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RESEARCH ARTICLE

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Phylogenetic lineages in *Entomophthoromycota*

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Key words

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Batkoa
Bayesian inference (BI)
Conidiobolus
Entomophthora
Entomophthorales
Entomophthoromycotina
maximum likelihood (ML)
phylogeny
Zoophthora

Abstract *Entomophthoromycota* is one of six major phylogenetic lineages among the former phylum *Zygomycota*. These early terrestrial fungi share evolutionarily ancestral characters such as coenocytic mycelium and gametangiogamy as a sexual process resulting in zygospore formation. Previous molecular studies have shown the monophyly of *Entomophthoromycota,* thus justifying raising the taxonomic status of these fungi to a phylum. Multi-gene phylogenies have identified five major lineages of *Entomophthoromycota*. In this review we provide a detailed discussion about the biology and taxonomy of these lineages: I) *Basidiobolus* (*Basidiobolomycetes*: *Basidiobolaceae*; primarily saprobic); II) *Conidiobolus* (*Entomophthoromycetes*, *Ancylistaceae*; several clades of saprobes and invertebrate pathogens), as well as three rapidly evolving entomopathogenic lineages in the family *Entomophthoraceae* centering around; III) *Batkoa*; IV) *Entomophthora* and allied genera; and V) the subfamily *Erynioideae* which includes *Zoophthora* and allied genera. Molecular phylogenic analysis has recently determined the relationships of several taxa that were previously unresolved based on morphology alone: *Eryniopsis, Macrobiotophthora*, *Massospora*, *Strongwellsea* and two as yet undescribed genera of *Basidiobolaceae*.

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INTRODUCTION

The phylum *Entomophthoromycota* (2012; see Table 1) is one of the largest groups of the early-diverging terrestrial fungi previously classified in the phylum *Zygomycota*. Using a multi-gene phylogeny of fungi from across all major lineages, James et al. (2006) showed that the *Zygomycota* was a non-monophyletic group and subsequent authors have worked to refine the classification of these early-diverging terrestrial fungi (Hibbett et al. 2007, Hoffmann et al. 2011). Gryganskyi et al. (2012) recently determined that the *Entomophthoromycota* constitutes a major monophyletic branch of these early-diverging fungi (Fig. 1). A phylogenetic examination of 46 slowly evolving and 107 moderately evolving, orthologous, protein-coding genes (Ebersberger et al. 2012) also suggests that the fungi included in *Entomophthoromycota* form a monophyletic group (although, unfortunately, insufficient data were available to include *Basidiobolus* in these protein-based analyses). The *Entomophthoromycota* currently includes more than 250 species that are mostly arthropod pathogens or soil- and litter-borne saprobes. This group is now distributed among three classes (Humber 2012) and six families: *Ancylistaceae*, *Basidiobolaceae*, *Completoriaceae*, *Entomophthoraceae*, *Meristacraceae* and *Neozygitaceae* (Humber 1989). In addition to the pathogens affecting arthropods, some *Entomophthoromycota* affect host organisms in other kingdoms. For example, *Ancylistes* species (*Ancylistaceae*) parasitize desmid algae, *Completoria complens* (the only species in *Completoriaceae*) parasitizes fern gametophytes and *Meristacrum* species (*Meristacraceae*) attack nematodes. Several *Conidiobolus* and *Basidiobolus* species can cause mycoses in vertebrates, including humans (Humber 1981, 1984a, Reiss et al. 2011). Some *Basidiobolus* species

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Table 1 New, phylogenetically based classification of entomophthoroid fungi (Humber 2012) including all genera and families treated by Humber (1989).

Phylum *Entomophthoromycota* Humber
- Class *Basidiobolomycetes* Humber
  - Order *Basidiobolales* Caval.-Sm.
    - Family *Basidiobolaceae* Claussen
      - *Basidiobolus* (plus undescribed new genera)
- Class *Neozygitomycetes* Humber
  - Order *Neozygitales* Humber
    - Family *Neozygitaceae* Ben Ze'ev, R.G. Kenneth & Uziel
      - *Apterivorax*, *Neozygites*, *Thaxterosporium*
- Class *Entomophthoromycetes* Humber
  - Order *Entomophthorales* G. Winter
    - Family *Ancylistaceae* J. Schröt.
      - *Ancylistes*, *Conidiobolus*, *Macrobiotophthora*
    - Family *Completoriaceae* Humber
      - *Completoria*
    - Family *Entomophthoraceae* Nowak.
      - Subfamily *Erynioideae* S. Keller
        - *Erynia*, *Eryniopsis* (in part), *Furia*, *Orthomyces, Pandora*, *Strongwellsea*, *Zoophthora*
      - Subfamily *Entomophthoroideae* S. Keller
        - *Batkoa*, *Entomophaga*, *Entomophthora*, *Eryniopsis* (in part), *Massospora*
    - Family *Meristacraceae* Humber
      - *Meristacrum, Tabanomyces*

Genera with uncertain status or excluded from phylum *Entomophthoromycota*:
- *Ballocephala* (excluded from *Meristacraceae*; reassigned to *Kickxellomycotina*; see Saikawa 1989)
- *Tarichium* (status uncertain: known from resting spores only; a mixture of fungi apparently referable to both *Entomophthoraceae* and *Neozygitaceae*)
- *Zygnemomyces* (excluded from *Meristacraceae*; reassigned to *Kickxellomycotina*; see Saikawa et al. 1997)

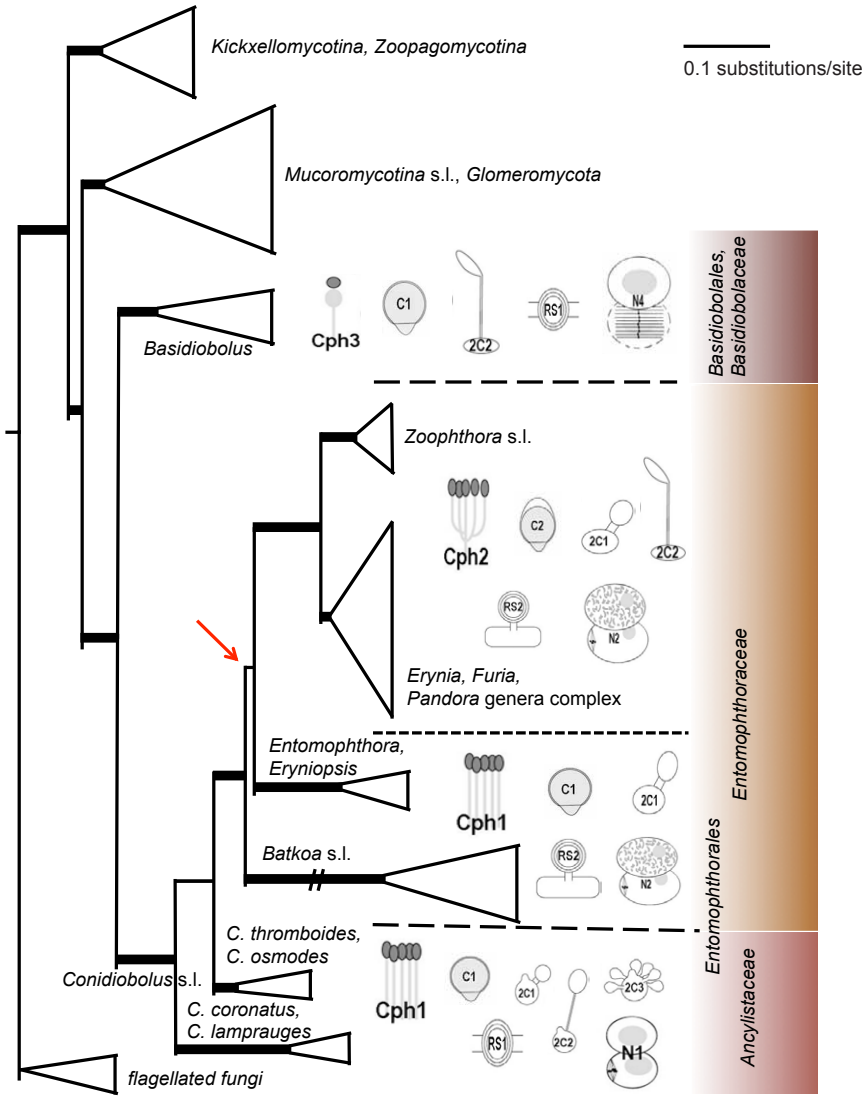

Fig. 1 Major molecular lineages in *Entomophthoromycota*, maximum likelihood phylogeny. Thickened branches have significant statistical support (ML bootstrap > 70 %, BI posterior probability > 95). Cph1-3 = types of conidiophores; C1-2 = types of primary conidia; 2C1-3 = types of secondary conidia; RS1-2 = types of resting spores; N1-2 = types of nuclear division; arrow indicates an unresolved relationship between the genus *Batkoa* and the entomophthoroid clade with insufficient statistical support for both ML and BI.

are best known as yeast-like endocommensals in the gut (or from faeces) of amphibians and reptiles.

