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Entomophthoromycota: a new phylum and reclassification for entomophthoroid fungi

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Abstract — One result of the recent phylogenetically based rejection of the phylum *Zygomycota* was the description of the subphylum *Entomophthoromycotina* (not assigned to any phylum) for fungi traditionally treated in the order *Entomophthorales*. More extensive gene-based analyses of these fungi suggest that they represent a monophyletic lineage distinct from all other fungi that deserves now to be recognized at the level of a new fungal phylum. These molecular data and further analyses of more traditional taxonomic criteria lead to this reclassification that still treats these fungi in six families but recognizes the new classes *Basidiobolomycetes*, *Neozygitomycetes*, and *Entomophthoromycetes* as well as the new order *Neozygitales*. *Ballocephala* and *Zygnemomyces* are excluded from *Entomophthorales* (*Meristacraceae*) and should be reclassified among the *Kickxellomycotina*.

Key words — *Zygomycetes*, sexuality, homothallism

Introduction

The reclassification of fungi by Hibbett et al. (2007) as the complement to a kaleidoscopic phylogenetic study all major fungal groups (James et al. 2006) validated the long-accepted sense that *Zygomycota* was polyphyletic, and recognized five taxa to replace this phylum: The phylum *Glomeromycota* accommodates arbuscular mycorrhizal fungi, and all other zygomycetes were distributed among subphyla *Entomophthoromycotina*, *Kickxellomycotina*, *Mucoromycotina*, and *Zoopagomycotina* without assignment to any phylum. It was assumed that subsequent research would determine whether any of these subphyla should be regrouped as part of an effort that would necessarily result in the recognition of one to four new phyla for these fungi.

The major characters traditionally used to classify the *Entomophthorales* have been thoroughly reviewed (summarized in Humber 1975, 1981, 1982,

1984) and were applied to six families in the last major reclassification of *Entomophthorales* (Humber 1989); this taxonomy is widely accepted despite a few minor differences in the treatments of some entomopathogenic genera (Bałazy 1993; Keller 1987, 1991, 1997; Keller & Petrini 2005). Until now, however, there have not been phylogenetic studies on a sufficiently broad range of their genes and taxa to propose a more contemporary revision.

The fungi in *Entomophthoromycotina* pose a few mycological puzzles (discussed below) for refining their current classification: Both the ultrastructure of the mitosis-associated organelle and early phylogenetic studies suggested that *Basidiobolus* has affinities with chytrid fungi and might better be excluded from *Entomophthorales*. Further, significant gaps in the gene-based understandings of entomophthoroid fungi exist because many taxa are very rarely collected and/or resist growth in vitro. Among these understudied taxa, *Neozygites* and related species represent the largest and most important 'black boxes' for which needed data remain unavailable.

The gene sequences now conceded to have taxonomic value for many fungi (nuclear rDNA genes, translation-elongation factor, β-tubulin, etc.) have been used in studies of a few entomophthoraleans in a more diverse set of fungi (Nagahama et al. 1995, Jensen et al. 2001, White et al. 2006) and for narrower studies of entomophthoralean species or species complexes (Jensen & Eilenberg 2001, Nielsen et al. 2001). A markedly different molecular approach comparing amino acid sequences for proteins (including some of the same proteins whose DNA sequences are widely used) has placed *Entomophthorales* outside of the true fungi (Einax & Voigt 2004, Liu & Voigt 2010, Voigt & Kirk 2011). While some skepticism about the meaning of such results based on amino acid sequences must be maintained, these findings do demonstrate the existence of distinct differences between all other groups of zygomycete fungi and *Entomophthoromycotina*. The amino-acid sequence-based 'exclusion' of *Entomophthorales* from the fungi echoes the hypotheses about placing *Basidiobolus* with watermolds but such 'anomalous' conclusions also suggest that molecular analyses based on highly limited inputs can yield results that have little sensibility from the broader perspective of the overall biology of the organisms analyzed.

