Diversity of Entomopathogenic Fungi: Which Groups Conquered the Insect Body?

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Abstract

The entomopathogenic fungi are organisms that evolved to exploit insects. They comprise a wide range of morphologically, phylogenetically, and ecologically diverse fungal species. Entomopathogenic fungi can be found distributed among five of the eight fungal phyla. Entomopathogens are also present among the ecologically similar but phylogenetically distinct Oomycota or water molds, which belong to a different kingdom, the Stramenopila. As a group of parasites, the entomopathogenic fungi and water molds infect a wide range of insect hosts, from aquatic larvae to adult insects from high canopies in tropical forests or even deserts. Their hosts are spread among 20 of the 31 orders of insects, in all developmental stages: eggs, larvae, pupae, nymphs, and adults. Such assortment of niches has resulted in these parasites evolving a considerable morphological diversity, resulting in enormous biodiversity, the majority of which remains unknown. Here we undertake a comprehensive survey of records of these entomopathogens in order to compare and contrast both their morphologies and their ecological traits. Our findings highlight a wide range of adaptations that evolved following the evolutionary transition by the fungi and water molds to infect the most diverse and widespread animals on Earth, the insects.

1. INTRODUCTION

The kingdom Fungi is one of the major groups of eukaryotic microbes in terrestrial and aquatic ecosystems (Mueller & Schmit, 2007). There are approximately 100,000 described species of fungi (Kirk, Cannon, Minter, & Stalpers, 2008), which only represents a fraction of the estimated diversity, considered to be between 1.5 and 5 million species (Blackwell, 2011; Hawksworth & Rossman, 1997). Importantly, one of the hallmarks of Fungi is their propensity to form intimate interactions with other groups of life on Earth (Vega & Blackwell, 2005). According to (Hawksworth, 1988), 21% of all described species of fungi are associated with algae as lichens and 8% form intimate relationships with plants as mycorrhiza. Few if any organisms in terrestrial ecosystems exist in nature in the complete absence of fungi, and for this reason fungi are essential players in the maintenance of ecosystem health (Braga-Neto, Luizão, Magnusson, Zuquim, & Castilho, 2008). Another group often considered when discussing fungi are the Oomycota. These are colloquially known as water molds and belong to a very distant kingdom—Stramenopila—(Alexopoulos, Mims, & Blackwell, 1996), more closely related to brown algae (Kamoun, 2003). However, it is appropriate to discuss oomycetes with fungi as they were long considered to be in the same group and exhibit very similar ecologies, acting as parasites of both plants and animals.

The insects, with over 900,000 described species, represent the most species-rich groups of eukaryotes (Grimaldi & Engel, 2005, p. 12). They are known to form intimate relationships with many fungal groups that include: mutualistic endosymbionts that assist in nutrition (Suh, McHugh, Pollock, & Blackwell, 2005), fungi as food sources that are farmed as crops by leaf cutter ants (Currie et al., 2003), vertically transmitted parasites (Lucarotti & Klein, 1988), commensals (DeKesel, 1996), and pathogens with pronounced effects on host populations (Evans & Samson, 1982, 1984). However, even though we know that many different fungal–insect associations do exist, this subject remains among the most understudied fields in fungal biodiversity and likely harbors one of the largest reservoirs of undocumented fungal species (Vega & Blackwell, 2005).

A prominent characteristic of insects is their chitinous exoskeleton, which the great majority of entomopathogenic fungi and Oomycota need to penetrate (Evans, 1988). Following entry, some groups (ie, *Metarhizium* and *Beauveria* in the order Hypocreales, phylum Ascomycota) are known to grow inside the host as yeast-like hyphal bodies, multiplying by budding (Prasertphon & Tanada, 1968). Others, for example, some species within the Entomophthoromycota, produce protoplasts (cells without cell walls) instead (Butt, Hajek, & Humber, 1996). A third group encompassing some species within Oomycota, Chytridiomycota and species within the genus *Entomophthora* that infect aphids are known to grow directly as hyphal filaments inside the host's body (Lucarotti & Shoulkamy, 2000; Roberts & Humber, 1981; Samson, Ramakers, & Oswald, 1979; Zattau & McInnis, 1987). The majority of entomopathogenic fungi kill their hosts before the spore production starts (as such they are termed hemibiotrophic). A few of them, especially some in the phylum Entomophthoromycota, sporulate from the living body of their hosts (and as such are termed biotrophic) (Roy, Steinkraus, Eilenberg, Hajek, & Pell, 2006). All of entomopathogenic oomycetes kill the host before transmission.

All entomopathogenic fungi and water molds are transmitted via spores. There are two types to consider. The sexual spores are actively released into the environment. By definition, zoospores are motile spores that swim and, in the case of pathogenic fungi, reach their target host actively, via a flagellum attached to the spore. Such motile spores occur in the Chytridiomycota and Oomycota. In other groups, sexual spores are named to link them to their groups: (zygo)spores, (basidio)spores, and (asco)spores belonging to respectively, the "zygomycetes," Basidiomycota and Ascomycota. Each of the three types exhibits unique traits (Fig. 1). The asexual mitotic spores (always called conidia regardless of taxon) are often passively released (Roberts & Humber, 1981). Spore morphology and their germination behavior have been heavily relied upon in the classification and systematics of different groups of fungi (Alexopoulos et al., 1996). We will discuss, later, the diversity of these varied spores separately for each major group of entomopathogenic fungi.

This review has multiple aims. The first is to ask which groups of Fungi and Oomycota evolved the ability to exploit the insect body. We will then explore the strategies these organisms employ for both infection and subsequent transmission. We view each group of entomopathogens within the ecological framework that is its insect host, an approach that has surprisingly not been previously considered in a broad sense. Our overarching aim is to provide a clearer understanding of the diversity and ecology of this important group of parasites, highlight lacunae in our knowledge, and motivate other studies. Before proceeding further, however, it is necessary to introduce each of the fungal and oomycete groups that are known to infect insects. This is because many groups presented here are generally unfamiliar.

2. THE MAJOR GROUPS OF ENTOMOPATHOGENIC FUNGI AND OOMYCETES

2.1 Oomycota

The species belonging to Oomycota were in the past considered among Fungi due to multiple ecological and morphological similarities. However, phylogenetic studies (James et al., 2006) confirmed earlier suggestions by some authors (Kreisel, 1969; Pringsheim, Pfeffer, & Strasburger, 1858; Shaffer, 1975) that these organisms are not Fungi. They were therefore placed in the Stramenopila, a kingdom containing morphologically diverse organisms such as Hyphochytriomycota and Labyrinthulomycota (Alexopoulos et al., 1996; Beakes, Glockling, & Sekimoto, 2012). Despite having been previously considered in the same group, the phylum Oomycota has a number of biological characters that distinguish them from Fungi. The first one is reproduction by biflagellate zoospore with a longer tinsel flagellum

