

Phylogenetic reclassification raises new respect—and a new phylum!—for Entomophthorales

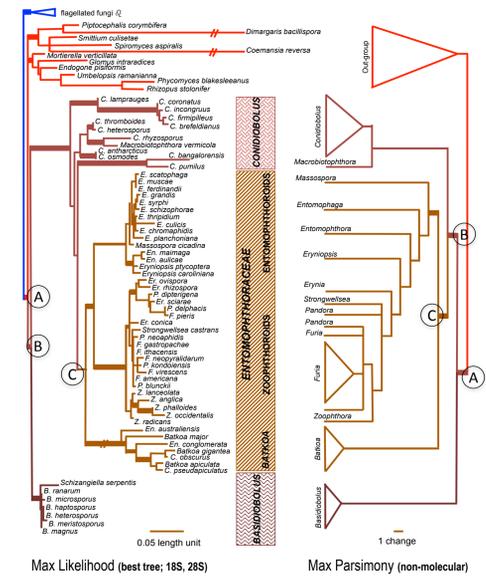
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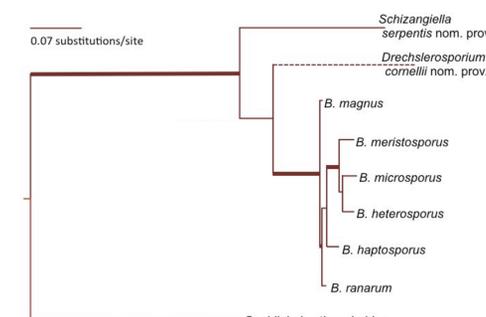
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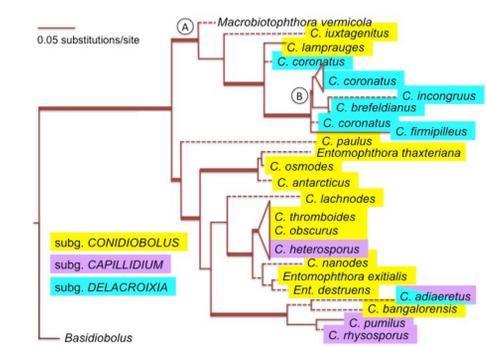
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THE MONOPHYLETIC PHYLUM ENTOMOPHTHOROMYCOTA. Comparison of trees from molecular (L) and morphological (R) character data. Thick branches are statistically supported. Major nodes show separation of entomophthoroid fungi (A) from all other fungi; Basidiobolomycetes (B) are basal to all other fungi in the phylum, and separation of the exclusively entomopathogenic Entomophthoraceae (C) from mostly saprobic taxa in Ancylistaceae. B=Basidiobolales, C=Conidiobolales, E=Entomophthorales, En=Entomophaga, Er=Erynia, F=Furia, P=Pandora, Z=Zoophthorales. (Gryganskyi et al. 2012a)



BASIDIOBOLUS LINEAGE (Basidiobolomycetes) ML analysis with LSU, SSU, RPB2, mtSSU. Thick branches are statistically supported. All taxa are united by cells with one large nucleus with a prominent central nucleolus and distinctive mitotic mechanism. (Gryganskyi et al. 2012b)



CONIDIOBOLUS LINEAGE (Ancylistaceae) ML analysis with LSU, SSU, RPB2, mtSSU. Thick branches are statistically supported. Conidiobolus is paraphyletic, and its current subgeneric taxonomy is not supported. Macrobiotophthora (A) is basal to Conidiobolus; C. adiaeretus produces all three types of secondary conidia and indicates microconidogenesis may be a paraphyletic character. (Gryganskyi et al. 2012b)

The earliest phylogenetic studies including entomophthoroid taxa suggested that they were not homogeneous. The much more extensive sampling and use of more genes here than in previous analyses confirm that the classically defined Entomophthorales is both monophyletic and distinct from all other zygomycetes. This justified raising the subphylum to phylum rank. There is an acute need for a kaleidoscopic survey and phylogenetic review of Conidiobolus. The existing subgeneric scheme for this genus is not supported, and we dare not guess about the number or circumscriptions of genera that will result from its reclassification.

The Entomophthoraceae is the largest and most taxonomically complex family of this phylum. More data (and better underlying identifications of a number of strains) will be needed to determine the extent to which the Batkoa lineage may be separate from the rest of the subfamily Entomophthoroideae. More species and isolates of several genera must be studied (especially species of Eryniopsis). The genera Erynioideae represent the most difficult problem using the current molecular data. Zoophthora is clearly supported as a separate genus, it is possible that Erynia might be supported, and Pandora and Furia may need to be combined; only the use of more collections and more genes will be able to resolve these questions.