A series of phylogenetic studies on entomophthoraleans (being prepared by A. Gryganskyi, R. Vilgalys, R. Humber, and other authors) incorporated more genes and a much broader range of entomophthoroid taxa than any earlier studies. These new analyses confirm the finding by James et al. (2006) that entomophthoroid fungi are a monophyletic group and that this group does include *Basidiobolus* and *Basidiobolaceae*. A reasonable integration of all results of traditional and phylogenetic analyses of entomophthoroid fungi suggests that they are distinct from all other fungi (including those in the other

unaffiliated zygomycete subphyla; Hibbett et al. 2007) and may occupy the most basal position among all non-flagellate fungi. The best-supported, most appropriate conclusion about the status of these fungi is, therefore, to recognize them as a new phylum in kingdom *Fungi*.

Materials & methods

Cultures and specimens used for the analyses discussed here are primarily from the USDA-ARS Collection of Entomopathogenic Fungal Cultures (ARSEF; http://www.ars.usda.gov/Main/docs.htm?docid=12125) and its associated herbarium. Unpublished molecular results of analyses of various genes are based on sequences of cultures obtained from ARSEF or isolated from nature and, in most cases, subsequently deposited in ARSEF. Other results involving reports on fungi and analyses using other isolates and specimens were completed at the Zoology Section of the Department of Agriculture and Ecology, University of Copenhagen, or at Agroscope FAL Reckenholz Eidgenössische Forschungsanstalt für Agrarökologie und Landbau (Zürich).

Major taxonomic issues affecting this reclassification

'Linkage' of *Basidiobolus* with flagellate fungi

The nuclei of entomophthoralean fungi and the details of their mitoses present a comparatively richer number and variety of characters than in most other fungal groups, and these nuclear characters are taxonomically informative, especially above the generic rank (Humber 1975, 1981, 1982, 1984, 1989). Mitosis by the huge nuclei of *Basidiobolus* species is sufficiently unusual to have been studied repeatedly (Olive 1907, Robinow 1963, Sun & Bowen 1972, Tanaka 1978). Among other surprises, the location of the mitosis-associated organelle in this genus is not fixed at the spindle poles and can even occur in the plane of the metaphase plate (Sun & Bowen 1972). The real controversies about this mitosis arose, however, when this organelle proved to be a short cylinder with a ring of 11–12 singlet microtubules (McKerracher & Heath 1985), and comparisons between this structure and centrioles were used to question the phylogenetic placement of this fungus; *Basidiobolus* remains the ONLY organism producing no flagellum in its life history for which microtubules are proven to be present in a mitosis-associated organelle. Nonetheless, any hypothesis that this organelle in *Basidiobolus* (but whose existence, location and ultrastructure remain unconfirmed from other fungi in its family) is linked with or derived from centrioles seems neither credible nor responsible: The 9×3 microtubular arrangement in centrioles and kinetosomes is invariant among ALL phyla of eukaryotes having flagella; conversely, no organelle with microtubules arranged like those in *Basidiobolus* is known from any other organism.

Later findings of gene sequence similarities between *Basidiobolus* and several chytrid and blastocladian watermolds (Nagahama et al. 1995, Jensen et al. 1998, Tanabe et al. 2000, White et al. 2006) have also been used to suggest

that *Basidiobolus* might not belong in *Entomophthorales*. However, other studies allied *Basidiobolus* with kickxelloid fungi from *Harpellales* (Keeling 2003, Tanabe et al. 2004) and one placed *Conidiobolus coronatus* (whose inclusion in *Entomophthorales* was never disputed) on a branch with the blastocladialean genus *Allomyces* (Tanabe et al 2005). These divergent findings underscore the peril of suggesting phylogenetic relationships among major fungal groups after comparing very limited sequence data and very sparse samplings of taxa within large and inherently diverse groups of fungi.

The recent survey of phylogenetic relationships within kingdom *Fungi* (James et al. 2006) supported the continued placement of *Basidiobolus* in *Entomophthorales*. Regardless of molecular or ultrastructural rationales to the contrary, hypotheses that *Basidiobolus* is not entomophthoroid are nonsensical if one considers the overall biology of these fungi. *Basidiobolus* and its relatives share many novel features in common with other entomophthoroid taxa despite the scant few pieces of data suggesting otherwise. It is necessary to bring a broader perspective to the uncertainties about *Basidiobolus*: Despite any and all evidence to the contrary, if this genus does not belong among the entomophthoroid fungi, then JUST WHERE AMONG FLAGELLATE (OR ANY OTHER) FUNGI SHOULD IT BE CLASSIFIED? The lack of any comparably well-supported answers for this question should quash any residual doubts about where *Basidiobolus* belongs.