The principal characters shared by most taxa of *Entomophthoromycota* (see Humber 2012, f. 2–4) include: 1) coenocytic vegetative cells (hyphae or shorter hyphal bodies); 2) sporulation by production of forcibly discharged dispersive or infective conidia (that may 'resporulate' to form secondary conidia); and 3) homothallic production of zygospores that function as resting spores to promote survival during unfavourable environmental conditions. It is important to note that the sexual nature of *Entomophthoromycota* zygospores has not been explicitly demonstrated since it is unknown whether karyogamy and meiosis actually occur in this spore type. In addition, some species of *Entomophthoromycota* make azygospores, which are thick-walled spores where no gametangial conjugations have been observed prior to spore formation but in which karyogamy and meiosis might still presumably occur.

The first molecular studies of early-diverging fungi were mostly based on a single gene locus (ITS-rDNA or a protein-coding gene) and suggested that the genus *Basidiobolus* was basal to and phylogenetically distant from the remainder of the *Entomophthorales* (Nagahama et al. 1995, Jensen et al. 1998, James et al. 2000, Tanabe et al. 2000, 2004, Keeling 2003, Liu & Voigt 2010, Voigt & Kirk 2011). Gryganskyi et al. (2012) recently showed that *Entomophthoromycota* is actually a monophyletic lineage that includes *Basidiobolus*. *Basidiobolus* is not closely related to any of the flagellate fungi (*Chytridiomycota* or *Blastocladiomycota*) as inferred by many of these early studies (Fig. 1). The aforementioned study also identified five major lineages of *Entomophthoromycota* that mostly correspond with traditional taxonomic groups within the group.

Previous molecular phylogenetic studies of *Entomophthoromycota* can be divided into three main groups based on the genetic information evaluated: 1) nuclear rDNA genes (18S, 28S or the whole operon); 2) protein-coding genes (actin and β-tubulin); and 3) multiple genes phylogenetic approach (rDNA, *RPB1, RPB2,* and α-transcription elongation factor). The study by Gryganskyi et al. (2012) discussed molecular data for more than a third of *Entomophthoromycota* taxa; all other molecular studies included fewer than 4 % of the described species. To date, only three studies (Nagahama et al. 1995, Jensen et al. 1998, Gryganskyi et al. 2012) were explicitly devoted to the molecular phylogeny of entomophthoroid fungi. Earlier molecular studies using only a single gene (Nagahama et al. 1995, Jensen et al. 1998, James et al. 2000, Tanabe et al. 2000, Schuessler et al. 2001) or only protein-coding genes (Keeling 2003, Einax & Voigt 2003, Tanabe et al. 2004, Liu & Voigt 2010, Voigt & Kirk 2011) suggest a polyphyletic nature for this fungal group

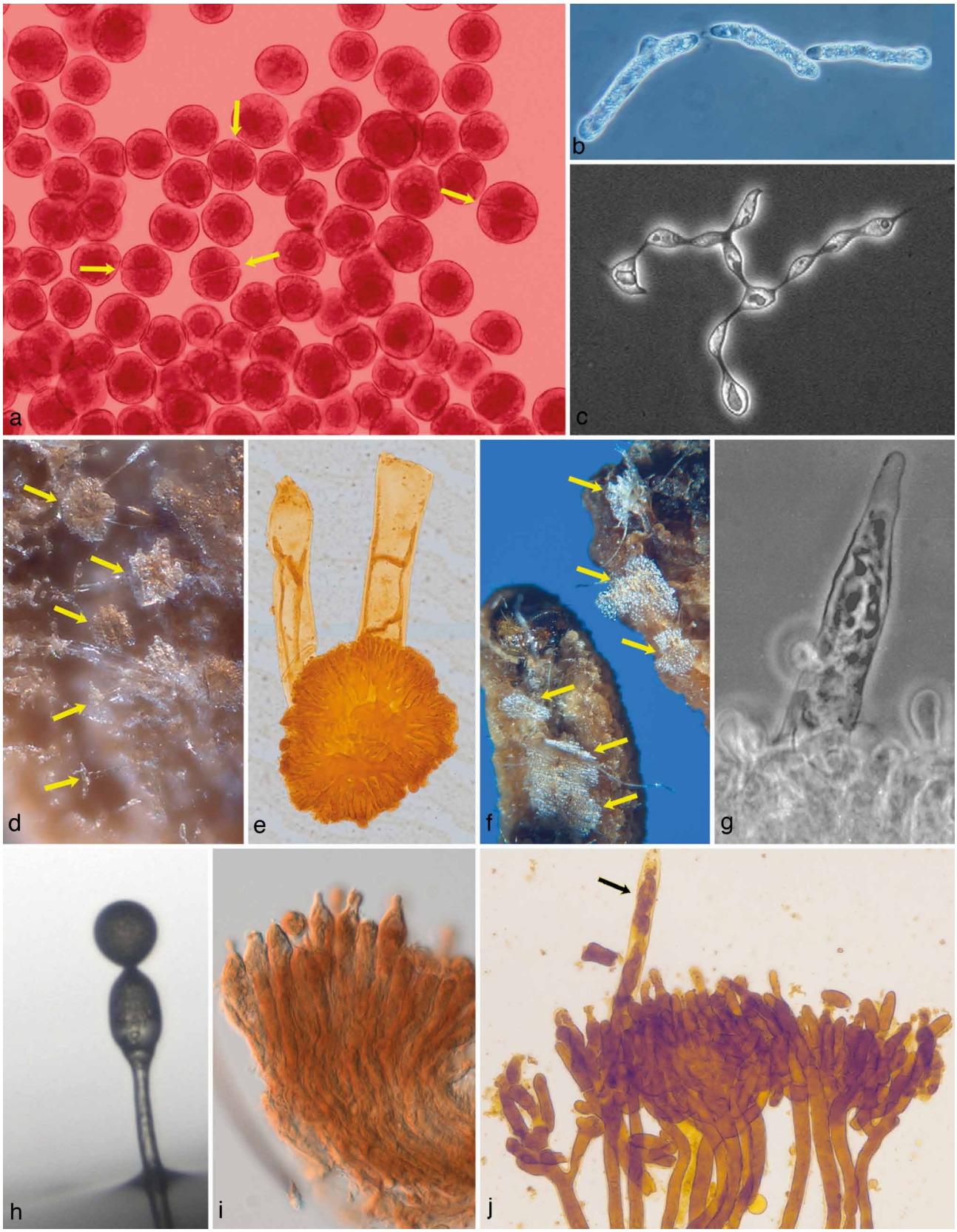

Fig. 2 Major characters of *Entomophthoromycota*. a–c. Vegetative growth: a. yeast-like growth of *Schizangiella* as uninucleate cells split internally (arrows indicate cleavage planes); b. wall-less, rod-like hyphal bodies of *Entomophthora muscae*; c. highly amoeboid protoplastic hyphal body of *Entomophaga ptychopterae*. – d–f. Rhizoids: d, e. disk-like terminal holdfasts (arrows) of *Pandora neoaphidis* from aphids; f. broad plates of holdfasts (arrows) apical on multihyphal pseudorhizomorphic rhizoids of *Zoophthora phytonomi*. – g. Cystidium of *Erynia aquatica* projecting from sporulating hymenium on infected mosquito. – h–j. Conidiophores: h. *Basidiobolus* conidiophore with subconidial swelling and globose conidium (note the base of cytoplasm in the swelling as it is pushed forward into the developing conidium); i. unbranched conidiophores of *Entomophthora* species; and j. digitately branched conidiophores and projecting cystidium (arrow) of *Zoophthora radicans*.

because *Basidiobolus* was phylogenetically distant from other *Entomophthoromycota*. The studies supporting the monophyletic interpretation of entomophthoroid fungi as traditionally recognised (e.g., James et al. 2006, Gryganskyi et al. 2012) were based on the analysis of multiple genes that included both nuclear rDNA and protein-coding genes. These phylogenetic studies clearly demonstrate that *Entomophthoromycota* is monophyletic and includes *Basidiobolus*, which should now end further speculation about phylogenetic 'links' between this genus and aquatic fungi. Nonetheless, future studies to explore the reasons for such spurious 'connections' might be useful and enlightening.

In all molecular phylogenetic studies to date, the obligately entomopathogenic taxa of *Entomophthoraceae* (including the *Batkoa*, *Entomophthora* and *Zoophthora* lineages) constitute the most derived and youngest members of *Entomophthoromycota*. The saprobic *Conidiobolus* group (*Ancylistaceae*) is basal to the *Entomophthoraceae* in all analyses. However, when multiple *Conidiobolus* species are included in analyses, this genus tends to break into two, three, or even four different clades, thus suggesting that *Conidiobolus* is a paraphyletic assemblage despite the overall morphological and ultrastructural similarities among its species (King 1976a, b, 1977). The phylogenetic analyses of James et al. (2006), White et al. (2006) and Gryganskyi et al. (2012) suggest that the *Basidiobolus* lineage is basal to the rest of the *Entomophthoromycota*.