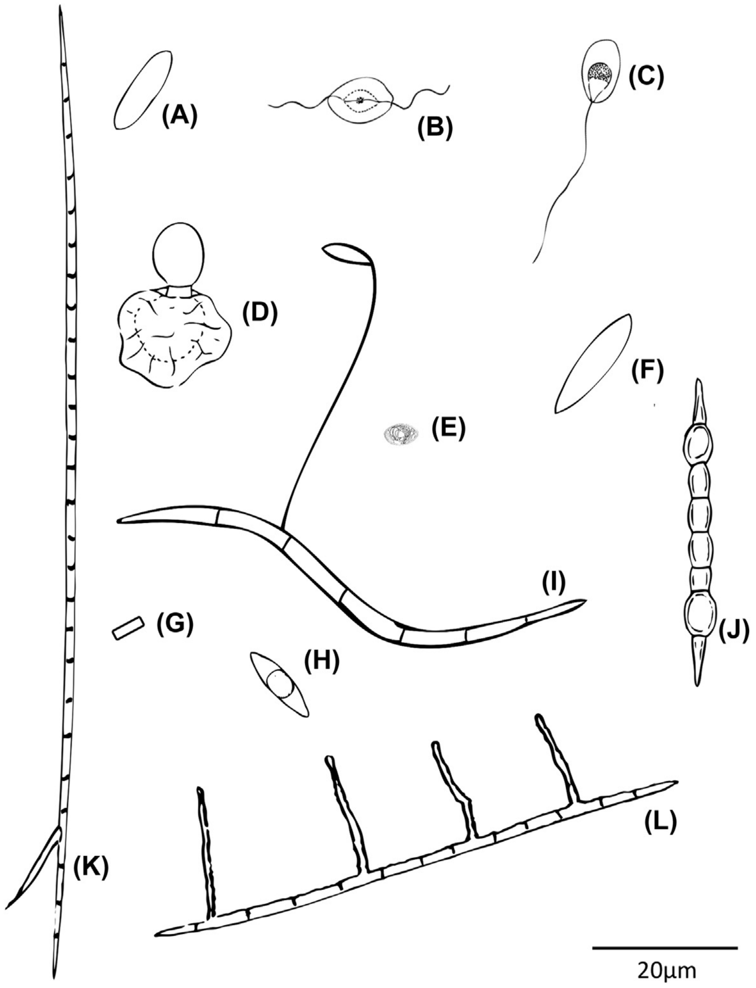


Figure 1 ***Spore diversity within entomopathogenic fungi (in μm).*** (A) Ascomycota—*Moelleriella sloaneae* (13–15 × 2.8–3 μm) (Chaverri, Liu, & Hodge, 2008); (B) Oomycota—*Lagenidium giganteum* (8–9 × 9–10 μm) (Couch, 1935); (C) Chytridiomycota—*Coelomomyces psophorae* (5 × 10) (Whisler, Zebold, & Shemanchuk, 1975); (D) Entomophthoromycota—*Entomophthora thripidum* (10–15 × 8–12 μm) (Samson et al., 1979); (E) Microsporidia—*Nosema hyperae* (3.1 × 1.7 μm) (Bulla & Cheng, 1977); (F) Basidiomycota—*Septobasidium maesae* (18–19.5 × 4–5 μm) (Lu & Guo, 2009); (G) Ascomycota—*Ophiocordyceps lloydii* (4 × 1 μm) (Kobayasi & Shimizu, 1978); (H) Ascomycota—*Hypocrella raciborskii* (10–16 × 2.5–4 μm) (Liu, Chaverri, & Hodge, 2006); (I) Ascomycota—*Ophiocordyceps camponoti-rufipedis* (80–95 × 2–3 μm) (Evans, Elliot, & Hughes, 2011); (J) Ascomycota—*Ophiocordyceps blattae* (40–60 × 4–6 μm) (Petch, 1924); (K) Ascomycota—*Ophiocordyceps camponoti-melanotici* (170–210 × 4–5 μm) (Evans et al., 2011); (L) Ascomycota—*Ophiocordyceps camponoti-novogranadensis* (75–95 × 2.5–3.5 μm) (Evans et al., 2011).

directed forward and a shorter whiplash flagellum directed backward (Fig. 1B) (Barr, 1992; Dick, 2001). They also reproduce by a thick-walled oospore, which is a structure not found in Fungi. At the cellular level, they possess mitochondria with tubular cristae, whereas the Fungi have mitochondria with flattened, plate-like cristae. Moreover, their cell walls contain cellulose, which is in contrast with Fungi that contain chitin as a cell wall component (Alexopoulos et al., 1996).

The Oomycota have evolved both parasitic and saprophytic (feeding from dead tissue and organic particles) lifestyles (Phillips, Anderson, Robertson, Secombes, & van West, 2008). As pathogens, oomycetes are able to infect a broad range of hosts such as algae, plants, protists, fungi, arthropods, and vertebrates, including humans (Kamoun, 2003). Certain genera are well-known plant pathogens, such as members of the genus *Phytophthora*, which was the causative agent of the Irish Potato Famine (Goss et al., 2014) and is currently causing Sudden Oak Death that affects millions of trees (Brasier, Denman, Brown, & Webber, 2004). Although better known as plant pathogens, the Oomycota do infect arthropods with records of infections on lobsters (Fisher, Nilson, & Shleser, 1975) and shrimps (Hatai, Rhoobunjongde, & Wada, 1992), as well as on insects (Pelizza, López Lastra, Maciá, Bisaro, & García, 2009; Samson, Evans, & Latgé, 1988; Seymour, 1984; Stephen & Kurtböke, 2011). For additional information about the entomopathogenic oomycetes, see Frances, Sweeney, and Humber (1989), Dick (1998), Scholte, Knols, Samson, and Takken (2004), Su, Zou, Guo, Huang, and Chen (2001) and Tiffney (1939).

2.2 Microsporidia

Traditionally, microsporidian species were classified within the phylum Apicomplexa as "sporozoan parasites" (Schwartz, 1998). However, an increasing number of studies are lending support to the hypothesis that Microsporidia are Fungi (Hibbett et al., 2007; Hirt et al., 1999; James et al., 2006). However, a conclusive resolution about microsporidians as an early lineage of Fungi will require further genetic studies from basal fungal taxa (James et al., 2006). Nevertheless, based on the studies mentioned earlier and the Microsporidia's ecological function as insect pathogens, we will include them among the entomopathogenic fungi in this study.

The most remarkable feature of this group is its unique spore—ranging from 1 to 40 μm (Wittner & Weiss, 1999, p. 8), which acts as a "syringe" injecting its protoplast material into the host (Keeling & Fast, 2002). Ohshima (1937) first suggested that the protoplasm is transmitted from the microsporidian spore to inside the host cell. We now know that this happens through the tube formed during adherence, which facilitates the subsequent discharge of the parasite's intracellular content to within the host's cell (Wittner & Weiss, 1999). The discharging of the polar tube occurs by breaking through the apex, which is the thinnest region of the spore wall. This event is compared by Keeling and Fast (2002) "to turning the finger of a glove inside-out."

The host range for most Microsporidia species is relatively restricted. They have been reported infecting a great number of domestic and wild animals such as fish (Kent, Shaw, & Sanders, 2014), amphibians, reptiles, birds (Kemp & Kluge, 1975), and mammals (Snowden & Shadduck, 1999), including some groups of humans, such as immunocompromised AIDS patients (Didier & Bessinger, 1999). Detailed studies on the biology and taxonomy of Microsporidia can be found in Bulla and Cheng (1977), Becnel and Andreadis (1999), Briano (2005), Lange (2010), Sokolova, Sokolov, and Carlton (2010), Kyei-Poku, Gauthier, Schwarz, and Frankenhuyzen (2011), Hossain, Gupta, Chakrabarty, Saha, and Bindroo (2012) and Vega and Kaya (2012).