Neozygitaceae: a special 'problem' in data gathering

The status of taxa in *Neozygitaceae* also presents a (temporary) problem for reclassifying the *Entomophthorales*. Very few cultures of *Neozygites* species are available in vitro, and, sadly, all current cultures of *Neozygites* are of mite pathogens with rough-walled, nearly globose zygospores. No gene-based data are available for *N. fresenii* [=*N. aphidis*, the type species] or other hemipteran pathogens that form ovoid, smooth-walled resting spores. A further taxonomic frustration is that these morphological and host differences suggest that *Neozygites* may eventually be split into two or more genera, but molecular data will be required to support such a decision. DNA-based evaluations of fungi from *Neozygitaceae* is encumbered by difficulties experienced in multiple laboratories to obtain clean DNA useful for amplifying and sequencing the genes needed to determine their phylogenetic relationships. While the recognition of *Neozygitomycetes* as a new class without supporting gene-based evidence may be dismissed by some as premature, such a treatment is the most reasonable for these fungi at this time, based on their known organismal biology (that integrates and represents a vastly larger proportion of the genome than those few genes now so widely treated as sufficient to complete high-level, phylogenetically sound reclassifications of virtually all other fungal groups).

Distinct differences between neozygitoid fungi and either basidioboloid or entomophthoroid taxa supports the description of three classes in this new phylum: As in basidioboloid fungi, neozygitoid fungi exert strong control in vegetative cells over nuclear number (usually four in *Neozygites*), have a central mitotic metaphase plate (Butt & Humber 1989), and, perhaps most significantly, a round of mitosis in gametangial cells precedes conjugation and zygosporogenesis while only one nucleus from each gametangium enters each zygospore (Keller 1997). As in entomophthoroid fungi, all neozygitoid taxa are obligate pathogens of insects or mites, and the nuclear membrane remains intact throughout mitosis. On the other hand, the chromosomes of neozygitoid fungi differ from basidioboloid and entomophthoroid fungi in being vermiform and of moderate size, condensing during mitosis but uncoiled (euchromatic) during interphase. *Neozygites* mitoses (Butt & Humber 1989) resemble those in animal or plant cells more closely than those in any other entomophthoroid fungi.

The presence of many novel characteristics shared among all of the fungi traditionally classified in order *Entomophthorales* underscores the need to keep these fungi together in a phylogenetically supported, coherent group and to pursue further studies to obtain more vital data about the genes, development, pathobiology, and other aspects for a better understanding of these fungi that can be very important naturally occurring biological control agents. Because these fungi occupy a very ancient position of the fungal tree of life it is also important to note that a better understanding should help to understand more about the enigmatic transition of fungi (as also occurred with plants and animals) from waterborne to terrestrial life forms.

Taxonomy

Entomophthoromycota Humber, **phyl. nov.** [Table 1]

MycoBank MB 564375

Vegetative growth as hyphae, hyphal bodies, or yeast-like; cells broad, walled or protoplastic. Conidiophores simple or digitate, each branch forming one conidiogenous cell and one conidium. Primary spores conidia, uni- to multinucleate, usually forcibly discharged; usually forming one or more types of secondary conidia. Resting spores homothallic zygospores or azygospores. Habit mostly as arthropod pathogens, but some saprobes or specialized phytopathogens.

Type genus: *Entomophthora* Fresen. 1856.

Conidiophores rise from mycelium or from body of host, usually with positive phototropic orientation, simple or apically (digitately) branched, with single conidiogenous cell on each branch giving rise to a single conidium or simple erect conidiophore becomes septate and each cell forms a single conidium. Primary conidia (not sporangia) with wall layers continuous with those on

TABLE 1. Proposed new classification for *Entomophthoromycota*.
New taxa described here are listed in ***boldface italics***.

PHYLUM *Entomophthoromycota* Humber, phyl. nov.
CLASS *Basidiobolomycetes* Humber, cl. nov.
ORDER *Basidiobolales* Caval.-Sm., Biol. Rev. 73: 246. 1998.
FAMILY *Basidiobolaceae* Claussen, Syllab. Pflanzenfam., Edn 9 & 10: 45. 1924.
Basidiobolus Eidam, Beitr. Biol. Pflanz. 4: 194. 1886.
Other new, undescribed genera (R.A. Humber, B. Huang & K. Hodge, unpublished).