The genetic evidence to date indicates that the great majority of genera and species in the family *Entomophthoraceae* (more than 180 obligately entomopathogenic species, see Index Fungorum; www.speciesfungorum.org/) form the core taxa for this order. The *Conidiobolus* lineage (*Ancylistaceae*) is comprised of 52–60 mostly saprobic species as well as the rare nematode pathogen *Macrobiotophthora* (Tucker 1984). Unfortunately, no gene sequences are yet available for any of the rarely collected species within the genus *Ancylistes*. All of the species in this genus are parasites of desmid algae and there are no reports that they have ever been grown in axenic culture. The *Basidiobolus* lineage (approximately 8–10 saprotrophic named and undescribed species in class *Basidiobolomycetes*) includes two as yet undescribed genera (Humber, unpubl. data), one of which is known so far only as a mycotic pathogen of snakes (Crispens & Marion 1975, Ippen 1980, Jessup & Seely 1981, Kaplan et al. 1983).

The purpose of this study is to describe each lineage from a phylogenetic perspective based on molecular data and to reveal the phylogenetic relationships within each lineage. The phylogenetic lineages are examined within a taxonomic framework intended to place the past, current, and future studies on the fungi of *Entomophthoromycota* in clearer perspective.

MATERIALS AND METHODS

In this study we used the same set of taxa, data and phylogenetic methods as described in Gryganskyi et al. (2012). We have added our own molecular data for several taxa: *Conidiobolus iuxtagenitus, C. lachnodes, C. paulus, Drechslerosporium cornellii* nom. prov., *Entomophaga australiensis* and we have also included sequences of *Pandora bullata* and *P. nourii* from Scorsetti et al. (2012). We used all available molecular data to combine the alignments for the separate analyses of each lineage: the molecular phylogenies of the *Basidiobolus* (with a total of 4 413 characters, 7 % genes missing) and *Entomophthora* (total of 2 826 characters, 43 % genes missing) lineages are based on five loci: LSU, SSU, *RPB2*, mtSSU, ITS. For the lineages centring on *Conidiobolus* (total of 3 173 characters, 30 % genes missing), *Batkoa* (total of 3 048 characters, 12.5 % missing genes) and *Zoophthora* (total of 3 076 characters, 3 % missing genes) we used four loci: LSU, SSU, *RPB2*, mtSSU. Sequence data and alignments of fungi are accessible in GenBank (Table 2) and TreeBASE (www.treebase.org/treebase-web/home.html).

RESULTS AND DISCUSSION

Our analysis for *Entomophthoromycota* identified five main phylogenetically identified lineages corresponding to the main genera *Basidiobolus*, *Conidiobolus*, *Batkoa*, *Entomophthora* and related genera in *Entomophthoroideae* and *Zoophthora* (*Entomophthoraceae* s.l. (a group of genera comprising the subfamily *Erynioideae*)). These lineages were identified in our previous multi-genic phylogenetic study (Gryganskyi et al. 2012). Lineages are named after the most species-rich genus in the group that also exhibits typical morphological and trophic characteristics. Most of these genera also constitute the majority of the taxa in the molecular dataset for their lineage. However, *Zoophthora* s. str. has a large number of species (Balazy 1993) but relatively few available DNA sequences.

I. *The basal Basidiobolus lineage*

The *Basidiobolus* lineage comprises all taxa of the class *Basidiobolomycetes*, which includes a single order and family, *Basidiobolales* and *Basidiobolaceae*, respectively. This clade occupies the most basal position on the phylogenetic tree for the phylum *Entomophthoromycota* (Fig. 1). The cardinal characteristics of this group include formation of uninucleate cells with very large nuclei (often exceeding 10 µm in length; Fig. 2a, b) containing a prominent central nucleolus, and a unique mode of mitosis; no stainable, condensed heterochromatin is present in interphasic nuclei (Humber 2012).

This lineage, which is the most distantly separated from the remainder of *Entomophthoromycota*, is strongly supported as a monophyletic group in all molecular analyses. The gene-based data distinguishes at least six species in *Basidiobolus* (Fig. 3) but *B. ranarum* has long been thought to be a globally distributed, poorly resolved species complex. There have been historical uncertainties about the taxonomy of *Basidiobolus* species except for the undisputed support for *B. microsporus* with a unique mode of secondary conidiogenesis. *Basidiobolus haptosporus*, *B. heterosporus* and *B. meristosporus* have been treated in the past as synonyms of the type species, *B. ranarum* (see Index Fungorum; www.speciesfungorum.org/).

The clarification of both generic and specific concepts within the class *Basidiobolomycetes* obviously needs further taxonomic study using both traditional and molecular approaches. The inclusion here of two still undescribed genera that are morphologically, developmentally, and genetically distinct from *Basidiobolus* further underscores the need for more intensive study of this group. One of these undescribed genera is known so far only as a pathogen of snakes to be described as *Schizangiella serpentis* nom. prov. (Humber, unpubl. data), whose vegetative stage is predominantly yeast-like (Fig. 2a). The other undescribed genus is *Drechslerosporium cornellii* nom. prov. (Huang, Humber & Hodge, unpubl. data), a saprobe from soil or plant detritus.

Future studies to clarify the taxonomy of the fungi in this lineage will need to incorporate data from a greater number and variety of genes. The accuracy of future phylogenetic analyses should be improved by incorporating results from molecular approaches that examine a higher level genomic expression than gene sequences. Such additional molecular approaches will include comparisons of amino acid sequences of key proteins (e.g., Voigt & Kirk 2011) and, possibly, might include matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) applications of universal protein profile-based mass spectroscopy. The