2.3 Chytridiomycota

The Chytridiomycota is the phylum suggested to be the earliest diverging lineage of the Fungi (James et al., 2006). There are reports of them dating from Lower Devonian (about 400 million years ago (mya)) (Taylor, Remy, & Hass, 1992) and a parasitic chytrid-like fungus dates from the Antarctic Permian (about 250—300 mya) (Massini, 2007). Chytridiomycota is the only phylum among the kingdom Fungi that possesses motile cells at least once in its life cycle. These zoospores are equipped with a single posteriorly directed whiplash flagellum, which reflects their aquatic life cycle (for details, see Barr and Désaulniers (1988)). They respond to chemical gradients allowing them to actively locate their hosts, which is especially important for species pathogenic on aquatic organisms (Sparrow, 1960). They can also adaptively respond to environmental changes (eg, fluctuations in heat and humidity) in ways that reduce water loss or the collapse of the cell (Gleason & Lilje, 2009). The zoospores of chytrids are functionally equivalent to motile spores in the Oomycota, and so this is an example of convergent evolution, as both groups are aquatic.

The majority of chytrids are found as saprophytic organisms, especially in freshwaters and wet soils, but there are also some marine species (Gleason et al., 2011). However, a significant number of species are known to be parasites of plants, animals, rotifers, tardigrades, protists, and also other fungi (Dewel, Joines, & Bond, 1985; Karling, 1946; Martin, 1978; Sparrow, 1960). Diseases of insects caused by chytrids seem to be comparatively rare (Karling, 1948). For further reading see Voos (1969), Whisler et al. (1975), Millay and Taylor (1978), and Padua, Whisler, Gabriel, and Zebold (1986).

2.4 "Zygomycetes"

The phylum Zygomycota was traditionally organized as a single phylum and two classes, Zygomycetes and Trichomycetes (Alexopoulos, 1962; Alexopoulos et al., 1996). Both classes share common features like coenocytic mycelium (ie, lacking regular septation), asexual reproduction usually by sporangiospores and absence of flagellate cells and centrioles (Alexopoulos et al., 1996). Their main general characteristic is the production of a thick-walled resting spore (ie, zygospore) within a commonly ornamented zygosporangium, formed after fusion of two specialized hyphae called gametangia (Alexopoulos et al., 1996). The phylum is ecologically very diverse, widely distributed, and very common, with most species occurring as saprotrophs in both soil and dung. Some of them are fast growing and they are often found colonizing bread, fruits, and vegetables.

However, despite being placed in a single group, molecular phylogenetic studies validated the long-suggested hypothesis concerning the polyphyly of Zygomycota species and recognized five monophyletic taxa to replace the phylum. Thus, species that form arbuscular mycorrhizal associations with plants were accommodated within the phylum Glomeromycota and all other taxa distributed among four subphyla: Entomophthoromycotina, Kickxellomycotina, Mucoromycotina, and Zoopagomycotina without placement to any phylum (Hibbett et al., 2007; James et al., 2006). Thereafter, Humber (2012) proposed a detailed morphological and ecological description of a new phylum: Entomophthoromycota Humber, to accommodate species previously assigned to Entomophthoromycotina. This study was supported by a comprehensive phylogenetic study of this new phylum, which demonstrated its monophyletic nature (Gryganskyi et al., 2012). In our study, we will use this modern classification, which is supported by morphological, ecological, and phylogenetic data.

The Kickxellomycotina, Mucoromycotina, and Zoopagomycotina are composed mostly of saprobes. However, some families within Zoopagomycotina are known to predate nematodes (Zhang & Hyde, 2014). These are the relatively well-known "nematode trapping fungi." Within Zoopagomycotina mycoparasitic species are more common (Alexopoulos et al., 1996). While Mucoromycotina is the largest and morphologically most diverse order within the zygomycetes, just one species of entomopathogenic fungi is assigned to the subphylum, ie, *Sporodiniella umbellata*, which occurs on various insects, notably membracids (plant-feeding insects in the order Hemiptera), in cocoa farms (Evans & Samson, 1977).

The trichomycetes, currently placed within subphylum Mucoromycotina, order Harpellales, are fungi that exclusively inhabit the guts of various arthropods (Horn & Lichtwardt, 1981). However, since the trichomycetes apparently do little, if any harm to their hosts under natural conditions (Horn & Lichtwardt, 1981) and the nature of their relationships is not fully understood, they will not be further discussed in this study.

One of the most important groups of all entomopathogens is Entomophthoromycota, which are mainly pathogens of insects. They exhibit specialized spore-producing cells (conidiophores) that have positive phototrophic growth. Their spores are usually discharged forcibly and this can occur by several different mechanisms, sometimes producing secondary and in some species tertiary conidia (ie, *Eryniopsis lampyridarum*, Fig. 2) (Humber, 2012; Thaxter, 1888). They frequently occur as epizootic events, killing a large number of insects in small patches of forest or agricultural systems (Roberts & Humber, 1981). For further reading see Nair and McEwen (1973), Humber (1976, 1981, 1982, 1984, 1989) and Scholte et al. (2004).

2.5 Basidiomycota

This group, together with Ascomycota, forms the subkingdom Dikarya, which exhibits a dikaryotic phase (Hibbett et al., 2007). They contain some of the most well-known fungi such as mushrooms, puffballs, earthstars, smuts, and rust fungi. The Basidiomycota are characterized by the formation of sexual spores called basidiospores, which are formed outside specialized reproductive cells called basidia. These spores are in most cases forcibly discharged by specialized structures (Pringle, Patek, Fischer, Stolze, & Money, 2005). Another important and unique trait for the group are clamp connections. Those are structures formed during the division of the nuclei on the tip of growing hyphae, which help to ensure the dikaryotic condition (Alexopoulos et al., 1996), and can be used to identify members of this phylum, even in fossil records (Krings, Dotzler, Galtier, & Taylor, 2011).

The basidiomycetes exhibit some important ecological traits. They colonize dead wood, decaying cellulose and lignin, also acting as leaf litter decomposers on the forest floor (Braga-Neto et al., 2008). Pathogenic basidiomycetes (ie, smut and rust fungi) are familiar scourges of plants,

