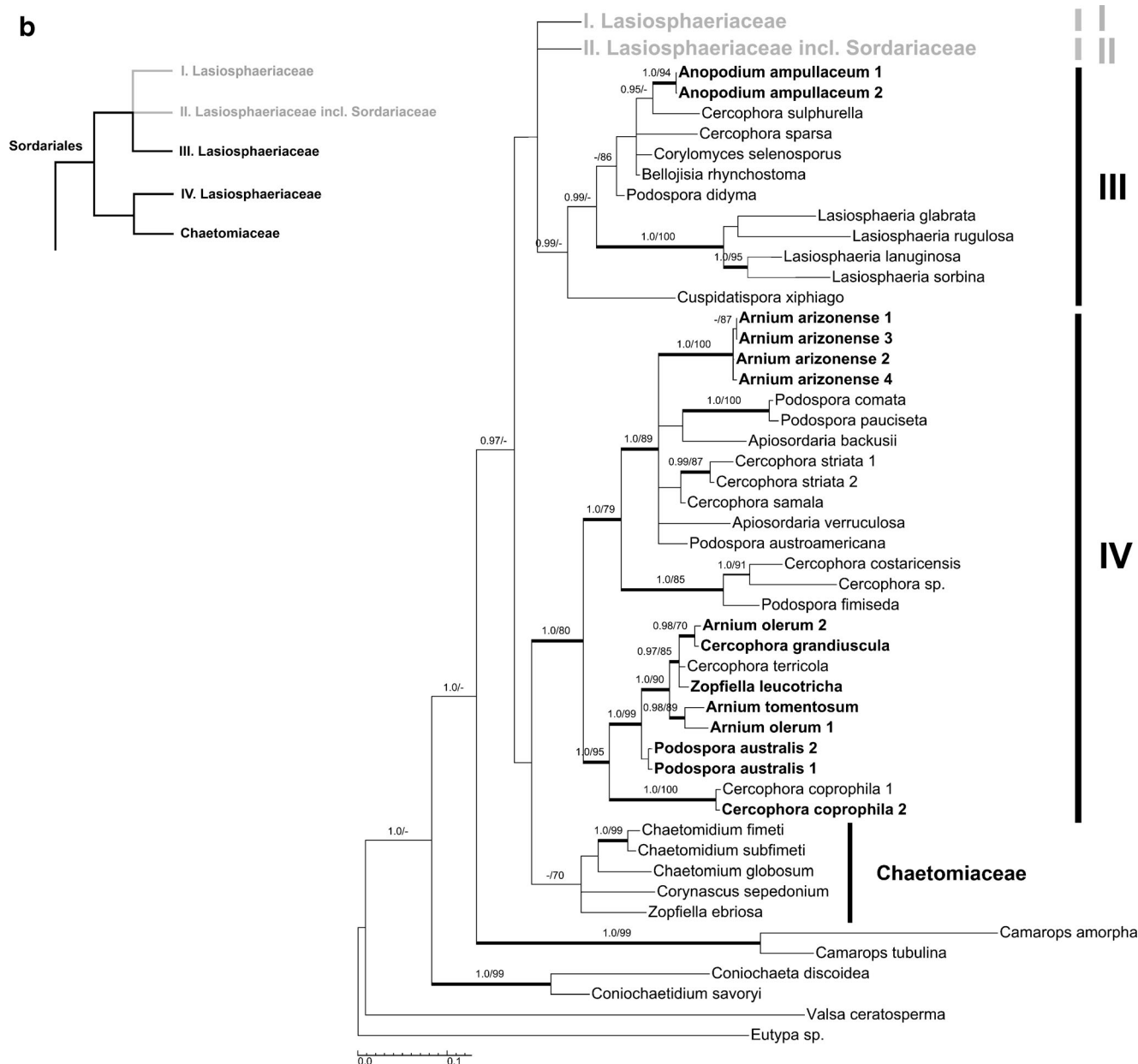


Fig. 1 (continued)

brown basal cell and a hyaline apSical pedicel (Fig. 2a-d). They resemble *Podospora* spores except the cells are in a reversed position (Lundqvist 1964a). Two collections of the generic type, *Anopodium ampullaceum*, were included in our molecular analyses and their closest relative is *Cercophora sulphurella* in clade III (Fig. 1b). Both species join the weakly supported clade containing *C. sparsa*, *Corylomyces selenosporus*, *Bellojisia rhynchostoma* and *Podospora didyma*. The sister taxa of this extended *Anopodium* clade are *Laiosphaeria* species. The latter all have a similar three-layered ascomal wall in which the outer wall layer is composed of hyphae that form a tomentum (Miller and Huhndorf