CLASS *Neozygitomycetes* Humber, cl. nov.
ORDER *Neozygitales* Humber, ord. nov.
FAMILY *Neozygitaceae* Ben-Ze'ev, R.G. Kenneth & Uziel, Mycotaxon 28: 321. 1987.
Apterivorax S. Keller, Sydowia 57: 47. 2005.
Neozygites Witlaczil, Arch. Mikr. Anat. 24: 601. 1885.
Thaxterosporium Ben-Ze'ev & R.G. Kenneth, Mycotaxon 28: 323. 1987.

CLASS *Entomophthoromycetes* Humber, cl. nov.
ORDER *Entomophthorales* G. Winter, Rabenh. Krypt.-Fl., Edn 2, 1(1): 74. 1880.
FAMILY *Ancylistaceae* J. Schröt., Nat. Pflanzenfam. 1(1): 92. 1893.
Ancylistes Pfitzer, Monatsb. Königl. Preuss. Akad. Wiss. Berlin: 396. 1872.
Conidiobolus Bref., Untersuch. Gesammtgeb. Mykol. 6: 37. 1884.
Macrobiotophthora Reukauf, Centrabl. Bakt., Abt 1, 63: 390. 1912.
FAMILY *Completoriaceae* Humber, Mycotaxon 34: 453. 1989.
Completoria Lohde, Tagebl. Versamml. Deutsch. Naturf. Aertze 47: 206. 1874.
FAMILY *Entomophthoraceae* Nowak., Bot. Ztg. 35: 35. 1877.
SUBFAMILY *Entomophthoroideae* S. Keller, Sydowia 57: 28. 2005.
Batkoa Humber, Mycotaxon 34: 446. 1989.
Entomophaga A. Batko, Bull. Polon. Acad. Sci. Sér. Biol. Sci. 12: 325. 1964.
Entomophthora Fresen., Bot. Ztg. 14: 883. 1856.
Eryniopsis Humber, Mycotaxon 21: 258. 1984, pro parte.
Massospora Peck, Rep. New York State Mus. 31: 44. 1879.
SUBFAMILY *Erynioideae* S. Keller, Sydowia 57: 33. 2005.
Erynia (Nowak. ex A. Batko) Remaud. & Hennebert, Mycotaxon 11: 333. 1980.
Eryniopsis Humber, Mycotaxon 21: 258. 1984, pro parte.
Furia (Batko) Humber, Mycotaxon 34: 450. 1989.
Orthomyces Steinkraus, Humber & J.B. Oliv., J. Invertebr. Pathol. 72: 5. 1998.
Pandora Humber, Mycotaxon 34: 451. 1989.
Strongwellsea A. Batko & Weiser, J. Invertebr. Pathol. 7: 463. 1965.
Zoophthora A. Batko, Bull. Polon. Acad. Sci. Sér. Biol. Sci. 12: 323. 1964.
FAMILY *Meristacraceae* Humber, Mycotaxon 34: 456. 1989.
Meristacrum Drechsler, J. Wash. Acad. Sci. 30: 250. 1940.
Tabanomyces Couch, RV Andrejeva, Laird & Nolan, Proc. Natl. Acad. Sci. USA 76: 2302. 1979.

conidiogenous cells, inner wall layer invaginating to form two-layered septum between conidium and conidiogenous cell; almost always forcibly discharged (several possible mechanisms are known). Secondary conidia formed by most taxa: if forcibly discharged from short secondary conidiophore then usually similar in shape to primary conidium; if passively dispersed from long, thin (capillary) secondary conidiophore then usually distinctly differing in morphology from primary conidium. Resting spores (when mature) with thick, distinctly 2-layered walls, colored or hyaline, outer layer surface smooth or variously decorated; formed as zygospores (after gametangial conjugation) or azygospores (with no conjugation) either in the axis of the parental cells or budded off laterally; nuclear number in mature spores varies from 2 (from initiation or progressively reducing to 2) to multiple; germinating directly by forming germ conidiophore and germ conidium (usually resembling a secondary spore type) or indirectly by forming a small germ mycelium and then germ conidia (usually like primary conidia). Habits: saprobes in soil or litter, primary pathogens of arthropods (insect, mites, spiders) or other soil invertebrates (nematodes, tardigrades), or highly specific phytopathogens (e.g., of desmid algae or fern gametophytes). Arthropod pathogens may form specialized organs: rhizoids with or without differentiated holdfasts may anchor host to substrate, and cystidia may perforate host cuticle and facilitate emergence of conidiophores.