Table 2 Accession numbers of *Entomophthoromycota* sequence data

Species, collection, strain	SSU	LSU	ITS	mtSSU	*RPB2*
Basidiobolus haptosporus ARSEF 261	JX242606	JX242586	EF392520	JX242626	EF392465
B. heterosporus ARSEF 262	–	EF392411			
	EF39252	–	EF392466		
B. heterosporus CBS 311.66	JX242607				
	JX242587	–	JX242627	–	
B. magnus CBS 205.64	JX242608	JX242588	NR_077175	JX242628	EF392479
B. meristosporus CBS 931.73	JX242609	JX242589	–	JX242629	–
B. meristosporus ATCC 14450	–	–	EF392533	–	EF392477
B. microsporus ARSEF 265	AF368505	DQ364202	EF392523	DQ364222	DQ364212
B. ranarum AFTOL 301	AY635841	DQ273807	AY997030	EF392490	DQ302777
Batkoa apiculata ARSEF 3130	DQ177437	EF392404	–	EF392513	EF392459
Bat. gigantea ARSEF 214	JX242611	JX242591	–	JX242631	EF392433
Bat. major ARSEF 2936	EF392559	EF392401	–	EF392511	EF392457
C. antarcticus ARSEF 6913	–	DQ364207	–	DQ364227	DQ364217
C. bangalorensis ARSEF 449	–	DQ364204	–	DQ364225	DQ364214
C. brefeldianus ARSEF 452	AF368506	EF392382	–	EF392495	
	EF392439				
C. coronatus AFTOL 137	AF113418	AY546691	AY997041	DQ364224	DQ302779
C. firmipilleus ARSEF 6384	JX242612	JX242592	–	JX242632	–
C. heterosporus ARSEF 6386	JX242613	JX242593	–	JX242633	–
C. incongruus NRRL 28636	AF113419	AF113457	–	–	–
C. iuxtagenitus ARSEF 6378	–	KC788410	–	–	–
C. lachnodes ARSEF 700	–	KC788408	–	–	–
C. lamprauges ARSEF 2338	AF296754				
	DQ364206	–	DQ364226	DQ364216	
C. nanodes CBS 183.62	JX242634	JX242594	–	JX242634	–
C. obscurus ARSEF 74	EF392541	EF392369	–	EF392485	EF392430
C. osmodes ARSEf 79	AF368510	EF392371	–	DQ364219	DQ364209
C. paulus ARSEF 450	–	KC788409	–	–	–
C. pseudapiculatus ARSEF 1662	EF392557	EF392398	–	EF392508	EF39245
C. pumilus ARSEF 453	JX242615	EF392383	–	EF392496	EF392440
C. rhysosporus ARSEF 448	AF368512	–	–	–	–
C. thromboides FSU 785	JX242616	JX242597	JN943012	JX242637	JX266783
Drechslerosporium cornellii, nom. nov. ARSEF 7942	KC788407	KC788411	–	KC788412	KC788413
Entomophaga aulicae ARSEF 172	EF392542	EF392372	–	EF392487	–
En. australiensis ARSEF 328	EF392546	EF392375	–	–	–
En. conglomerata ARSEF 2227	AF368509				
	–	–	–	–	
En. destruens CBS 208.65	JX242617	JX242598	–	JX242638	JX266784
En. maimaiga ARSEF 1400	EF392556	EF392395	–	EF392505	–
Entomophthora chromaphidis ARSEF 1860	AF353725	–	GQ285848	–	–
E. culicis ARSEF 387	AF368516				
	–	–	–	–	
E. exitialis CBS 180.60	JX242618	JX242599	–	JX242639	–
E. ferdinandii ARSEF 6918	–	–	GQ285860	–	–
E. ferdinandii KVL 99-87	–	GQ285882	–	–	–
E. grandis ARSEF 6701					
	–	–	GQ285863		
	–	–			
E. muscae AFTOL 28	AY635820	DQ273772	AY997047		
	AFToL Database	DQ302778			
E. planchoniana ARSEF 6252	AF353724	GQ285878	GQ285856	–	–
E. scatophaga ARSEF 6704	–	DQ481226	DQ481219	–	–
E. schizophorae ARSEF 6817	–	DQ481228	DQ481221	–	–
E. syrphi ARSEF 5595	–	DQ481230	DQ481223	–	–
E. thripidum ARSEF 6518	AF296755	–	–	–	–
E. thaxteriana CBS 181.60	JX242619	JX242600	–	JX242640	–
Er. conica ARSEF 1439	AF368513	EF392396	–	EF392506	EF392452
Er. ovispora ARSEF 400	EF392549	EF392381	–	JX242641	EF392438
Er. rhizospora ARSEF 1441	AF368514	EF392397	–	EF392507	EF392453
E. sciarae ARSEF 1870	AF368515	EF392399	–	EF392509	EF392455
Eryniopsis caroliniana ARSEF 640	EF392552	EF392387	–	EF392500	EF392444
Eryn. ptycopterae KVL 48	AF052403	–	–	–	–
Furia americana ARSEF 742	EF392554	EF392389	–	–	EF392446
F. gastropachae ARSEF 5541	EF392562	EF392407	–	EF392516	EF392462
F. ithacensis ARSEF 663	EF392553	EF392388	–	EF392501	EF392445
F. neopyralidarum ARSEF 1145	AF368518	EF392394	–	EF392504	EF392451
F. pieris ARSEF 781	AF368519	EF392390	–	EF392502	EF392447
F. virescens ARSEF 1129	EF392555	EF392393	–	EF392503	EF392450
Macrobiotophthora vermicola ARSEF 65	AF052400				
	–	–	–	–	
Massospora cicadina ARSEF 374	EF392548	EF392377	–	EF392492	–
Pandora bullata ARSEF 116	HQ677592	–	–	–	–
P. blunckii ARSEF 217	JX242621	EF392374	–	–	EF392434
P. delphacis ARSEF 581	EF392551	EF392386	–	EF392499	EF392443
P. dipterigena ARSEF 397	AF368522	EF392380	–	EF392565	EF392437
P. kondoiensis CBS 642.92	JX242622	JX242603	–	JX242642	JX266788
P. neoaphidis ARSEF 3240	EF392560	EF392405	–	EF392514	EF392460
P. nouryi ARSEF 366	HQ677594	–	–	–	–
Schizangiella serpentis, nom. nov. ARSEF 203	AF368523	EF392428	EF392538	EF392488	EF392481
Strongwellsea castrans ARSEF 3485	AF052406	–	–	–	–
Zoophthora anglica ARSEF 396	AF368524	EF392379	–	EF392493	EF392436
Z. lanceolata ARSEF 469	EF392550				
	EF392385	–	EF392498	EF392442	
Z. occidentalis ARSEF 207	JX242623	EF392402	–	JX242643	EF392458
Z. phalloides ARSEF 2281	EF392558	EF392400	–	EF392510	EF392456
Z. radicans ARSEF 388	JX242624	JX242605	–	JX242644	
	–				
Z. radicans ARSEF 4784	EF392561	EF392406	–	EF392515	EF392461

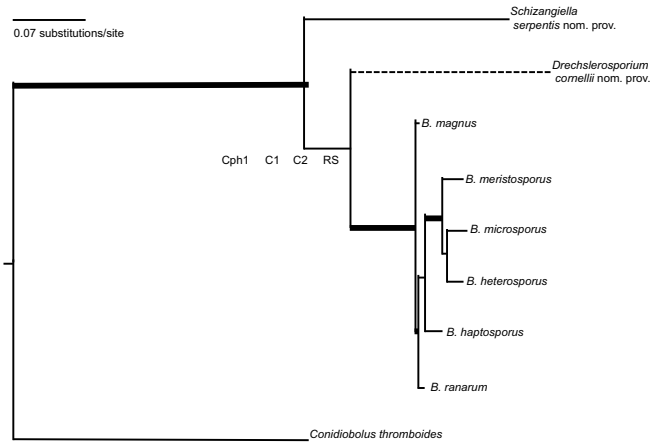

Fig. 3 Maximum likelihood phylogeny of *Basidiobolomycotina*: *Basidiobolus* and the still formally undescribed genera *Schizangiella* and *Drechslerosporium* (LSU, SSU, *RPB2*, mtSSU, ITS). Thickened branches have statistically significant statistical support (ML bootstrap > 70 %, BI posterior probability > 95). Cph1 = unbranched conidiophores; C1 = primary conidia; C2 = secondary conidia; RS = resting spores.

taxonomic uses of MALDI-TOF for fungi are new and promising (Horka et al. 2012, Schrödl et al. 2012, Wieser et al. 2012), and will be used in Brazil to distinguish species of common and important hypocrealean entomopathogens from *Metarhizium* and *Beauveria* (RB Lopes; Embrapa-Cenargen; pers. comm.). MALDI-TOF remains to be explored with entomophthoroid fungi but could become an important and versatile tool to support many diverse aspects of the taxonomy and applied uses of fungal entomopathogens.

The placement of *Basidiobolus* in relation to all other fungi has been notoriously problematic. Initial analyses of 18S rDNA sequences grouped *Basidiobolus* together with flagellate fungi and outside the *Entomophthorales* (Nagahama et al. 1995, Jensen et al. 1998). A later, more comprehensive analysis of the rDNA operon (18S, 28S and 5.8S) grouped *Basidiobolus* with *Olpidium brassicae* in a position basal to the other *Entomophthorales* (White et al. 2006). The result of this study separated a mite-parasitic *Neozygites* species from the other *Entomophthorales*. A kaleidoscopic six-gene analysis of fungi placed *Basidiobolus* in the traditionally recognised *Entomophthorales* but also placed *Olpidium brassicae* on the same phylogenetic branch (James et al. 2006). While any 'meaning' for this pairing of *Basidiobolus* and flagellate fungi still deserves exploration with a much more balanced, comprehensive analysis involving more genes, no traditional taxonomic characters account for or corroborate such an unexpected and seemingly anomalous genomic suggestion. The 'relatedness' of *Entomophthorales* to distinctly non-fungal groups and, in fact, the removal of *Entomophthorales* (other than *Basidiobolus*) from the true fungi, has been inferred from amino acid sequences of protein-coding genes (Liu & Voigt 2010, Voigt & Kirk 2011). Despite all of these other results, multi-gene phylogenetic analyses of rDNA, mtSSU, and *RPB2* sequences confirm the monophyletic status of *Entomophthoromycota* and separate them from the flagellate fungi that more limited, earlier studies treated as allied with *Basidiobolus* (Gryganskyi et al. 2012).

II. *The Conidiobolus lineage and its conundrum*

The *Conidiobolus* lineage is composed of species of the *Ancylistaceae* (*Entomophthoromycetes*: *Entomophthorales*) in the genera *Conidiobolus* (which is shown here to be paraphyletic)