2005). *Cercophora sulphurella* fits morphologically into this group with its typical yellowish-green tomentum, along with *B. rhynchostoma* (Réblová 2008) and *C. selenosporus* (Stchigel et al. 2006). *Corylomyces selenosporus* has olivaceous–yellow, tomentose perithecia and olivaceous–yellow mycelium (Stchigel et al. 2006), and *B. rhynchostoma* has perithecia with a yellowish inconspicuous tomentum and a subiculum of yellowish to pale olivaceous–brown hyphae around the base (Réblová 2008). *Anopodium* species on the other hand have glabrous or hairy perithecia, as do *P. didyma* and *C. sparsa*. A feature that is shared by both the extended *Anopodium* clade and the *Laiosphaeria* clade is the presence

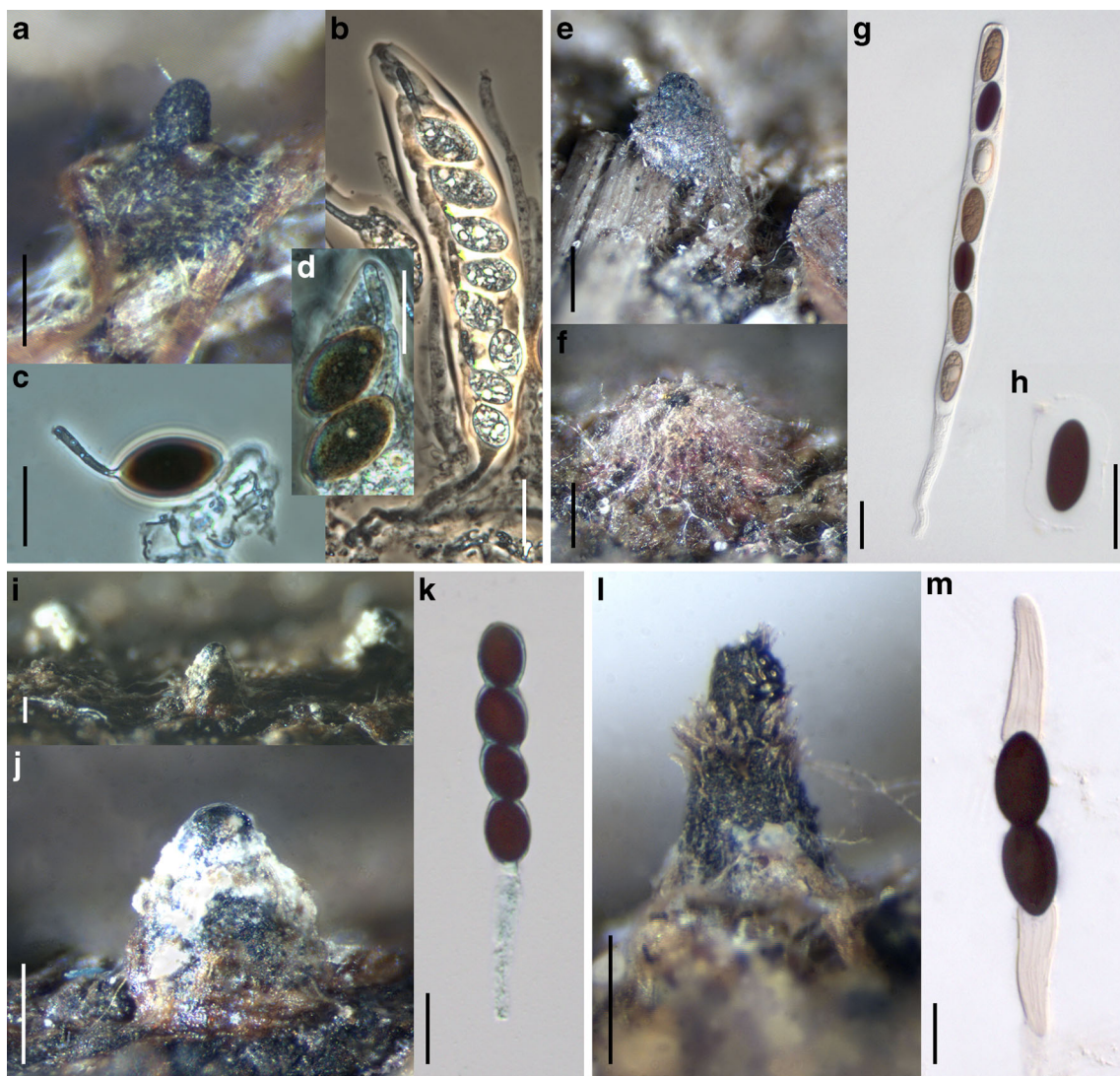


of yellow pigment. It occurs in the membranaceous, transparent peridium of both *A. ampullaceum* (Lundqvist 1964b) and *P. didyma* (Mirza and Cain 1969). *Cercophora sparsa* and *C. sulphurella* both have yellow centrum pigments (Hilber and Hilber 1979).

Within this extended *Anopodium* clade are taxa exemplifying a range of ascospore types: *Podospora didyma* with the typical dark brown apical cell and a hyaline basal pedicel, *Anopodium* with inverted *Podospora*-like spores, *Cercophora* spores with a long basal pedicel, and the dark brown navicular, lunate to reniform ascospores of *Corylomyces* and *Bellojisia*.