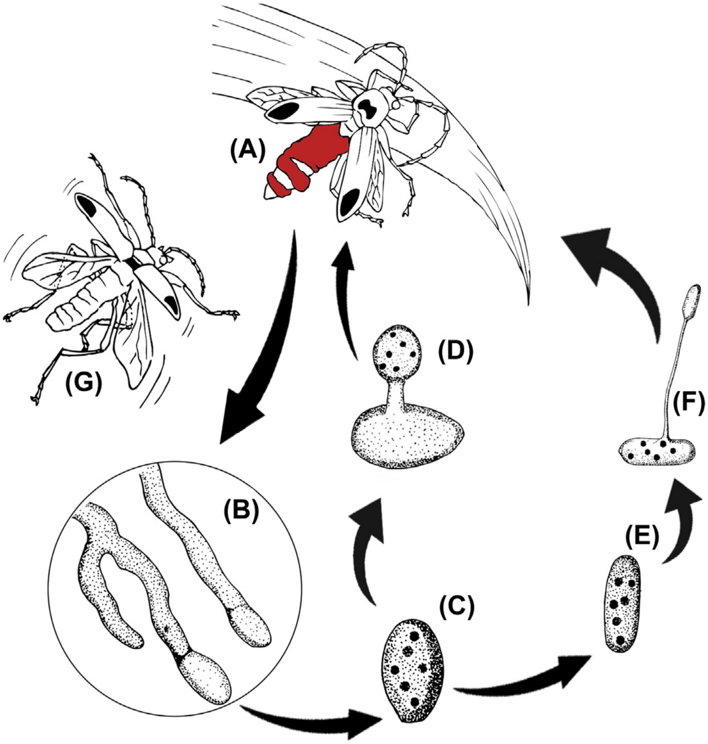


Figure 2 ***Entomophthoromycota*****—Eryniopsis lampyridarum.** (A) Cantharid beetle infected by *E. lampyridarum* died with its mandibles attached to flowering plants or grass. The elytra and wings gradually open as the fungus grows through the host's body; (B) Conidiophores emerges directly from the host's body; (C) Primary conidium; (D) Primary conidium bearing mature secondary conidium at the tip; (E) Secondary conidium; (F) Secondary conidia eventually will produce capilliconidia in absence of a suitable host; (G) Another cantharid beetle will get infected if it touches the exposed fungal hymenium. *Humber, R. A. (1984). Eryniopsis, a new genus of the Entomophthoraceae (Entomophthorales).* Mycotaxon, 21, *257—264, Roy, H. E., Steinkraus, D. C., Eilenberg, J., Hajek, A. E., & Pell, J. K. (2006). Bizarre interactions and endgames: entomopathogenic fungi and their arthropod hosts.* Annual Review of Entomology, 51, *331—357, and Thaxter, R. (1888).* The Entomophthoreae of the United States.

responsible for huge losses in agriculture. In addition, forest environments are also attacked by species like *Armillariella mellea*, which attack trees and *Heterobasidion annosum*, attacking specifically conifers (Kendrick, 2000). As animal pathogens, some species in the anamorphic genus *Nematoctonus* (linked to the teleomorphic genus *Hohenbuehelia*) are known to attack nematodes (Barron & Dierkes, 1977). A few genera are known as pathogens of insects, which infect scale insects (ie, *Septobasidium* and *Uredinella*, order

Septobasidiales) and termite eggs (ie, *Fibularhizoctonia*, order Atheliales, attacking eggs of the termite genus *Reticulitermes*).

The Septobasidiales exclusively attack scale insects (Hemiptera, Diaspididae) (Evans, 1989). The order includes two genera of entomopathogens: *Uredinella*, attacking single insects, and *Septobasidium*, attacking whole colonies of plant-feeding insects, with as many as 250 insects infected by one fungus (Couch, 1938). This character is one of the most remarkable differences between both genera, but morphological differences also exist. For example, the presence of a binucleate uredospore in *Uredinella* does not occur in *Septobasidium* (Couch, 1937). Due to this trait, *Uredinella* was described as "a new fungus intermediate between the rusts—a plant pathogen—and *Septobasidium,*" exhibiting traits of both (Couch, 1937).

Another group within Basidiomycota was described on termite eggs (Matsuura, Tanaka, & Nishida, 2000). This fungus was found living inside the nest of termites, among their eggs, which they occasionally consume. The authors identified this fungus, based on molecular studies, within the order Atheliales, as being a species very close related to *Fibularhizoctonia* sp. (asexual state of the genus *Athelia*), however, not describing them formally (Matsuura et al., 2000). For more details and species descriptions see Couch (1938), Matsuura (2005, 2006), Yashiro and Matsuura (2007), Lu and Guo (2009), Matsuura, Yashiro, Shimizu, Tatsumi, and Tamura (2009) and Matsuura and Yashiro (2010).

2.6 Ascomycota

The phylum Ascomycota is the largest group in kingdom Fungi with about 64,000 species described (Kirk et al., 2008). The majority of them are filamentous, producing regularly septate hyphae. They are characterized by the formation of sexual spores (ie, ascospores) in sac-like structures, singularly called an ascus. As the most speciose group of fungi, it is not surprising that they also have diverse ecological breadth comprised by decomposers, plant pathogens, human and animal pathogens, as well as being known to form mutualistic relationships (ie, lichens) (Alexopoulos et al., 1996).

The majority of entomopathogenic species within Ascomycota have a well-developed parasitic phase that infects the host's body. Furthermore, after killing the insect, this group is able to colonize the cadaver switching to saprophytic nutrition (hemibiotrophic), maintaining hyphal growth, even after the host's death (Evans, 1988). According to the same author, these entomopathogens would have evolved and diversified in early, moist

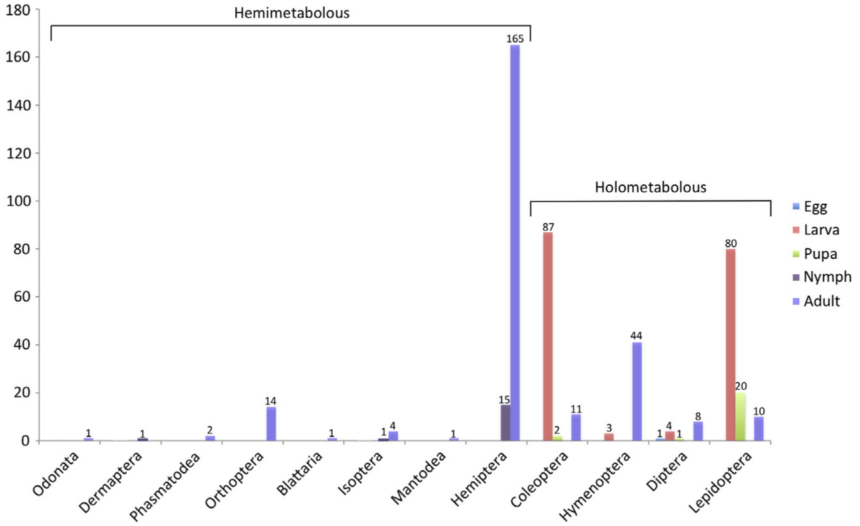


Figure 3 ***Hypocreales teleomorphic species.*** Hypocreales teleomorphic species numbers (y-axis) and their distribution across the different orders of insects, further divided into the stage of host development attacked. The Holometabolous orders have complete development with a larval stage, whereas the Hemimetabolous orders have an incomplete development with no distinct larval stage, but rather nymphs.

tropical forests, particularly rainforests. They are known to attack a wide range of different hosts (Fig. 3). The great majority of entomopathogenic ascomycetes form their spores inside structures called perithecia, a subglobose or flask-like ostiolate ascoma that contains many asci (Evans et al., 2011; Kirk et al., 2008; Kobayasi, 1941; Kobayasi & Shimizu, 1978). There is a wide diversity of spore types and shapes (Fig. 1). The phylum ranges from insect pathogens such as Pleosporales, Myriangiales, and Ascosphaerales, which have relatively few species, to the biggest group of entomopathogens, the hyperdiverse Hypocreales (Samson et al., 1988).