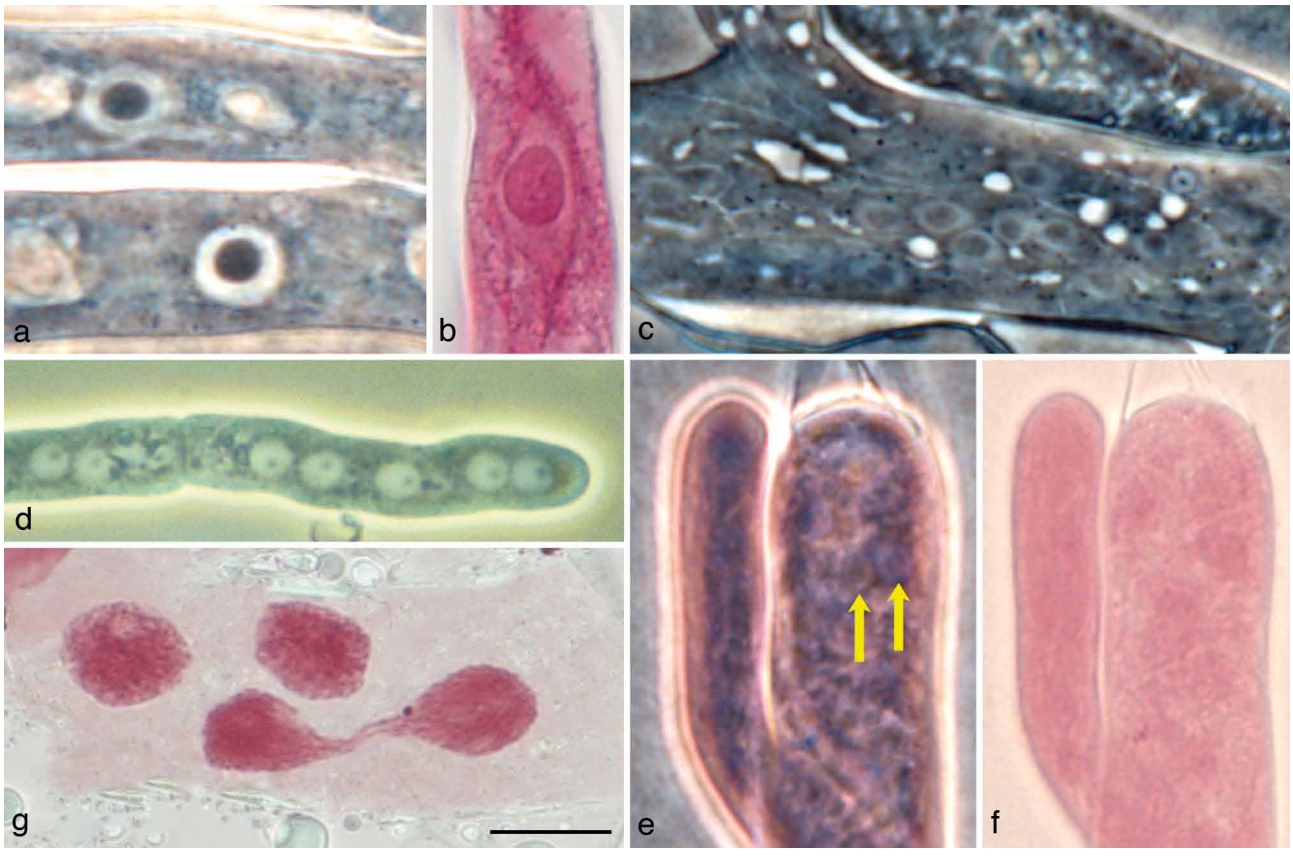

Fig. 4 Major characters of *Entomophthoromycota*. Nuclei (all shown at the same magnification). a, b. *Basidiobolus* sp. (*Basidiobolaceae*); living nuclei seen by phase contrast (a) and stained in aceto-orcein (b) have no interphasic heterochromatin. – c. *Neozygites floridana* (*Neozygitaceae*) hyphal body nuclei with a small nucleolus and no interphasic heterochromatin. – d–f. *Conidiobolus* sp. (*Ancylistaceae*); living nuclei seen by phase contrast (d) with central nucleolus and heterochromatin-free nucleoplasm, and stained in aceto-orcein and observed with phase contrast (e; yellow arrows indicating two nuclei) and bright-field optics (f; with nuclei typically not visualized in this family). – g. *Pandora neoaphidis* (*Entomophthoraceae*) nuclei in aceto-orcein show strongly stained, granular heterochromatin both in interphase (above) and mitosis (below, mid-anaphase).

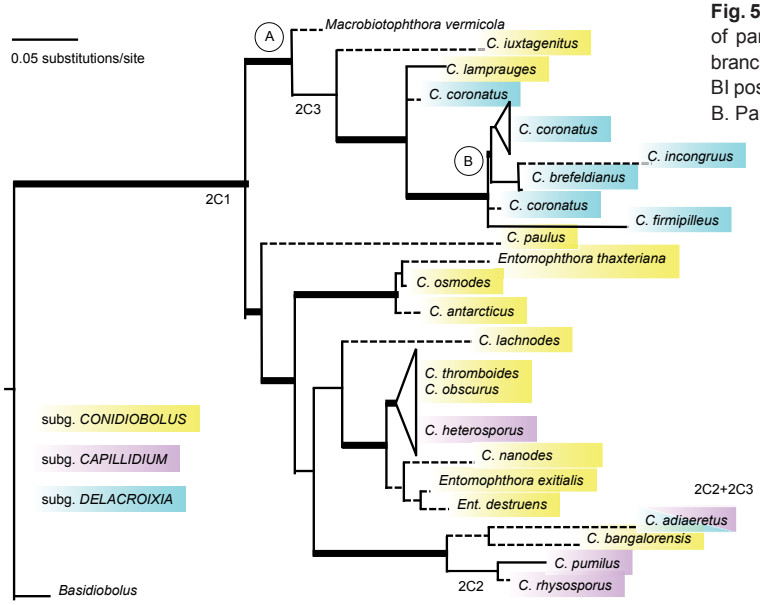

Fig. 5 Maximum likelihood phylogeny of *Ancylistaceae*, with demonstration of paraphyly in *Conidiobolus* s.l. (LSU, SSU, *RPB2*, mtSSU). Thickened branches have statistically significant statistical support (ML bootstrap > 70 %, BI posterior probability > 95). A. Basal position of genus *Macrobiotophthora*; B. Paraphyly of *C. coronatus*. 2C1-3 = types of secondary conidia.

and *Macrobiotiophthora.* These taxa occupy a position between the *Basidiobolus* lineage and the more highly derived taxa of the core *Entomophthoraceae*. These taxa all produce coenocytic mycelium or hyphal bodies, and nuclei that are mostly 2.5–4 µm diam (very small for entomophthoroid fungi; see Fig. 4d) and a prominent central nucleolus and no significantly stainable quantity of interphasic heterochromatin (Fig. 4d). The primary conidia of all species of the *Ancylistaceae* are globose to pyriform, multinucleate, and forcibly discharged by papillar eversion (Humber 1989). Their resting spores (zygospores or azygospores) form in the axis of the parental cell. The morphological, developmental, and genetic characters of the rarely collected fungus *Macrobiotophthora vermicola*, a nematode pathogen that is available in culture, clearly place this taxon in the ancylistaceous lineage. Unfortunately, no species from this family's type genus, *Ancylistes*, which parasitizes desmid algae, has ever been cultured and there are no recently collected specimens available for DNA extraction.

The gene-based data for the fungi in this lineage (Fig. 1, 5) highlight the underlying taxonomic problems in the genus *Conidiobolus*. Our four-gene phylogeny of the complete set of fungi (Fig. 1) clearly demonstrated the distribution of *Conidiobolus* species in two clades. The analysis with more species, but fewer genes in Fig. 5 suggests that *Conidiobolus* breaks into at least three groups. The *C. coronatus* group is distinct from other *Conidiobolus* subclades and includes at least four additional taxa: *C. brefeldianus*, *C. firmipilleus*, *C. incongruus* and *C. lamprauges*. *Macrobiotophthora vermicola*, a soil-dwelling pathogen of nematodes, is also allied with this clade of soil and litter inhabiting fungi. *Conidiobolus coronatus* is a very widely distributed and common species, which can easily be isolated from soil or plant detritus obtained in many types of habitats. Nonetheless, *C. coronatus* is also a weak pathogen of diverse insects. Two species of *Conidiobolus*, *C. coronatus* and less commonly *C. lamprauges*, can sometimes infect humans and other mammals (Humber et al. 1989, Reiss et al. 2011). A second, well-supported clade comprised of *C. pumilus* and *C. bangalorensis* was only recovered as a long branch in the taxon-rich phylogeny of the genus (Fig. 5) because 18S and 28S were the only genes available for these two species. A third *Conidiobolus* group, including *C. thromboides* and *C. osmodes,* was well supported in the four-gene analysis (Fig. 1). However, when more species were included in the analysis (Fig. 5), *C. thromboides* and *C. osmodes* were separated into different subgroups with good statistical support. Many species from this subclade are also known as insect pathogens, mostly on aphid hosts.

Our ancestral state reconstruction and comparisons of morphological and ultrastructural similarities of this genus with other lineages of *Entomophthoromycota* suggest that the most ancestral fungi of the class *Entomophthoromycetes* (Table 1) may have very closely resembled the extant taxa now classified in *Conidiobolus* (Humber 1984a, Gryganskyi et al. 2012).

The taxonomic heterogeneity (paraphyly) of *Conidiobolus* demonstrated in our analyses is exemplified, in part, by the inclusion of '*Entomophthora*' species on the tree in Fig. 5. These seemingly misplaced taxa (whose sequences were obtained from GenBank) were identified before the Batkoan reclassifications of these fungi (see Humber 1989). This occurred at a time when virtually all entomopathogenic entomophthoraleans were automatically treated in *Entomophthora*. Each of these species is now correctly recognised as ancylistaceous (not entomophthoraceous) and placed in *Conidiobolus* (Ben-Ze'ev & Kenneth 1982; Balazy 1993). The last major revision of *Conidiobolus* species (King 1976a, b, 1977) was morphologically based and remains difficult to interpret; identifications of most species with the aforementioned revision remain equivocal or provisional, mainly because so few adequately informative characters were then recognised.