*Apodospora* The genus comprises species that have one-celled, dark brown ascospores with an apical germ pore and

a gelatinous sheath and asci with an apical ring (Fig. 2e-h) (Bell et al. 2008; Cain and Mirza 1970; Lundqvist 1972). The ascospore morphology is reminiscent of *Sordaria* but *Apodospora* differs in the apical location of the germ pore as well as an ascomatal peridium composed of smaller-sized cells (Lundqvist 1972). The species included in our study (*A. gotlandica*, *A. peruviana* and the generic type, *A. simulans*) have ascomata that are all more or less glabrous with a reddish-brown peridium. The genus forms a well-supported monophyletic group in clade II but without higher-level support (Fig. 1a). The unsupported clade II is an assemblage of taxa in a number of morphologically diverse genera that often have branch support for several species. Molecular data confirms the morphological viewpoint given



**Fig. 2** *Anopodium ampullaceum*, **a** ascoma, **b** immature ascospores in the ascus, **c** ascospore, **d** ascospores position in the ascus. (Coll. Krays 699, UPS). *Apodospora simulans*, **e** ascoma, **f** ascoma, **g** ascus, **h** ascospore with gelatinous sheath. (e Coll. TRTC156582, TRTC. f–h Coll. Krays 701, UPS). *Fimetariella rabenhorstii*, **i** ascomata, **j** ascoma, **k**