3. METHODS

3.1 Search Strategy

We are interested in determining which species of fungi and water molds successfully conquered the insect body. As such, our basic unit of analysis is the species name (binomial). The repositories for such names are electronic databases such as Mycobank (http://www.mycobank.org) and Index Fungorum (http://www.indexfungorum.org) (Fig. 4). MycoBank is owned by the International Mycological Association and is an online database aimed as a service to the mycological and scientific community by documenting mycological nomenclatural novelties (new names and combinations) and associated data, for example, descriptions and illustrations. The Index Fungorum, another global fungal database, coordinated and supported by the Index Fungorum Partnerships, contains names of fungi (including yeasts, lichens, chromistan fungal analogues, protozoan fungal analogues, and fossil forms) at all ranks.

All groups (phyla) of fungi were investigated separately. Once we found a phylum containing at least one entomopathogenic species (six in total), a thorough search was made within such phylum, narrowing until the entomopathogenic genera and finally identifying all species recorded as insect pathogens in each phylum.

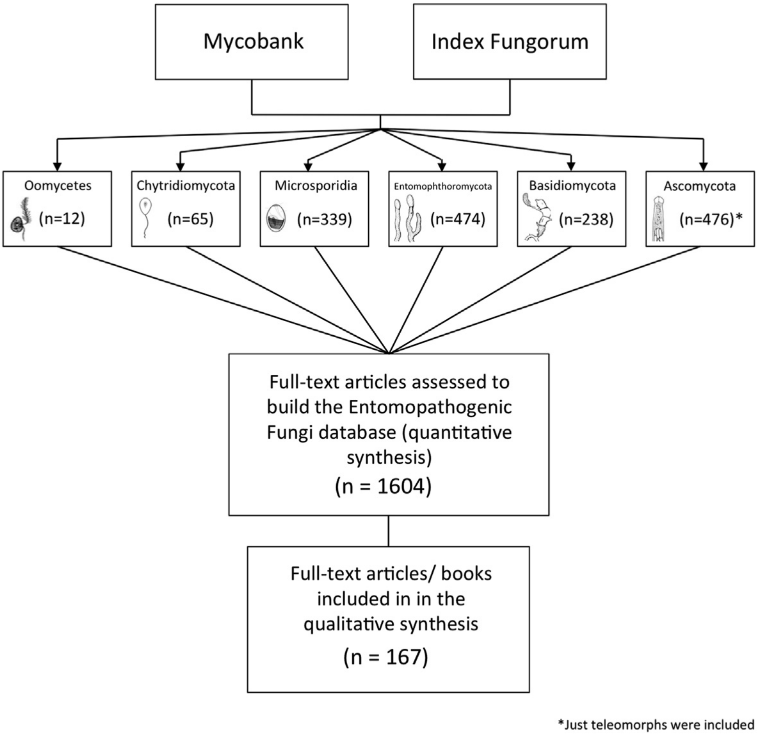


Figure 4 Flowchart shows the main sources consulted (Mycobank and Index Fungorum), the fungal/oomycetes phyla found infecting insects, and the number of species and sources consulted in this study.

3.2 Dealing With Name Changes

Species names are often not static and can change as taxonomists reorganize synonyms and as we advance with molecular phylogeny. We matched old records of entomopathogenic fungi with their current valid names, avoiding any duplicated record for the same organism.

3.3 Determining Host Associations

To determine the host association we first consulted the original formal description. This information is available on both Mycobank and Index Fungorum.

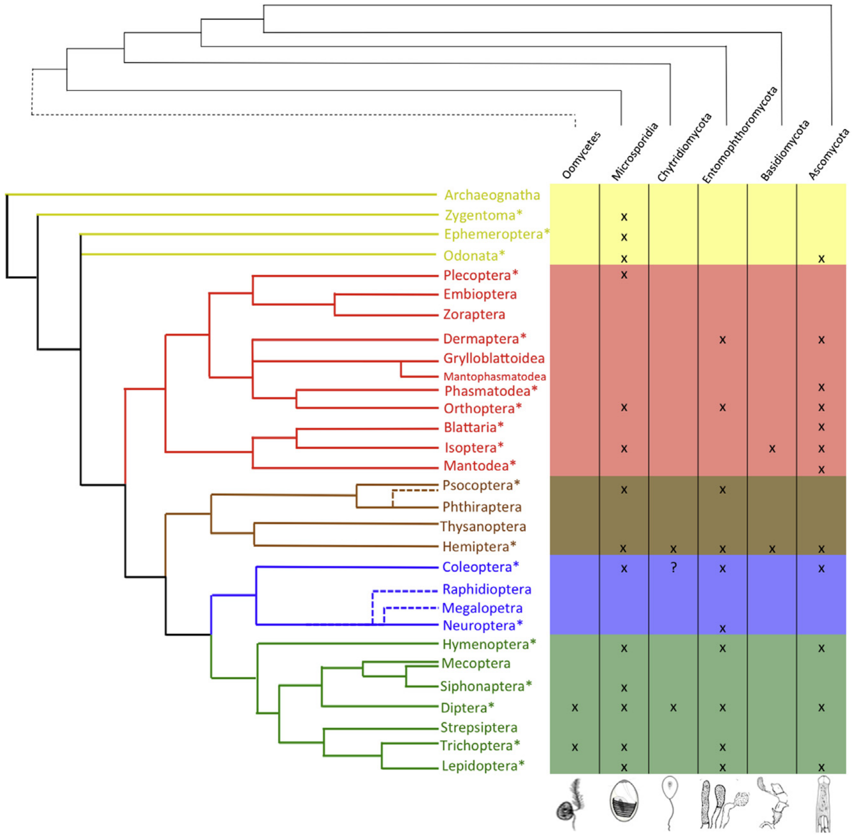
The complete list of original descriptions and references—that are not in the text—are listed within the tables organized by phyla, in the supplementary materials with species names, host association, and original reference(s).

3.4 Monographs and Atlases

We also consulted monographs and atlases of insect pathogenic fungi, eg, *Atlas of Entomopathogenic Fungi* (Samson et al., 1988), the monograph of hypocreloid fungi (Chaverri et al., 2008), *The Microsporidia and Microsporidiosis* (Wittner & Weiss, 1999), *The Genus Coelomomyces* (Couch & Bland, 1985), the *Genus Septobasidium* (Couch, 1938) among others. These were also useful for discovering both host associations and ecological aspects of the fungi reviewed herein.

4. RESULTS

We found 20 of 30 orders (sensu Grimaldi and Engel (2005)) of insects infected by fungi and Oomycetes (approximately 65% of insect orders are infected; Fig. 5). Microsporidia infects 14 orders of insects, Ascomycota (mostly Hypocreales) and Entomophthoromycota infect 13 and 10 orders of insects, respectively. Chytrids infect three, Basidiomycota and Oomycota, each infect two orders. There are several genera that are pathogenic to insects (Fig. 6). Later we examine incidences of parasitism for each of these six phylum focusing on the morphological and ecological traits they evolved to make them efficient and specialized parasites of insects.