Ben-Ze'ev & Kenneth (1982) divided *Conidiobolus* into three subgenera based on the types of secondary conidia (SC) formed by these species. Type I SC (Fig. 6d) are forcibly discharged conidia formed singly on primary conidia, Type II SC (Fig. 6e, f) are elongated, passively-dispersed capilliconidia formed on elongated conidiophores, and Type III SC (Fig. 6g) are multiple microconidia (6–20) forcibly discharged from a single primary conidium. As described by Ben-Ze'ev and Kenneth (1982) the subgenus *Conidiobolus* forms only Type I SC, the subgenus *Capillidium* forms both Type I and Type II SC and the subgenus *Delacroixia* forms both Type I and Type III SC. This subgeneric taxonomy was significantly challenged when Callaghan et al. (2000) demonstrated that *C. adiaeretus* alternatively produces all three types of secondary conidia depending on the environmental conditions. The subgeneric boundaries of Ben-Ze'ev & Kenneth (1982) are not supported by our molecular results, suggesting that the ability to form different types of secondary conidia is more fluid than was previously thought.

No meaningful phylogenetic reclassification of *Conidiobolus* will be possible until the genotypes of all available ex-type

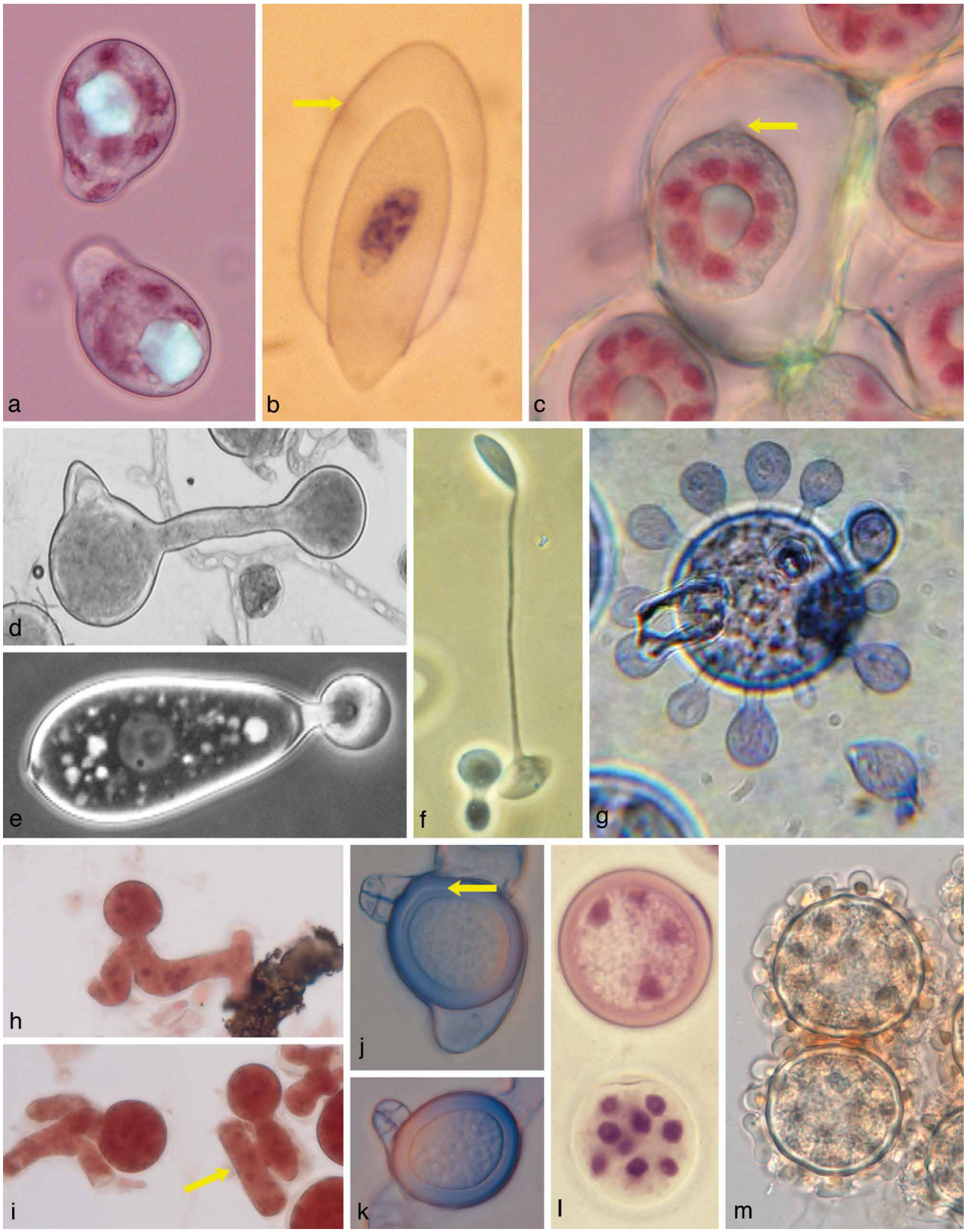

Fig. 6 Major characters of *Entomophthoromycota*. a–c: Primary conidia: a. pyriform multinucleate conidia of *Entomophaga aulicae*; b. uninucleate bitunicate conidium (arrow, outer wall layer can lift away from inner layer) of *Zoophthora radicans*; c. campanulate (bell-shaped) multinucleate conidium of *Entomophthora muscae* with apiculus (arrow), broad basal papilla, and embedded in quantity of residual cytoplasm discharged with the conidium. – d–g: Secondary conidia: d. single (Type I) replicative conidium of *Conidiobolus* sp.; e. Type II capilliconidium with terminal mucoid droplet (right) of *Basidiobolus*; f. Type II capilliconidium (developing) on capillary conidiophore of *Zoophthora radicans*; g. multiple microconidia (Type III) produced by *Conidiobolus coronatus*; note discharged microconidium at lower right. – h–m: Zygosporogenesis and zygospores: h, i. developing zygospores of *Z. radicans* bud off from gametangia; note apical budding in (i) from gametangium with a median conjugation bridge (arrow). – j, k: *Basidiobolus* zygospores showing characteristic 'knees' and (arrow in j) separation of the outer (zygosporangial) and inner (zygosporic) wall layers; l. immature (below, multinucleate and thin-walled) and more mature (above, with fewer nuclei and notably thickened wall) resting spores of *Z. radicans*; m. highly decorated (bullate) outer (zygosporangial) wall layer on resting spores of *Pandora bullata.*

cultures for species of this genus can be examined in parallel with detailed morphological and developmental studies. However, an unavoidable problem must be solved first: The first two described species, *C. utriculosus* and *C. minor,* have not been isolated or collected since Brefeld described them in 1884, and there appears to be no herbarium material of either taxon. Most students of entomophthoroid fungi believe that Brefeld's species probably represent the primary conidia (*C. utriculosus*) and secondary microconidia (*C. minor*) of the fungus now universally recognised as *Conidiobolus coronatus*. Until the nomenclatural status of the type species of *Conidiobolus* can be resolved by its recollection (but there is no adequate basis to identify *C. utriculosus* if it were found again) or, more probably, officially eliminated by the formal conservation of the generic name with a new (and properly typified) type species, it will not be possible to undertake any revision of the taxonomy of this large and important but heterogeneous constellation of species.

III. *The Batkoa lineage (Entomophthoraceae)*

Although the statistical support is weak in both Maximum Likelihood and Bayesian analyses for a separate lineage that includes only the genus *Batkoa*, these is little doubt that the species included in Fig. 7 form a natural grouping that is distinct from the remainder of taxa in the *Entomophthoraceae*. As with all species of *Entomophthoraceae,* members of the genus *Batkoa* are all obligatory entomopathogens. They share the synapomorphy of forming large nuclei that are readily stainable due to the presence of large quantities of granular-appearing heterochromatin during interphase (Fig. 4g, 6a–c,h,i,l).

Batkoa was segregated from *Entomophaga* by Humber (1989a) on the basis of its formation of globose to subglobose conidia, the distinctively narrowed extension of the conidiogenous cell before conidial formation, and the ability in most species to produce thick rhizoids with discoid terminal holdfasts (Fig. 2d, e). Molecular data are available for *B. apiculata*, *B. gigantea* and *B. major* (Fig. 7) but not for the other seven recognised species.

The fungi included in this lineage in Fig. 7, identified as species of *Entomophaga* or *Conidiobolus* reflect historically based misidentifications. A similar situation led to the apparent inclusion of *'Entomophthora'* species in *Conidiobolus* lineage. The most common species of *Batkoa*, pathogens of aphids and other hemipterans, have globose conidia indistinguishable in size and shape from those of several common species of *Conidiobolus*, such as *Conidiobolus obscurus* (Fig. 5, 7). These *Conidiobolus* species are also aphid pathogens. Both genera belong in different families but the morphological similarity of their conidia led to misidentifications prior to the recognition (Humber 1989a) of the nuclear characters (compare Fig. 4d, g). Nonetheless, the seemingly chaotic placement of names within the *Conidiobolus* and *Batkoa* lineages underscores the need for a thorough, molecular-based revision of these genera. A concerted attempt to re-examine a wide range of isolates and specimens from the world's culture collections and herbaria also is necessary. Such study would be also able to address whether the *Batkoa* lineage truly stands apart from the other fungi originally placed in the subfamily *Entomophthoroideae* (Keller & Petrini 2005). This lineage is also provisionally placed in Table 1 and by Humber (2012).