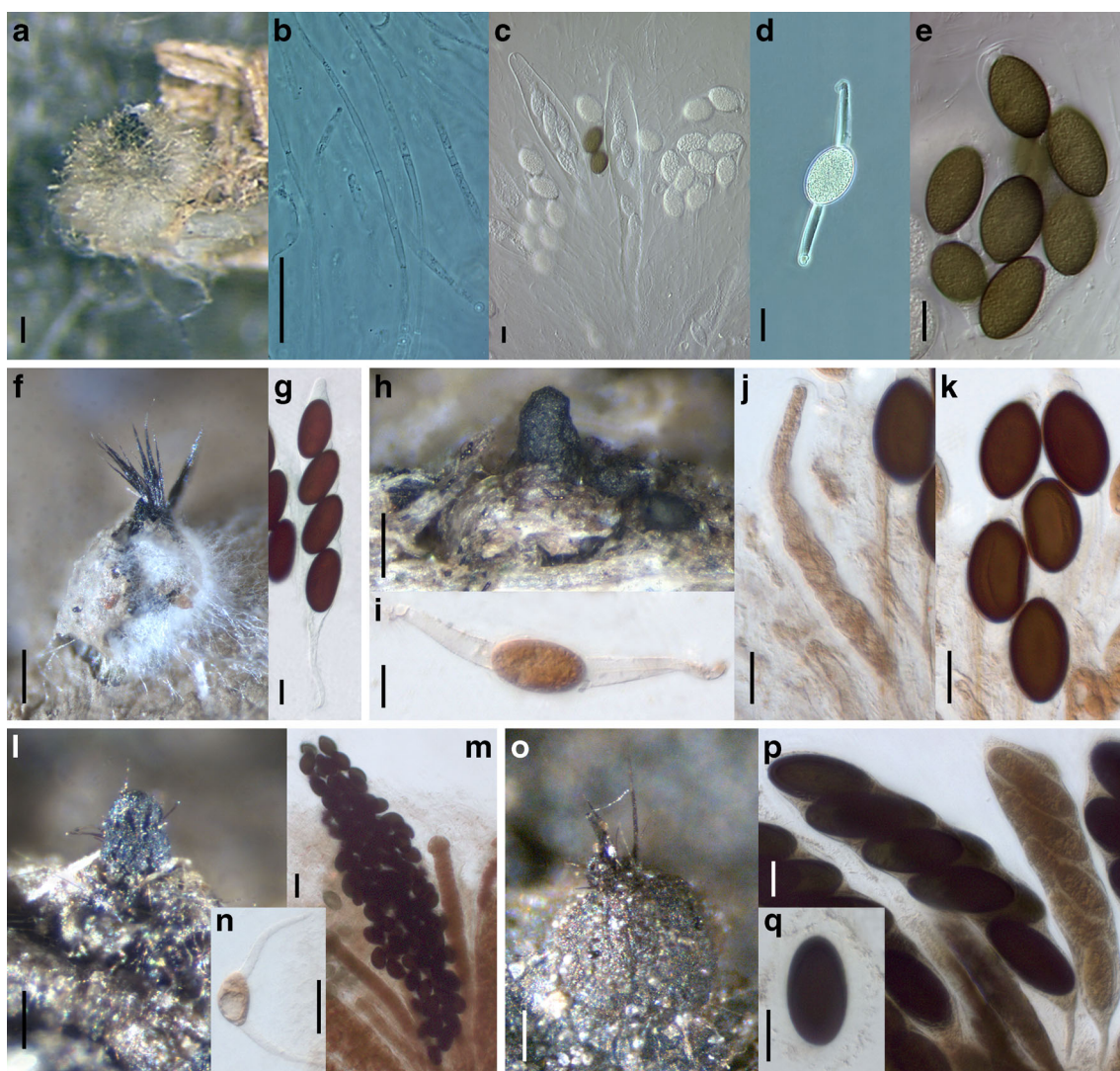
ascus with ascospores. (i–j Coll. F-644,827, UPS. k Coll. TRTC156796, TRTC). *Zygospermella insignis*. **l** ascoma, **m** ascospore with gelatinous appendages. (l Coll. Krays 718, UPS. m Coll. TRTC156807, TRTC). Scale bars: **a**, **e–f**, **i–j**, **l**=200  $\mu$ m, **b–d**, **g–h**, **k**, **m**=20  $\mu$ m

by Lundqvist (1972) that *Apodospora* does not belong within the *Sordariaceae*. Additional taxa included in the genus based on morphology are *A. viridis*, a species with yellow-green peridium and papillae around the neck, as well as *A. bulgarica*, characterized by olive-brown peridium and rigid hairs around the neck. Their inclusion in the genus must be further investigated with molecular data.

**Arnium** The genus *Arnium* is morphologically very diverse, and therefore not surprisingly, found in three of the four clades in the phylogenetic tree (Fig. 1, Fig. 3). The ascomatal peridium varies from membranaceous and light coloured to coriaceous or carbonaceous and opaque in different species. Various hairs or bristles may cover the ascomata, and short

inconspicuous tubercles occur around the neck in certain species (Krug and Cain 1972; Lundqvist 1972). Some species have asci with an apical ring. The ascospores are dark brown, single-celled or with a transverse septum and often provided with one gelatinous appendage at each end or covered with a striated sheath (Lundqvist 1972).