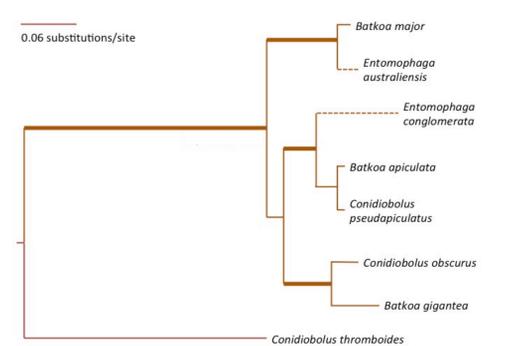
The systematics of entomophthoralean fungi has changed dramatically since the time when nearly all entomopathogens in this group (except, notably, for the cicada-pathogenic species of *Massospora*—were treated as *Entomophthora* species. The pace of taxonomic improvements heated up dramatically in the mid-1960's when the Batkooan classification offered so many radical changes from the existing taxonomy of these fungi that his scheme was effectively ignored in print by all other students of these fungi, and barely even mentioned in conversations despite the recognition that the Batkooan classification included many good decisions as well as some that were clearly wrong. Batko's classification was ignored—neither praised, condemned, nor corrected—until entomophthoralean taxonomy boiled over in the 1980's with the publication of several competing large-scale attempts to reclassify these fungi (by Remaudière and colleagues in Europe, by Humber and, separately, Tucker in the US, and by Ben-Ze'ev and Kenneth in Israel) that spawned serious disagreements in print, and also some loud ones in person. After the dust settled and tempers cooled, a six-family classification with a number of new and modified genera (Humber 1989) gained nearly universal acceptance.

The Entomophthorales entered the era of phylogenetic systematics with an immediate challenge to the integrity of the order thanks to single-gene analyses that suggested the *Basidiobolus* was more closely related to chytrid fungi than to the *Entomophthorales* (Nagahama et al 1995). The addition of more genes to the analyses, particularly under the global All-Fungal Tree of Life project, led James et al. (2006) to suggest that *Basidiobolus* was, indeed, a member of *Entomophthorales*. Molecular studies on these fungi were uniformly based until this year on limited numbers of genes and on very limited samplings of taxa from among entomophthoroid fungi. New analyses (Gryganskyi et al. 2012a,b) using multiple genes and an unprecedented number of entomophthoralean taxa unambiguously confirm several key points about the systematics of these fungi:

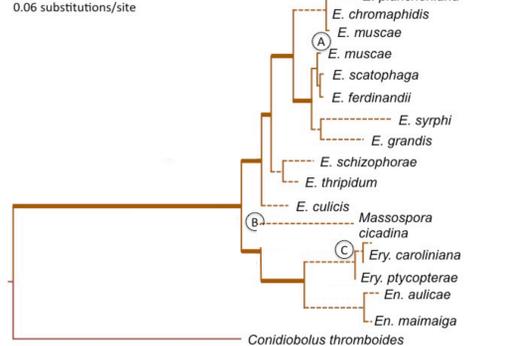
- The Entomophthorales as traditionally classified and as recognized by Hibbett et al. (2007) as the subphylum *Entomophthoromycotina* is a monophyletic group distinct from all other fungi.
- This confirmation justifies their treatment as a new phylum and the newly adjusted classification (Humber 2012) at the right.
- No cultures of other material was available for *Completoriaceae* or *Meristacraceae*. Few data were available for *Neozygitaceae* but their pertinent sequences, while difficult to obtain from the few cultured taxa, clearly exclude these fungi from the three well studied families.
- *Basidiobolus* (and all *Basidiobolaceae*) occupy a basal position in the phylum. *Conidiobolus* (the major genus of *Ancylistaceae*) proved to be paraphyletic and needs a new gene-based classification to replace its current, unsupported subgenera; *Ancylistaceae* is basal to the large, complex, and wholly entomopathogenic family *Entomophthoraceae*.
- Within the *Entomophthoraceae*, a separate subfamily for *Massospora* subfamily (Keller & Petrini 2005) is not supported. *Batkoa* may (or may not) represent a new subfamily. The currently recognized generic limits among zoophthoroid genera (subfamily *Erynioideae*) are proven to need more intensive study and, except for *Zoophthora*, are unsupported as currently recognized.