Primary and secondary conidia are the major spore forms in this phylum and constitute the primary basis for the taxonomy of these fungi. The resting spores are formed much less commonly than are conidia. The majority of species are pathogens of arthropods although pathogens of other soil invertebrates (nematodes and tardigrades) or of plants (desmid algae or fern gametophytes) are rare. The primary habit (especially in *Basidiobolaceae* and *Ancylistaceae*) may be in soil and plant detritus, but some species in these groups are best known as colonists of amphibian and reptile guts (*Basidiobolus*) or as facultative or obligate entomopathogens (*Conidiobolus*).

Any continued use of subphylum *Entomophthoromycotina* Humber (Hibbett et al. 2007: 517) is now superfluous until any future decision divides *Entomophthoromycota* into subphyla. This reclassification does not take up the phylum *Basidiobolomycota* Doweld (2001; LXXVII) because Doweld's name was proposed as part of a general reclassification of all fungi that does not agree with current understandings of fungal biology and relationships and, as circumscribed, *Basidiobolomycota* and the class *Basidiobolomycetes* Doweld (2001: LXXVII) used fragmentary knowledge of characters that may not apply to all taxa intended to be included while failing to account in any way for most taxa specifically included in this circumscription of phylum *Entomophthoromycota*; also see discussion below for class *Basidiobolomycetes*.

Basidiobolomycetes Humber, **cl. nov.**

MycoBank MB 564376

Differs from *Entomophthoromycetes* and *Neozygitomycetes* by unusually large nuclei (often ≥10 µm long) with a large central nucleolus that is the major feature of uninucleate cells. Mitoses involve barrel-shaped spindles, mitotic organelles incorporating microtubules (but not centrioles) but not always located at the spindle poles, and the nuclear content isolated from the cytoplasm by a layer of nuclear and cytoplasmic membrane fragments.

Type genus: *Basidiobolus* Eidam 1886.

Vegetative cells uninucleate, as regularly septate mycelium or yeast-like cells cleaved from contents of a parental cells (e.g., so-called 'Darmform' growth). Mitosis begins with fragmentation of nuclear membrane and aggregation of these and other membranes around a nuclear zone; chromosomes numerous, tiny, condensed and aligned on central metaphase plate (usually embedded inside the nucleolus) in association with a barrel-shaped spindle, chromosomes uncoil during interphase. Conidiogenous cell (conidiophore) simple but with bulbous apical swelling immediately below developing conidium. Conidia uninucleate, globose, with small conical basal papilla (projecting into spore body but everting during discharge), unitunicate (wall layers not separable). Conidia discharge forcibly by rocket-like ejection when central circumscissile weakness of the subconidial swelling ruptures; the upper portion of the swelling discharges together with conidium but may detach during flight. Secondary conidia (if formed) usually elongate, often curved, with or without an apical mucoid droplet, formed apically on an elongated capillary conidiophore, passively dispersed. Resting spores (RS) usually zygospores, formed homothallically in axis of parental cells; gametangial nuclei undergo mitosis before conjugation but only one nucleus from each cell enters the zygospore. Mature zygospores have thick, bi-layered walls; RS germinate by direct formation of germ conidium (usually a secondary conidial type: elongate, passively dispersed from a capillary conidiophore).

The foremost diagnostic character for basidioboloid fungi is their huge nucleus (often ≥10 µm in length) with a prominent central nucleolus that is the major feature of uninucleate cells (either as a broad, septate mycelium or cells cleaving internally in yeast-like growth mode). There is no staining of interphasic nuclei (nor, in any obvious manner, of mitotic chromosomes) in *Basidiobolomycetes* in aceto-orcein or other nuclear stains. The individual volumes of these nuclei may be many times greater than the entire cells of most ascomycete yeasts), and their mitoses are unusual for more than just the microtubular nucleus-associated organelle (McKerracher & Heath 1985): As mitosis begins, the nuclear envelope breaks down but the endoplasmic reticulum and other membranous cell components cluster around the nuclear zone so that the

spindle and chromosomes remain well isolated from the cytoplasm despite the fragmentation of the nuclear envelope; the corollary effect of this membrane organization is that mitotic nuclei 'disappear' when viewed with light microscopy (Robinow 1963).