Included in the analyses are two *Arnium* species with perithecia forming a tomentum, the type species *A. olerum* (Fig. 3a-e) and *A. tomentosum*. Both species belong to clade IV (Fig. 1b) and are related to the tomentose species *C. coprophila* and *C. grandiuscula*. *Arnium olerum* is characterized by a greyish-whitish tomentum, which is also found in *C. coprophila* and *C. grandiuscula*, while *A. tomentosum* has an olivaceous-brown tomentum. We have included two



**Fig. 3** *Arnium olerum*, **a** ascoma, **b** paraphyses, **c** asci, **d** immature hyaline ascospore with gelatinous appendages, **e** ascospores. (Coll. SMH3253 3, F). *A. arizonense*, **f** ascoma, **g** asci with ascospores. (f Coll. F66864, S, g Coll. TRTC38138, TRTC). *A. mendax*, **h** ascoma, **i** immature ascospore with gelatinous appendages, **j** ascus, **k** ascospores, (h Coll. F66872, S, i-k Coll. TRTC156621, TRTC). *A. leporinum*, **l** ascoma,

**m** asci and ascospores, **n** immature ascospore with gelatinous appendages. (l Coll. F-007,119, UPS, m-n Coll. TRTC156605, TRTC). *Echria macrotheca*, **o** ascoma, **p** asci, **q** ascospore with gelatinous sheath. (o Coll. F-644,828, UPS, p-q Coll. TRTC54023, TRTC). Scale bars: **a, f, h, l, o**=200  $\mu$ m, **b-e, g-i-k, m-n, p-q**=20  $\mu$ m

representatives of *A. olerum*, one of which, (SMH3253.3) is a sister taxon to *A. tomentosum*. The molecular data suggest that either these two species may be one entity or one of these specimens is misidentified. Given the morphological and apparent molecular similarity of these two species, additional collections should be sought for sequencing, perhaps including the RPB2 gene in a potentially more-informative dataset. The other *A. olerum* (1), originating from CBS 120,012, groups with *Cercophora grandiuscula* (CBS 120,013).

*Arnium arizonense* also belongs to clade IV (Fig. 1b, 3f-g) and four representatives of the species form a well-supported clade. It clusters within an internally unresolved but significantly supported clade of *Apiosordaria backusii*, *A. verruculosa*, *Cercophora samala*, *C. striata*, *Podospora austroamericana*, *P. comata*, and *P. pauciseta*. This mixture of taxa contains a variety of morphological characteristics with no discernable pattern of similarities. The well-supported sister group to this overall clade is a clade comprising two *Cercophora* species and the type species of *Podospora*, *P. fimiseda*. Miller and Huhndorf (2005) distinguished this group of taxa with ascomata possessing pseudo-bombardioid walls. *Arnium ontariense*, a taxon sharing a similar wall structure, most likely belongs in this clade, however, no sequences and no recent collections of this species were available for inclusion in this study.

Several *Arnium* species have ascomata that are either nearly glabrous or have flexuous hairs, and the ones included in our study (*A. caballinum*, *A. inaequilaterale*, *A. japonense*, *A. mendax*, *A. sudermanniae*) all occur in an unsupported group in clade II (Fig. 1a). Within this clade, *Arnium mendax* (Fig. 3h-k) and *A. inaequilaterale* form a moderately-supported sister clade to two taxa distinguished by their areolate peridia, *Cercophora ambigua* and *C. areolata*, although this distinctive wall structure is not found in these two *Arnium* species. *Arnium caballinum* and *A. sudermanniae* occur in a well-supported clade with three collections of *Arnium leporinum* and a collection named as *A. japonense*. *Arnium leporinum* differs morphologically from the former two *Arnium* species by ascomata that are covered with rigid hairs around the neck and multi-spored asci with a range of 64–128 ascospores (Fig. 3l-n). The original description gives *A. japonense* as having ascomata without neck hairs, 256 spores, and occurring on hare dung (Furuya and Udagawa 1976). From the molecular data it appears that the *A. japonense* specimen from mouse dung is actually *A. leporinum*.