	Oomycetes	Microsporidia	Chytridiomycota	Entomophthoromycota	Basidiomycota	Ascomycota
Archaeognatha						
Zygentoma*		x				
Ephemeroptera*		x				
Odonata*		x				x
Plecoptera*		x				
Embioptera						
Zoraptera						
Dermaptera*				x		x
Grylloblattoidea						
Mantophasmatodea						
Phasmatodea*						x
Orthoptera*		x		x		x
Blattaria*						x
Isoptera*		x			x	x
Mantodea*						x
Psocoptera*		x		x		
Phthiraptera						
Thysanoptera						
Hemiptera*		x	x	x	x	x
Coleoptera*		x	?	x		x
Raphidioptera						
Megalopetra						
Neuroptera*				x		
Hymenoptera*		x		x		x
Mecoptera						
Siphonaptera*		x				
Diptera*	x	x	x	x		x
Strepsiptera						
Trichoptera*	x	x		x		
Lepidoptera*		x		x		x

Figure 5 ***Insect orders × fungal phyla (and oomycetes): the parasitic relationship between entomopathogens and their hosts.*** On the left, the phylogeny of insect orders (*adapted from Grimaldi, D. & Engel, M. S. (2005). Evolution of the Insects. Cambridge University Press.*); on the top the phylogeny of fungal phyla and oomycetes (*adapted from James, T.Y., Kauff, F., Schoch, C.L., Matheny, P.B., Hofstetter, V., Cox, … Miadlikowska, J. (2006). Reconstructing the early evolution of fungi using a six-gene phylogeny. Nature, 443(7113), 818–822.*); the table shows which fungal group infects each insect order. The uncertainty of a record is denoted with a question mark.

4.1 Incidence of Disease on Insects Caused by Fungus and Oomycetes

4.1.1 Oomycetes

The entomopathogenic oomycetes are comprised of 12 species distributed among six genera: *Lagenidium* (one species, *Lagenidium giganteum*), *Leptolegnia* (two species, *Leptolegnia caudata* and *Leptolegnia chapmanii*), *Pythium* (three

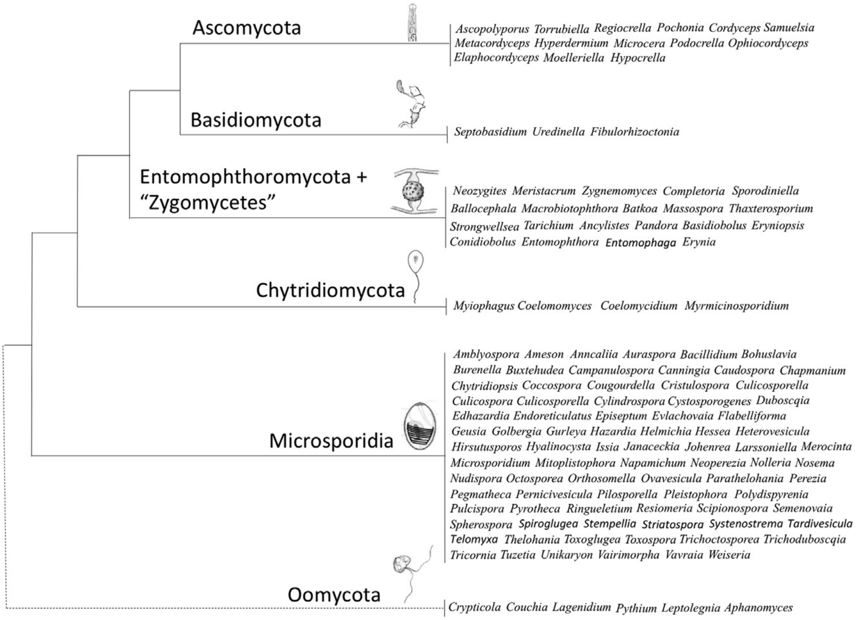


Figure 6 Diversity of genera of entomopathogens across Fungi and oomycetes.

species, *Pythium carolinianum*, *Pythium sierrensis*, and *Pythium flevoense*); *Crypticola* (two species, *Crypticola clavulifera* and *Crypticola entomophaga*); *Couchia* (three species, *Couchia amphora*, *Couchia linnophila*, and *Couchia circumplexa*), and *Aphanomyces* (one species, *Aphanomyces laevis*). They have been discovered attacking species of mosquito in the following genera: *Aedes*, *Anopheles, Chironomus, Culex, Forcipomyia, Glyptotendipes, Mansonia, Ochlerotatus, Pentaneura, Polypedilum, Tendipes*, and *Uranotaenia* (Martin, 1981, 2000; Scholte et al., 2004).

Oomycetes infections have been recorded from mosquito larvae in freshwater, primarily in well-aerated streams, rivers, ponds, lakes (Alexopoulos et al., 1996), and even treeholes (Saunders, Washburn, Egerter, & Anderson, 1988) and water that collects on leaf axils (Frances et al., 1989). A single example of oomycetes infecting a nondipteran was *Crypticola entomophaga*, which was described attacking caddis flies (Trichoptera), which are also aquatic (Dick, 1998).

Among the entomopathogenic oomycetes, the most well-known and broadly studied species is *L. giganteum* Couch, a facultative mosquito larvae parasite (see Fig. 7) (Scholte et al., 2004). In an experimental study researchers described the swimming behavior of zoospores towards the surface

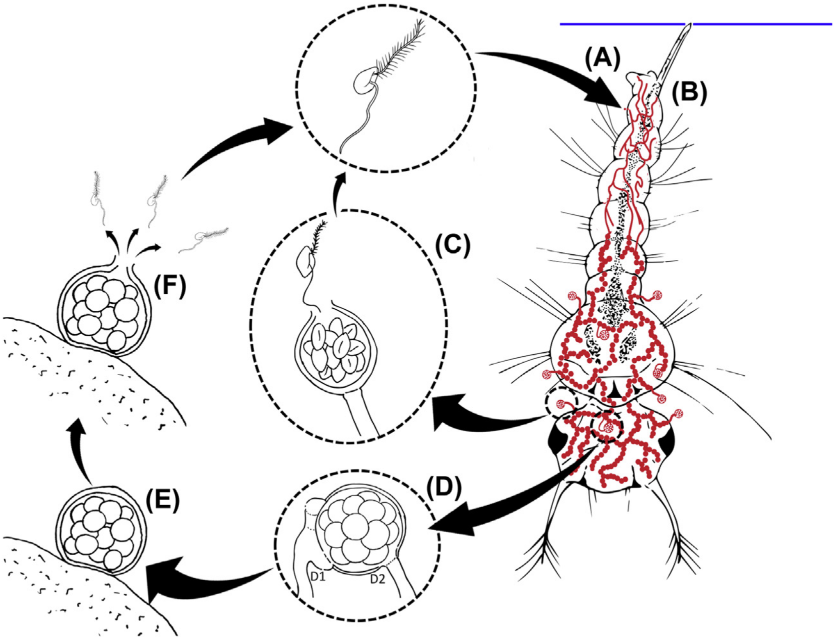


Figure 7 ***Oomycetes*—Lagenidium giganteum.** (A) Zoospore (n) adhere and penetrate the cuticle, starting the infection; (B) Mycelium starts to grow and proliferates within the larva's body; (C) Zoosporangium releasing asexual zoospores; (D) Oospore (Sexual part of the cycle)—(D1) Antheridium fertilizing (D2) oogonium; (E) Oospore detaches from the hyphae and settles down in the environment; (F) Releasing of sexual zoospores (2n).

of the water (Golkar et al., 1993). Their results suggested that spore-swimming behavior seems to be due to the cell shape and location of center of gravity rather than a sensory response.