Zygosporogenesis in *Basidiobolus* (Eidam 1886) is also very distinctive as short beak-like, lateral projections form at the septum between gametangial cells; gametangial nuclei move into the beaks, undergo mitosis, and the (uninucleate) beak cells are walled off before the septum dissolves and zygosporogenesis proceeds; remnants of these 'beaks' often remain visible on mature zygospores.

As noted in the discussion for the new phylum, two available names for this new class were not adopted: *Bolomycetes* Cav.-Smith (Cavalier-Smith 1998: 243) was based mainly on the microtubular mitotic organelle and 'beaked' zygospores in *Basidiobolus*. This mitotic organelle is not confirmed as present in all taxa in the *Basidiobolaceae* (including at least two still undescribed new genera), and the zygospores of some basidiobolaceous fungi are not 'beaked' as in *Basidiobolus*. *Basidiobolomycetes* Doweld (2001: LXXVII) was proposed as a nomen novum for *Bolomycetes* and cited Cavalier-Smith's description for this class; Doweld neither placed nor mentioned other entomophthoralean fungi in any rank in his general reclassification of fungi.

Neozygitomycetes Humber, **cl. nov.**

MycoBank MB 564377

Differs from *Basidiobolomycetes* and *Entomophthoromycetes* by vermiform, moderately sized chromosomes that condense during mitosis on a central metaphase plate but uncoil during interphase. Nuclear numbers in vegetative cells and conidia are low and apparently controlled at (3)-4-(5).

Type genus: *Neozygites* Witlaczil 1885.

Vegetative cells are rod-like hyphal bodies, walled or protoplastic, usually with 4 (3–5) nuclei, elongating until ± synchronous mitosis; daughter cells separate by splitting of septum. Nuclear number in all cell types strongly regulated; usually 4 (3–5) in vegetative cells and conidia, 2 in resting spores. Mitoses intranuclear, ± synchronous in any cell; nuclei fusoid at metaphase with central, fusoid spindle; no nucleus-associated mitotic organelle observed; chromosomes uncoil (euchromatic) during interphase. Conidiophores simple; forming apical conidiogenous cell and one conidium. Primary conidia subglobose to broadly ovoid, basal papilla short, comparatively flat; forcibly discharged to short distance by papillar eversion. Secondary conidia usually form quickly after primary conidial discharge, most commonly form as capilliconidia (that are the primary infective units). Resting spores bud from short conjugation bridge between rounded-up hyphal bodies (gametangia)

after preconjugational mitosis in contacting gametangia; zygospore receives one nucleus from each gametangium; only outer wall layer is melanized. Mature resting spores with two adjacent round fenestrae ('holes' through outer wall layer) and raised ridge of gametangial wall remnants between them.

Melanization of all spore types is a major feature of *Neozygitomycetes*. Primary and secondary conidia are pale, smoky gray; individual resting spores are much more strongly colored, and dark gray to black in mass.

Neozygitales Humber, **ord. nov.**

MycoBank MB

Order having all characteristics of class *Neozygitomycetes*.

Type genus: *Neozygites* Witlaczil 1885.

This order has all characters of class *Neozygitomycetes* (which includes only a single order and family).

Entomophthoromycetes Humber, **cl. nov.**

MycoBank MB 564381

Differs from *Basidiobolomycetes* by lack of uniformly uninucleate cells, nuclear morphology, details of mitoses, and modes of zygosporogenesis; and from *Neozygitomycetes* by cells not having uniformly small numbers of nuclei, details of mitoses, and lack of melanization of all spore types.

Type genus: *Entomophthora* Fresen. 1856.