Four collections of *Arnium hirtum* are found on two separate branches within clade I (Fig. 1a). The two representatives from Scotland are strongly supported in a clade with *Immersiella caudata* and *I. immersa*. They fit Lundqvist's (1972) description of *A. hirtum* well by having ascospores with the same large variation in size, rigid ascomatal hairs and a few swollen cells in the neck region, unlike the palisade of

slightly swollen cells found in the neck region of *I. caudata* and *I. immersa*, which give these two species a sulcate appearance (Miller and Huhndorf 2004). The two North American specimens of *A. hirtum* are sister taxa to *Podospora intestinacea* and differ from the reported description of *A. hirtum* by having ascomata with longer necks that lack hairs, but are similar in having ascospores with inflated or distally swelling appendages. In both of these characteristics, the North American specimens of *A. hirtum* are similar to *P. intestinacea*. To clarify which of these two sets of *A. hirtum* specimens represent the taxon and which characteristics indicate relatedness, additional collections from the type locality should be sequenced. *Arnium cirriferum* also belongs to clade I, but due to low support values its closest relatives are uncertain (Fig. 1a).

The majority of species in *Arnium* have spores with two gelatinous appendages, one at each end of the spore. Lundqvist (1972) established the name *Echria* for a section of *Arnium* species that instead have spores covered with a fringed sheath or numerous filaments. We include two representatives of this group, which resolve in clade I (Fig. 1a). Since these two species are separate from the generic type species, *Arnium olerum* (clade IV), we recognize a separate genus utilizing the existing section name. Therefore we raise Section *Echria* to genus level and transfer the two species as new combinations. Comparing the two species, *Echria gigantospora* has larger, fusiform spores, with a roughened spore wall, and is described from submerged wood, while *E. macrotheca* (Fig. 3o-q) has smaller, ellipsoidal spores, and is coprophilous (Raja and Shearer 2006). The two species form a strongly supported monophyletic clade, with *E. gigantospora* nested within *E. macrotheca*, suggesting they may constitute a single species. They are morphologically similar in that both have ascomata covered with long pointed tufts of agglutinated rigid hairs. Additional species like *A. ditremum* may belong to *Echria* based on morphological similarities, but these taxonomic changes await the future addition of molecular data.

*Fimetariella* Lundqvist (1964b) and Krug (1995) circumscribed the genus mainly based on the characteristic one-celled, dark brown ascospores with a gelatinous sheath, two terminal germ pores, and several scattered smaller pores (Fig. 2i-k). The ascospores are four or eight in the ascus and frequently develop with half the number of spores reversed in position with respect to the germ pore. Our phylogeny places *Fimetariella rabenhorstii* on a long branch in clade II with significant support as a sister taxon to *Podospora appendiculata* and *Cercophora scorteae* (Fig. 1a). Both *P. appendiculata* and *C. scorteae* have pseudo-bombardioid walls with short, brown, hyaline-tipped setae (Miller and Huhndorf 2005), and they show no obvious similarity to the sometimes white-powdered, ± glabrous, membranaceous

peridium of *F. rabenhorstii*. Also found in clade II is *Bombardioidea*, which occurs on an unsupported sister clade to *F. rabenhorstii*. *Bombardioidea* also possesses small, minor ascospore pores similar to those of *F. rabenhorstii* (Lundqvist 1972) making their putative, relative positions in the phylogeny intriguing. Lundqvist (1972) speculated of a potential relationship between the two taxa based on similarities in long-stipitate asci, ascospore pores and filiform paraphyses and the data suggests this possibility.

*Zygospermella* The characteristics of *Zygospermella* are perithecia with pointed, verrucose setae around the neck, a pseudoparenchymatous peridium with scattered reddish crust in the outer layer, and dark brown, two-celled ascospores with gelatinous caudae (Fig. 2l-m) (Lundqvist 1969). Similar neck setae are found in a few other taxa including *Zygospermella striata* and *Cercophora muskokensis* and on the surface of the ascomata in *Arnium imitans*, none of which were available for sequencing. Three collections of the type species, *Z. insignis* were included in our analyses and they find placement in clade I grouping with moderate support with *Cercophora sordarioides* (Fig. 1a). This clade is a moderately supported sister group to the *Lasiosphaeria* clade. While ascomata with pointed setae are found in *Lasiosphaeria hispida*, they differ from the setae in *Zygospermella* by being smooth and mostly covering the entire ascomata. The sister taxon, *C. sordarioides*, lacks setae and shows no obvious similarities.

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