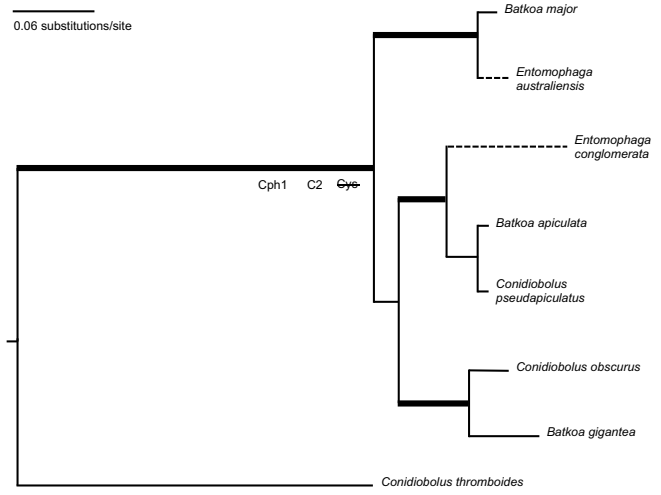

Fig. 7 Maximum likelihood phylogeny of *Entomophthoraceae* and taxonomic confusion within the genus *Batkoa* (LSU, SSU, *RPB2*, mtSSU). Thickened branches have statistically significant statistical support (ML bootstrap > 70 %, BI posterior probability > 95). Cph1 = unbranched conidiophores; C1 = primary conidia; C2 = secondary conidia; Cys = cystidia or pseudocystidia.

IV. *The Entomophthora lineage (Entomophthoraceae subfamily Entomophthoroideae)*

The *Entomophthora* clade (Fig. 8) is the most morphologically diverse of the lineages recognised here and includes *Entomophthora muscae,* which is a common pathogen of adult cyclorrhaphan flies and is the type species for the *Entomophthoromycota*. This group contains genera of the *Entomophthoraceae* with variously shaped (but rarely elongated), multinucleate conidia borne on unbranched conidiophores (Fig. 2i). The most taxon-rich genera treated here are morphologically distinct and constitute the two main branches on the tree. *Entomophthora* species have uniquely shaped campanulate conidia (Fig. 6c) with rhizoids formed in some species whereas *Entomophaga* species have ovoid to pyriform conidia (Fig. 6a) and never form rhizoids (Fig. 8). This lineage also includes the genera *Entomophthora*, *Entomophaga*, two species of *Eryniopsis*, whose generic circumscription and status need to be re-examined, and *Massospora*. Other *Eryniopsis* species, including the type, *E. lampyridarum*, may not belong in this subfamily. Humber (1984a) noted that the *Entomophthoraceae* splits into distinctive generic groups, one with multinucleate, unitunicate conidia on unbranched conidiophores and the other with uninucleate, bitunicate conidia on digitately branched conidiophores, produced in all genera except *Strongwellsea*. Keller & Petrini (2005) formalised these generic groupings as the

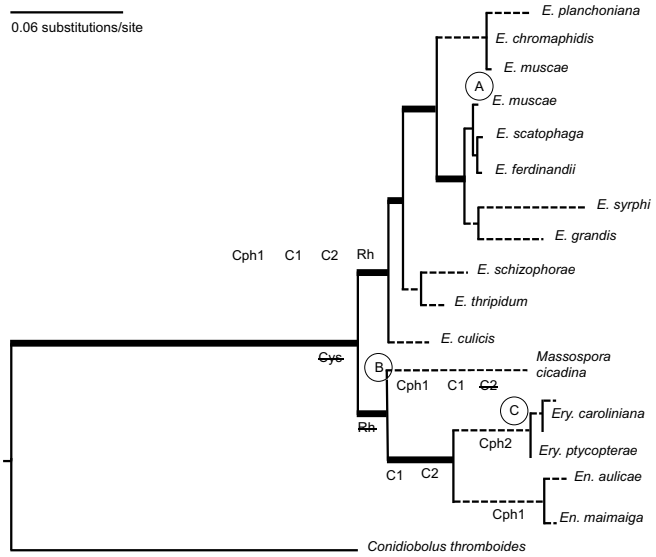

Fig. 8 Maximum likelihood phylogeny of *Entomophthoraceae* subfamily *Entomophthoroideae* (LSU, SSU, *RPB2*, mtSSU, ITS). Thickened branches have statistically significant statistical support (ML bootstrap > 70 %, BI posterior probability > 95). A. Paraphyly in *Entomophthora muscae* species complex; B. *Massospora* is part of *Entomophthoroideae*; C. *Eryniopsis* (in part) belongs in *Entomophthoroideae*. Cph1-2 = unbranched and branched conidiophores, respectively; C1 = primary conidia; C2 = secondary conidia; Cys = cystidia or pseudocystidia; Rh = rhizoids.

subfamilies *Entomophthoroideae* and *Erynioideae*, respectively. They also separated *Massospora* into a monogeneric subfamily *Massosporoideae* but this third subfamily is not supported in recent analyses (Humber 2012, Gryganskyi et al. 2012). One unexpected result of our analysis of the *Entomophthora* species is that those that are pathogens of flies, including (*E. ferdinandii*, *E. grandis*, *E. muscae*, *E. scatophagae* and *E. syrphi*, are scattered across four branches of the dendrogram in Fig. 8, despite their morphological similarities and closely related host insects.

The extraordinary genus *Massospora* is also included in the *Entomophthora* lineage. This genus consists of more than dozen species pathogenic to adult gregarious cicadas, *Hemiptera*: *Cicadidae* (Soper 1974) whose development is restricted to the terminal abdominal segments and whose dispersal is exclusively from living cicada hosts. Only two *Massospora* species have been grown in vitro, but it appears that the only culture now available may be of the type species, *M. cicadina*. The vegetative development *of M. cicadina* as wall-less hyphal bodies is indistinguishable from that *Entomophthora* species (Fig. 2b), so it is not altogether surprising that the result of our phylogeny places *M. cicadina* in the middle of the *Entomophthoroideae.* While biologically interesting, the unusual sporulation of these fungi from living hosts is not unique: *Entomophthora thripidum* and all *Strongwellsea* species also sporulate from living hosts. Our results do not support the inclusion of this genus by Keller & Petrini (2005) into its own subfamily *Massosporoideae* (also see Humber 2012).

V. *The Zoophthora s.l. lineage (Entomophthoraceae subfamily Erynioideae)*

Batko (1964) described *Zoophthora* but soon split this genus into four subgenera (Batko 1966) that were, in turn, raised to the genus level by Humber (1989). The later author separated these genera primarily based on rhizoid and cystidial morphology. *Zoophthora* s.str., which is restricted to species that form passively dispersed secondary capilliconidia on elongated capillary conidiophores (Fig. 6f), appears to be the most derived of the taxa studied here, and *Zoophthora* is the only taxon that is unambiguously supported as distinct at the currently recognised generic level (Fig. 9). The genus *Erynia* may not be supported here as monophyletic although most of its species seem to form the earliest diverging clade within the zoophthoroid lineage. Representatives of the genera *Furia* and *Pandora* appear on multiple branches of the tree. Our phylogenetic analyses suggest that the recognition of separate genera for *Erynia*, *Pandora,* and *Furia*, which are recognised, based on rhizoid and cystidial morphology may not be valid. The genus *Strongwellsea* is unique because: 1) sporulation is from an intra-abdominal hymenium of unbranched (rather than digitately branched; see Fig. 2j) conidiophores; and 2) conidia are discharged through a gaping, fungus-generated hole in the abdominal cuticle of living muscoid flies (Humber 1976). The one species included in our analyses, *Strongwellsea castrans*, clustered with species of *Pandora* and *Furia*, as suggested by Humber (1982) based on overall morphology and development. The results of Table 1 indicate that species of *Eryniopsis* (Humber 1984b, Keller 1991) could be included in both subfamilies of the *Entomophthoraceae*. The taxonomy of *Eryniopsis* must be revised since it was described exclusively based on morphological criteria. *Eryniopsis* is an artificial group of species with simple or basally dichotomous conidiophores, plurinucleate conidia, and elongated unitunicate conidia that were not accommodated in any other genus. The molecular data included are based only on entomophthoroid species placed in this genus, *Ery. caroliniana* and *Ery. ptycopterae*. The latter species is now classified in *Entomophaga* (Hajek et al. 2003). No molecular data are available for *Ery. longispora*. Its conidial and rhizoidal morphology would place it in *Erynia* except that its conidia are plurinucleate and unitunicate rather than uninucleate and bitunicate (Fig. 6b) in members of the *Zoophthora/Erynia/Furia/Pandora* clade. Molecular data and cultures are not available for the type species *Ery. lampyridarum.* Similarly, the rare monotypic fungus *Orthomyces* has never been available for molecular study. This genus resembles *Zoophthora* but has shorter, thicker secondary capillary conidiophores forcibly discharging globose conidia (Steinkraus et al. 1998).