Vegetative growth as coenocytic mycelium or rod-like to variably shaped hyphal bodies, walled or naturally protoplastic; if wall-less, rod-like to highly variable in shape and/amoeboid. Conidiophores simple or digitately branched, each branch with a single apical conidiogenous cell, or (in *Meristacraceae*) an unbranched erect, septate conidiophore forming one conidium per cell. Conidia unitunicate (wall layers not separating in liquid) or bitunicate (with separable outer wall layer); variously shaped, uni- to multinucleate, with basal papilla flat, conical or rounded; forcibly discharged by papillar eversion in most genera. Secondary conidia more or less similar in shape to primary conidia and forcibly discharged if formed on short secondary conidiophore, or elongate and passively dispersed if formed on elongated capillary secondary conidiophore. Nuclei (interphase) with small nucleolus, interphasic heterochromatin present in *Entomophthoraceae* but absent in all other families; mitoses intranuclear, with small lateral metaphase plate lateral; interphasic chromosomes are partly condensed (heterochromatic) and stain readily in *Entomophthoraceae* but euchromatic (uncoiled and nonstaining) in other families. Resting spores globose to subglobose, formed as zygospores or azygospores. Habit obligately pathogenic for invertebrates (*Entomophthoraceae*, *Meristacraceae*, some *Ancylistaceae*), saprobic (some *Ancylistaceae*), or phytopathogenic (*Completoria* [*Completoriaceae*] and *Ancylistes* [*Ancylistaceae*]).

This class includes all members of *Ancylistaceae*, *Entomophthoraceae*, *Completoriaceae*, and *Meristacraceae* but omits those entomophthoralean taxa reassigned here to *Basidiobolomycetes* or *Neozygitomycetes* (Table 1) or removed from *Entomophthoromycota* as noted below.

Genera incertae sedis:

Eryniopsis Humber, Mycotaxon 21: 258. 1984.

All species are in *Entomophthoraceae* but would appear to be a mix of taxa representing both subfamilies *Erynioideae* and *Entomophthoroideae*; the type species, *E. lampyridarum*, has morphological characters suggestive of both subfamilies and cannot be placed in either without molecular studies.

Tarichium Cohn, Beitr. Biol. Pflanzen 1: 69. 1870.

This form genus for species known only from resting spores apparently represents a mix of species attributable to *Neozygitaceae* (especially species pathogenic to mites) and *Entomophthoraceae*. No new species should be added to this genus; DNA-based studies and morphological re-evaluations should allow most species to be recognized as synonyms of other species or transferred to other genera in *Entomophthoraceae* and *Neozygitaceae*.

Taxa inadoptata vel excludenda:

Massosporoideae S. Keller, Sydowia 57: 44. 2005.

This subfamily (accommodating only the genus *Massospora*) seems not to be supported by phylogenetic evidence and is treated as a synonym of subfamily *Entomophthoroideae*.

Ballocephala Drechsler, Bull. Torrey Bot. Club 78: 199. 1951.

Zygnemomyces K. Miura, Rep. Tottori Mycol. Inst. 10: 520. 1973.

These two genera are excluded from *Meristacraceae* and reassigned to *Kickxellomycotina* Benny (Hibbett et al. 2007) based on the bifurcate septa with lenticular plugs in their vegetative hyphae (Saikawa 1989; Saikawa et al. 1997).

Discussion

The terms 'mitospore' and 'meiospore' are not used in characterizing taxa of *Entomophthoromycota*. They were originally adopted to describe ascomycete and basidiomycete spores, and are not applicable to entomophthoroid fungi because the reproductive products and life histories of entomophthoroid fungi are not strictly comparable with those of the *Dikarya*: The thin-walled primary conidia (the basis for entomophthoroid taxonomy) are produced by the vegetative cells of these fungi, usually forcibly discharged, and usually able to produce one or more subsequent forms of secondary conidium if conidia do not germinate by producing a germ tube. Entomophthoroid resting spores may be conventionally sexual in nature (zygospores in which it may be assumed, although not yet proven, that karyogamy and meiotic

divisions occur) in *Basidiobolomycetes* and *Neozygitomycetes*; for taxa in the *Entomophthoromycetes*, and especially those in *Entomophthoraceae*, it was noted that the MORPHOLOGICAL events of sexuality (the presence or absence of gametangial conjugations define zygo- and azygosporogenesis, respectively) and the GENETIC events of sexuality (karyogamy and meiosis, that presumably happen during resting spore germination) may be completely independent processes (Humber 1981, McCabe et al. 1984). Entomophthoroid fungi may be the only fungi in which the morphological and genetic definitions of sexuality (or their absences) are present in all possible permutations and without the routine linkage between the morphological and genetic events of sexuality that is taken for granted in virtually all other types of organisms.