Other oomycete genera like *Leptolegnia*, *Pythium*, *Crypticola* and *Aphanomyces* have received only limited attention (Scholte et al., 2004). Within the genus *Leptolegnia*, only *L. caudata* de Bary (Bisht, Joshi, & Khulbe, 1996) and *L. chapmanii* Seymour (Seymour, 1984) have been isolated from insects (Scholte et al., 2004). The life cycle of *L. chapmanii* was presented by Zattau and McInnis (1987), who reported that species infecting *Aedes aegypti*, the yellow fever and dengue disease vector. Effects of *L. chapmanii* on other aquatic invertebrates such as Odonata, Thichoptera, Coleoptera, Plecoptera, and Cladocera were tested, though no infections were observed, suggesting specificity (McInnis & Schimmel, 1985). Members of the genus *Pythium* are species spread across the world, occurring mostly as soil-inhabiting organisms or plant pathogens (Alexopoulos et al., 1996). Three species of *Pythium* are known to infect insect larvae (Phillips et al., 2008). *Aphanomyces* was recorded causing seasonal epizootic in insectaries (Seymour & Briggs, 1985), but few studies were published about this genus infecting insects.

4.1.2 Microsporidia

Microsporidia is a group of pathogens comprising 143 genera (Sprague & Becnel, 1999) with more than 1200 species (Wittner & Weiss, 1999). Among those, 69 genera were recorded infecting insects, attacking 12 orders (Fig. 5). According to Becnel and Andreadis (1999), the majority (42 of 69 genera) infect Diptera; five genera infect Ephemeroptera and Coleoptera; four genera infect Lepidoptera, followed by Trichoptera, infected by three, Orthoptera, Odonata, and Siphonaptera each infected by two genera; and Thysanura, Hymenoptera, and Isoptera with one genus of Microsporidia infecting each of them. These accounts are data based on described genera that possess an insect as type-host. Hence, this number certainly will increase in future publications.

As will be discussed further, the dipterans are the only insect group infected by five different groups of Fungi/oomycetes (only the Basidiomycota have not been recorded as pathogens of Diptera). Among the 42 microsporidian genera attacking Diptera, the largest, most widespread, and common is *Amblyospora* (Andreadis, 1985), which is known to infect 79 species of Diptera in 8 genera (Becnel & Andreadis, 1999). This genus of Microsporidia exhibits a complex life cycle, which requires an intermediate copepod host and two mosquito generations in order to complete its full life span (Sweeney & Becnel, 1991).

Another important group among the entomopathogenic Microsporidia is the genus *Nosema* (see Fig. 8). Some authors consider them the most important and widely distributed genus (Tsai, Lo, Soichi, & Wang, 2003), being responsible for the majority of microsporidian infections in Lepidoptera species (Tsai et al., 2003). A good example of their ecological and economical importance occurs with the species *Nosema bombycis* and *Nosema ceranae* that infect bees and are known to be responsible for great losses in apiculture (Higes, Martín, & Meana, 2006). These infections are restricted to the midgut epithelial cells of bees and occur by ingestion of spores by adults (Fig. 8). Once in the midgut, the spores are chemically stimulated to trigger the polar tube, which penetrates the host's cells, starting the infection processes (de Graaf, Raes, & Jacobs, 1994). Infectious spores are then

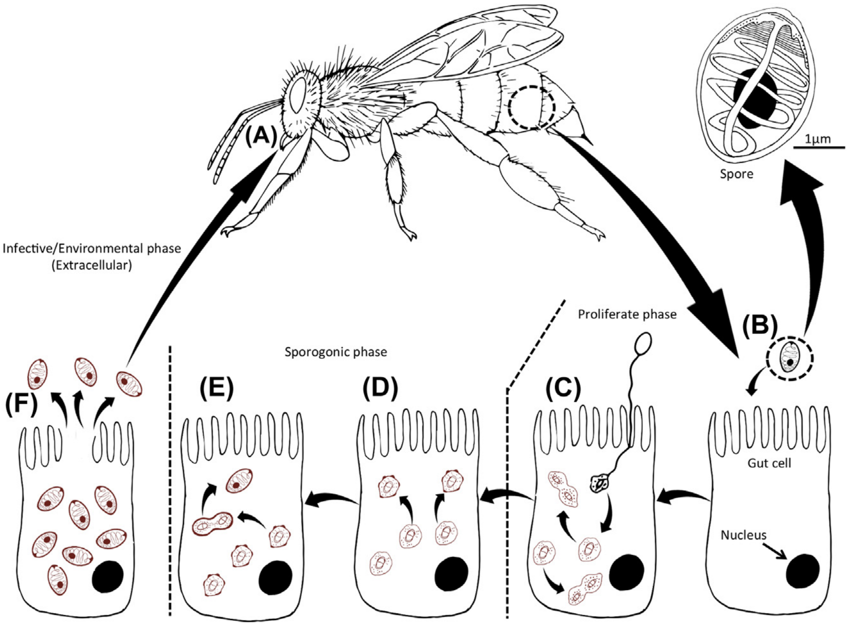


Figure 8 ***Microsporidia*—Nosema *sp.*** (A) Ingestion of spores; (B) Spore reaches the gut of the bee and is activated by its environment, triggering the polar tube to inject the sporoplasm into the host's cell; (C) Cellular multiplication (proliferate phase); (D and E) Transition from sporoplasm to spore; (F) Spores are released into the gut again, and will be spread in the bee's feces or will reinfect the same individual.

released with the feces and due to the characteristic thick three-layered wall structure, they are well adapted to resist in the environment until they are ingested by another adult bee (Wittner & Weiss, 1999).

4.1.3 Chytridiomycota

Among the chytrids, there are four genera that are entomopathogenic: *Myiophagus* (one species, *Myiophagus* cf. *ucrainicus*), *Coelomycidium* (one species, *Coelomycidium simulii*), *Myrmicinosporidium* (one species, *Myrmicinosporidium durum*), and the most diverse genus *Coelomomyces* (63 species). Most of the chytrid infections in insects have been recorded for Diptera.

The genus *Myiophagus* was described infecting dipteran pupae (Petch, 1948) and scale insects (Karling, 1948; Muma & Clancy, 1961). Doberski and Tribe (1978) reported *Catenaria auxiliaris* on coleopteran larvae, although, they are not sure if the colonization occurred after the larva's death (saprophytism) or if, in fact, parasitism occurred, leading to the death of the larvae (in Fig. 5 the uncertainty of this record is denoted with a question mark). Thus, since this relationship is not proven yet, we will not consider *C. auxiliaris* among the chytrids that parasitize insects.

The genus *Coelomycidium* is known to attack a specific group within Diptera order, the black flies (Simuliidae) (Jitklang, Ahantarig, Kuvangkadilok, Baimai, & Adler, 2012; McCreadie & Adler, 1999). This disease is identified by the observation of the larvae filled with spherical sporangia throughout the body cavity (Kim, 2011).

One group deserves special mention because of the effect they have on insect reproduction and behavior. The *Coelomomyces* species (Fig. 9) are relatively well known because their hosts are important human disease vectors (*Simulium* and the mosquitoes *Anopheles*, *Culex*, and *Aedes*). Species within this genus can infect eggs (Martin, 1978), larvae (the most common type of infection, see details in Travland (1979)), and adults (Lucarotti & Klein, 1988). In some species (ie, *Coelomomyces psophorae*, Fig. 8) a copepod is required to complete the whole life cycle (Whisler et al., 1975).