The resolution of generic classification within this complex and species-rich subfamily will almost certainly require more complete samplings of the included taxa and more genes. More than 70 % of the included taxa have not yet been studied molecularly. At this point, however, it would appear that there is excellent support for *Zoophthora* as a distinct genus, characterised mainly by its secondary capilliconidia and the mostly conical papillae of the conidia. None of the scant molecular evidence now available suggests that *Strongwellsea* is not a distinct and valid genus (Humber 1982). The available molecular data do suggest that *Pandora* and *Furia* may need to be combined into a single genus, and that *Erynia*, most of whose species affect hosts in

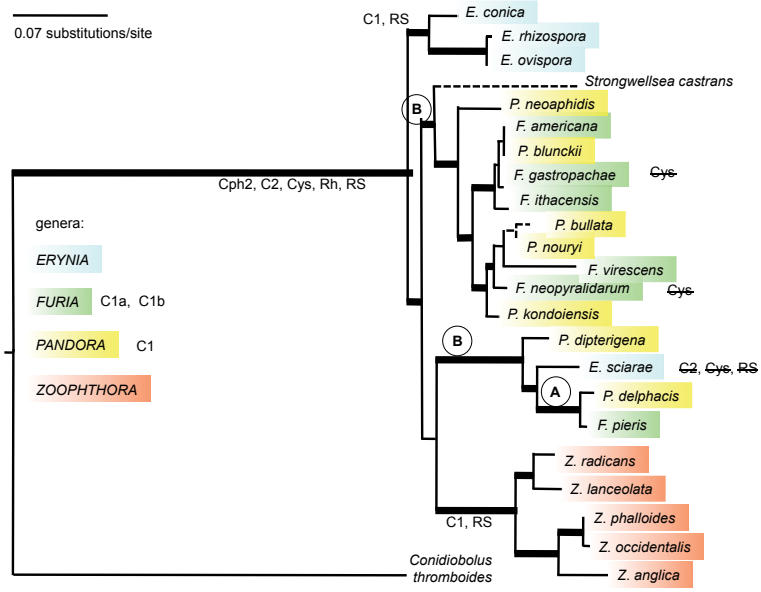

Fig. 9 Maximum likelihood phylogeny of *Entomophthoraceae* subfamily *Erynioideae* and relationships among the principal genera of this group (LSU, SSU, *RPB2*, mtSSU). Thickened branches have statistically significant statistical support (ML bootstrap > 70 %, BI posterior probability > 95). A. Presence of *Erynia* in unresolved *Furia*/*Pandora* complex (B); Cph2 = branched conidiophores; C1a-b = type of primary conidia; C2 = secondary conidia; RS = resting spores; Cys = cystidia or pseudocystidia; Rh = rhizoids.

distinctly wet habitats, may be supported as a distinct genus based on molecular, morphological, and developmental studies.

A major presumptive lineage still 'missing' from this overview

The genus *Neozygites* (*Neozygitomycetes*: *Neozygitales*: *Neozygitaceae*) contains 23 described species, all of which are pathogens of either aphids or mites. None of the species of *Neozygites* now available in culture in vitro – *N. floridana*, *N. parvispora* and *N. tanajoae* – were included in this analysis because their only available sequences (18S rDNA) could not be aligned adequately with sequences from other entomophthoroid fungi. When 18S rDNA from *Neozygites* species were included in the computations, our analyses yielded no statistically or phylogenetically meaningful results, which place *Neozygites* outside the *Entomophthoromycota*.

Such a placement outside of the *Entomophthoromycota* may have resulted from a long-branch attraction (Bergsten 2005) that artificially groups distantly related taxa – e.g., the grouping of *Neozygites* with *Dimargaris* (*Kickxellomycotina*) (White et al. 2006). For now, the taxonomic position of *Neozygites* remains unverified until additional sequence data are available.

All of the cultured *Neozygites* species with any molecular data are pathogens of mites. Neither cultures nor any molecular data are available for any *Neozygites* species – pathogens of aphids, including *N. fresenii*, the type species of this genus. There are some distinct and consistent differences in zygospore morphology between the mite (globose, rough-surfaced) and aphid (ovoid, smooth-surfaced) parasites from genus *Neozygites* that might still need to be recognised at the generic level.

Further needs for taxonomic research on entomophthoroid fungi

The recognition of several genetically supported lineages within the *Entomophthoromycota* broadly supports the traditionally based classification of entomophthoroid fungi. The patterns of phylogenetic relationships among *Entomophthoromycota* reflect the previously inferred general evolutionary trend for a transition from saprobic to weak or facultative or obligately associations with invertebrates (Humber 1984a, 2008). The *Basidiobolus* lineage is generally saprobic or associated with arthropods for phoresis, possibly only very rarely in any sort of pathogenic association, commensally in the intestines of some poikilothermic vertebrates, to the comparatively rare mycotic associations with vertebrates observed in both *Basidiobolus* and *Schizangiella*. The *Conidiobolus* lineage is also primarily composed of saprobic taxa with some species acting as occasional pathogens of arthropods or known only as entomopathogens; within the *Ancylistaceae*. However, the genera *Macrobiotophthora* and *Ancylistes* (*Ancylistaceae*) are known only as pathogens of nematodes and desmid algae, respectively. All taxa of the *Entomophthoraceae* including *Batkoa*, *Entomophthora* and *Zoophthora* lineages, are obligatorily entomopathogenic.

Careful, traditionally based studies of type specimens, ex-type cultures, and taxonomic concepts form the indispensible foundation upon which molecular taxonomic studies can make reasonable progress. Genera discussed here that should be revised based on analysis of both molecular and traditional characters include *Conidiobolus*, *Batkoa*, and the *Zoophthora/Erynia/Furia/Pandora* complex. One additional genus, *Tarichium*, has not been mentioned because it is reserved for several dozen named species known so far only from their resting spores. Other taxa at every taxonomic rank in this phylum are based in large part on their conidial reproduction. Future molecular and traditional revisionary studies will reveal *Tarichium*, a genus that comprises a mix of species from both the *Neozygitaceae* (especially the mite pathogens) and *Entomophthoraceae* (Humber, unpubl. data). A revision based on both traditional and molecular taxonomic methods may reveal that the affinities of most species of *Tarichium* to currently accepted, valid genera.

The greatest emphasis in phylogenetic studies of *Entomophthoromycota* has been based on nuclear genes. Little sequence data from mitochondrial genes is available. All available evidence suggests that sexuality in the *Entomophthoromycota* is exclusively homothallic, it has also been believed that all reproduction and, therefore, phylogenetic radiation of these fungi is clonal. Heterothallic sexuality (with mating types and routine outcrossing) is the standard mode of sexuality both below and above the *Entomophthoromycota* on the All-Fungal Tree of Life (James et al. 2006). More intense genomic studies of entomophthoroid fungi (including, of course, whole genome sequences that are currently in progress or planned for some taxa within this phylum) may provide some insight into why sexuality within this phylum appears to be exclusively homothallic.

The most pressing requirements for clarification of the taxonomy of the *Entomophthoromycota* are to include more species and genera in the analyses, with a special need to include the phylum's rarest and most unusual fungi, many of which have never been cultured. Representatives of two of the six families of entomophthoroid fungi are among this list of taxa most needed for inclusion in future datasets. The rarest of these may be *Completoria complens*, the sole species in *Completoriaceae*, an intracellular parasite of fern gametophytes. The species of *Meristacrum* and *Tabanomyces* (*Entomophthorales*: *Meristacraceae*) are pathogens of nematodes and tabanid fly pupae, respectively. The transfer *Ballocephala* and *Zygnemomyces* (*Meristacraceae*, *Entomophthorales*) to the *Kickxellomycotina* based on their septal ultrastructure (Saikawa 1989, Saikawa et al. 1997) was made by Humber (2012) and it is being followed by us.

The fact, that *Entomophthoromycota* consists of several fungal taxa whose systematics conform to modern phylogenetic taxonomic standards is both daunting and exciting. The *Entomophthoromycota* is an important group because of its potential for microbial biocontrol of invertebrate pests. These fungi also occupy pivotal position on the Fungal Tree of Life, at precisely the point basal to virtually all other terrestrial fungi, where the aquatic fungi began to exploit terrestrial habitats and hosts.

The closest relative of *Microsporidia* might be the *Entomophthoromycota*. The *Entomophthoromycota* phylogenetically are among the oldest extant nonflagellate fungi it should be recognised that these organisms have acquired many extraordinary survival strategies and unexpected surprises in their biologies. The *Entomophthoromycota* should be better appreciated and intensively studied by more mycologists and entomologists.

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