No zygomycetous or flagellate fungi produce spores that can accurately be referred to as meiospores in the sense of basidiospores and ascospores. The proven or presumably 'sexual' spores of fungi below the subkingdom *Dikarya* are thick-walled, environmentally resistant spores (zygospores, azygospores, resistant sporangia, etc.) that go through a quiescent dormancy before germinating to undergo a type of sporulation that is neither functionally nor developmentally comparable to the basidiospores and ascospores that are the direct and obligatory developmental products of the cells in which karyogamy and meiosis occur.

To obtain clean DNA and good sequence data from entomophthoroid fungi may be more difficult than for many, much more extensively studied fungal groups. Part of this difficulty might involve the physical organization of the genome in these fungi that might lead to overlapping but divergent sequences for some 'needed' genes. Chromosomal counts for *Basidiobolus* (which was long treated as including only one species, *B. ranarum*) have ranged from about 60 (Olive 1907) to several hundred (Sun & Bowen 1972) based on kinetochore counts in serial sections for transmission electron microscopy. These high numbers suggest that polyploidization events may have occurred repeatedly in *Basidiobolus*. This possibility seems to be verified by genetic studies showing multiple, genetically distinct allelic forms in *B. ranarum* for elongation-translation factor genes that usually occur in single copies in the genome (Henk & Fisher 2012). The few chromosome counts for entomophthoraceous entomopathogens also suggest a tendency to towards polyploidy: While the nature of chromosomes and mitoses may not facilitate chromosomal counts in *Entomophthoraceae* (Olive 1906, Humber 1975), the few published numbers in various taxa are 8, 12 (or more), 16, and 32 (see Humber 1982). No genetic studies like those of Henk & Fisher (2012) are available for *Entomophthoraceae* but no genetic studies of this family with techniques ranging from allozyme polymorphisms (Hajek et al. 1990; B. May, personal communication) to the latest gene sequencing efforts suggest that these fungi simultaneously harbor

multiple allelic variants at single loci; there is no indication that vegetative nuclei of these fungi are either diploid or include heterologous sets of chromosomes.

The interpretation of such seemingly numerous chromosomes in some taxa in *Entomophthoromycota* becomes more problematic in the absence of evidence suggesting that any putatively sexual reproduction in this phylum is heterothallic rather than strictly homothallic. No data support the invocation of heterothallic sexuality (even if outbreeding events were extremely rare) to explain Henk & Fisher's (2012) conclusions about the *Basidiobolus* genome. Except for gametangial fusions during zygosporogenesis, no cellular fusions (even between the naturally protoplastic vegetative cells of some of the pathogenic taxa) are known from cultures or natural collections of any entomophthoroid fungus; such fastidious behavior by these fungi precludes consideration of heterokaryosis and parasexuality as a mechanism to increase or to sustain gene flow among taxa in *Entomophthoromycota*.

It is important to note again that the *Entomophthorales* as traditionally recognized (Humber 1989) is the same group reclassified here except for the removal of *Ballocephala* and *Zygnemomyces* to *Kickxellomycotina* based on the bifurcate, plugged septa in their vegetative hyphae (Saikawa 1989, Saikawa et al. 1997). Despite earlier doubts about retaining *Basidiobolus* in *Entomophthorales*, molecular studies of more genes and a broader spectrum of entomophthoraleans (A. Gryganskyi, R. Vilgalys, and R. Humber, unpublished) confirm that this order, as historically treated, is monophyletic. These fungi exemplify yet another major group for which the traditional, pre-molecular classification has been fundamentally confirmed (although amplified and adjusted) rather than overturned by phylogenetic analyses. Phylogenetic techniques must not be allowed to override or to supplant the existing knowledge about groups of organisms despite the vital inputs, seductively authoritative-looking dendrograms, and current pre-eminence among taxonomy methodologies. The best role for phylogenetic techniques should be as partners with the much broader (and usually older) perspectives gained by a thorough understanding of the overall biology as the means to determine the most sensible and best supported organismal classifications.

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Gryganskyi AP, Humber RA, Smith ME, Miadlikovska J., Wu S, Voigt K, Walther G, Anishchenko IM, Vilgalys R. 2012. Molecular phylogeny of the Entomophthoromycota. Mol. Phylog. Evol. 65: 682-694. http://dx.doi.org/10.1016/j.ympev.2012.07.026

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