REFERENCES

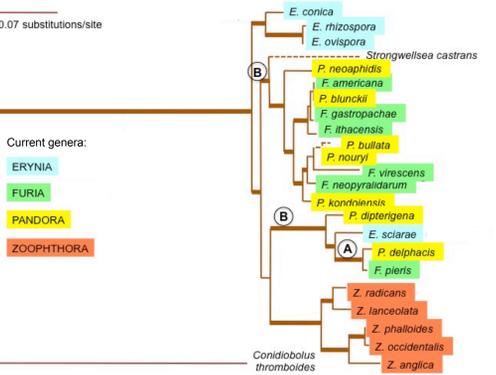
Gryganskyi AP, Humber RA, Smith ME, Miallikovska J, Wu S, Voigt K, Walter G, Anischenko IM, Vilgalys R. 2012. Molecular phylogeny of *Entomophthoromycota*. *Mol. Phylog. Evol.*: in press.
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BATKOA LINEAGE (Entomophthoroideae) ML analysis of LSU, SSU, RPB2, mtSSU. Thick branches are statistically supported. Conidiophores are unbranched; all produce secondary conidia; no cystidia are formed. Mixed identifications of taxa indicate strains identified before description of *Batkoa*. (Gryganskyi et al. 2012b)



ENTOMOPHTHORA LINEAGE (Entomophthoroideae) ML analysis of LSU, SSU, RPB2, mtSSU, ITS. Thick branches are statistically supported. *Entomophthora muscae* complex (A) is paraphyletic; *Massospora* (B) belongs in *Entomophthoroideae*; status of *Eryniopsis* (C) needs re-examination. (Gryganskyi et al. 2012b)



ZOOPHTHOROIDE LINEAGE (Erynioideae) ML analysis of LSU, SSU, RPB2, mtSSU. Thick branches are statistically supported. *Erynia sciarae* (A) in *Furia/Pandora* complex may reflect misidentification; *Furia* and *Pandora* (B) do not have molecular support; *Zoophthora* does seem to have good molecular support. (Gryganskyi et al. 2012b)

ABSTRACT: The recent phylogenetic studies and reclassifications produced by the global All-Fungal Tree of Life study recognized the *Entomophthorales* (as historically treated, with *Basidiobolus* remaining in this order) as a new subphylum, *Entomophthoromycotina*, that was not placed in any phylum. Subsequent phylogenetic analyses of the broadest range of entomophthoroid taxa and more genes than in any previous studies confirmed the monophyletic nature of these fungi and their distinctness from all other groups formerly classified in the phylum *Zygomycota*. As a lead-in to the publications of these molecular and traditional taxonomic analyses, the subphylum is now formally raised to phylum level as the *Entomophthoromycota*, and its included fungi are distributed among the classes *Basidiobolomycetes*, *Neozygitomycetes*, and *Entomophthoromycetes*; the genera *Balloecephala* and *Zygnemomyces* were removed from the family *Meristacraceae* (*Entomophthorales*) and reassigned to the subphylum *Kickxellomycotina*.

PHYLUM Entomophthoromycota Humber, phyl. nov.

- CLASS Basidiobolomycetes Humber, cl. nov.**
ORDER Basidiobolales Cavalier-Smith
FAMILY Basidiobolaceae Claussen
Basidiobolus Eidam
Schizangiella Humber, B. Huang & Hodge (unpubl. new genus)
Drechslerosporium B. Huang, Humber & Hodge (unpubl. new genus)
- CLASS Neozygitomycetes Humber, cl. nov.**
ORDER Neozygiales Humber, ord. nov.
FAMILY Neozygitaceae Ben-Ze'ev, R.G. Kenneth & Uziel
Apterivorax S. Keller
Neozygites Wiltaczil
Thaxterosporium Ben-Ze'ev & R.G. Kenneth
- CLASS Entomophthoromycetes Humber, cl. nov.**
ORDER Entomophthorales G. Winter
FAMILY Ancylistaceae J. Schröter
Ancylistes Pfitzer
Conidiobolus Brefeld
Macrobiotophthora Reukauf
FAMILY Completoriaceae Humber
Complectoria Lohde
FAMILY Entomophthoraceae Nowakowski
SUBFAMILY Entomophthoroideae S. Keller
Batkoa Humber
Entomophaga A. Batko
Entomophthora Fresenius
Eryniopsis Humber (in part; see subfam. *Erynioideae*)
Massospora Peck
SUBFAMILY Erynioideae S. Keller
Erynia (Nowakowski ex A. Batko) Remaudière & Hennebert
Eryniopsis Humber (in part; see subfam. *Entomophthoroideae*)
Furia (Batko) Humber
Orthomyces Steinkraus, Humber & J.B. Oliver
Pandora Humber
Strongwellsea A. Batko & Weiser
Zoophthora A. Batko
- FAMILY Meristacraceae** Humber
Meristacrum Drechsler
Tabanomyces Couch, RV Andrejeva, Laird & Nolan

Taxa with uncertain status, not accepted, or excluded from *Entomophthoromycota*:

- Subfamily Massosporoideae** Keller – Not accepted; without molecular support
Tarichium Cohn – A form-genus; mixture of species of *Entomophthoraceae* and *Neozygitaceae*
Eryniopsis Humber – Heterogeneous; species probably belong in separate subfamilies
Balloecephala Drechsler } Moved from *Meristacraceae* to subphylum *Kickxellomycotina*
Zygnemomyces Miura } due to septal ultrastructure (Saikawa 1989; Saikawa et al. 1997)