In some cases, the fungus does not kill the larvae. Rather, the chytrid remains inside the insect as it passes through the larval and pupal stages before maturing in the ovaries of adult females (Lucarotti, 1992). Once there and after the first mosquito's blood meal, the hypha matures to become sporangia, which is the fungal structure responsible for producing zoospores (Lucarotti & Shoulkamy, 2000). Thus, instead of laying eggs, the mosquito will 'lay' sporangia full of zoospores, ready to infect new larvae (Lucarotti & Klein, 1988). Fatefully, the fungus is reintroduced at the mosquito's breeding site by its own host.

4.1.4 Entomophthoromycota

The phylum Entomophthoromycota is composed mostly of pathogens of insects, with few pathogens of other invertebrates, desmid algae, and fern gametophytes, and some that live a saprophytic life (Humber, 2012). The entomopathogenic species are distributed among 19 genera: *Entomophthora, Conidiobolus, Entomophaga, Erynia, Meristacrum, Neozygites, Strongwellsea* and *Massospora, Pandora, Eryniopsis* (Fig. 2), *Batkoa, Tarichium, Completoria, Ballocephala, Zygnemomyces, Ancylistes, Macrobiotophthora, Thaxterosporium*, and *Basidiobolus*. It is difficult to say how many species of entomopathogens exist since many of these genera also infect different groups of hosts. In addition, the group is in constant taxonomic flux (Humber, 2012). However, since the scope of this work is not to provide a complete list of all species within

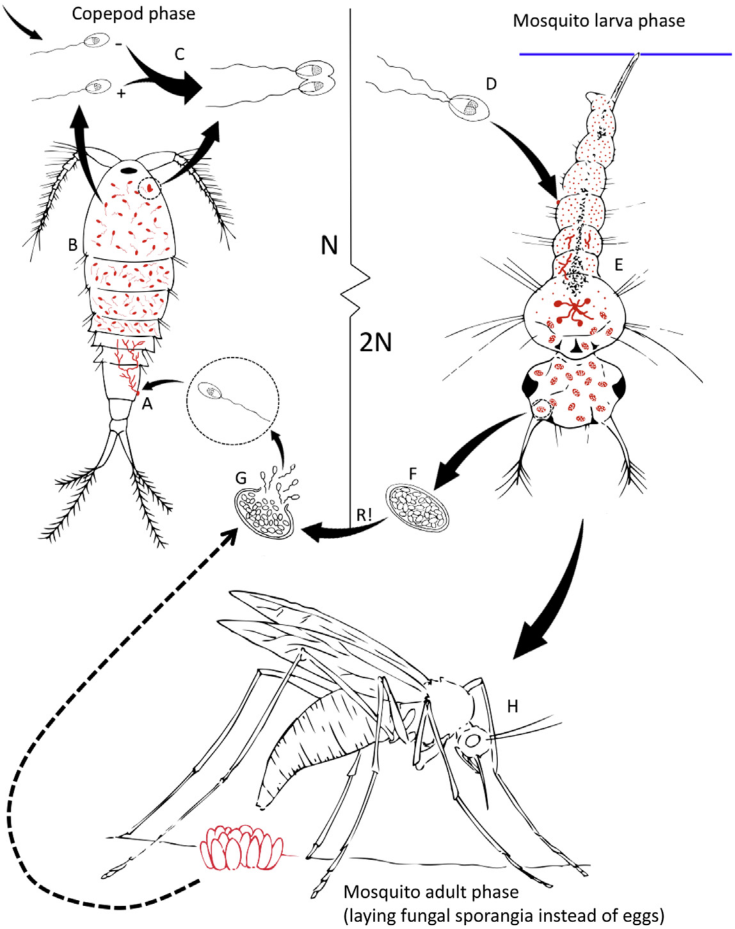


Figure 9 ***Chytridiomycota*** **Coelomomyces psophorae.** (A) Zoospores attach and penetrate the copepod cuticle; (B) Development of the gametophytic phase and dispersion of gametes into the environment; (C) Fusion of compatible gametes, inside the copepod or in the environment (plasmogamy); (D) Formation of zygote (kariogamy = 2n) and attachment to the cuticle of the mosquito larva; (E) Colonization and development of the sporophytic phase and formation of sporangium; (F) Resting sporangium released into the environment after the larva's death; (G) Meiosis and release of asexual zoospores; (H) If the larvae reach the adult stage, the fungus will migrate to the ovaries. Instead of laying eggs, the mosquito will lay fungal sporangia.

each group, but to present the diversity of morphologies and strategies to infect their hosts, we will provide a broad overview of entomopathogenic species among Entomophthoromycota, presenting some representative examples of their diversity.

The entomophthoroid fungi are well known as insect pathogens. This group attacks mainly adult insects, although two species of *Entomophthora* (*Entomophthora aquatic*a and *Entomophthora conglomerata*) and *Erynia aquatica* are known to infect aquatic larval stages of mosquitoes (Scholte et al., 2004). Transmission within entomophthoroid fungi is via forcibly discharged spores into the environment, with the exception of one single genus (*Massospora*) that releases the spores passively, with the host still alive (Humber, 1981; Thaxter, 1888) (Fig. 10). In addition to *Massospora*, other groups like *Strongwellsea* and certain species of *Entomophthora*, *Erynia*, and *Entomophaga* (in addition, to the Ascomycete *Lecanicillium longisporum*) produce spores before the host death, in or on their living bodies (Roy et al., 2006). These fungi are characteristically biotrophics, consuming the host when they are still alive with no somatic growth after its death. This is one of the major differences when compared with hypocrealean fungi (discussed further in the Ascomycota section), which are all hemibiotrophic,

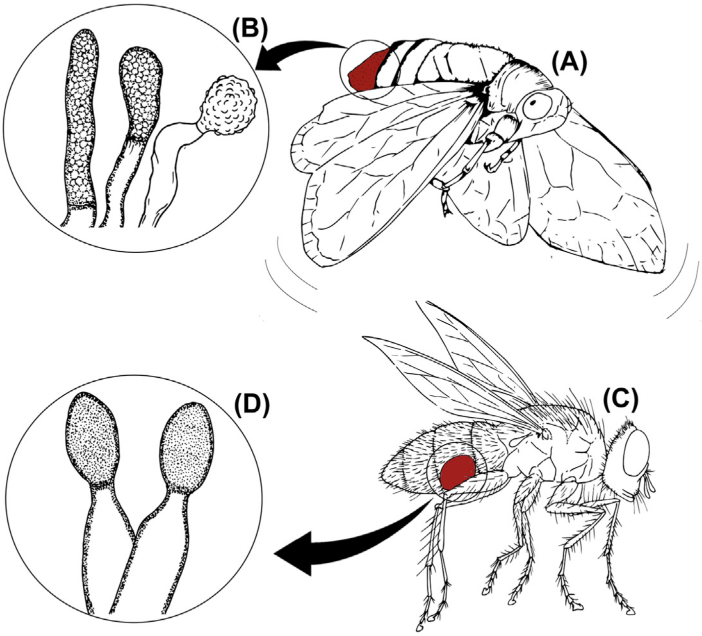


Figure 10 ***Entomophthoromycota*—Massospora cicadina *(A and B),* Strongwellsea castrans *(C and D).*** (A) A living cicada flying and dispersing spores while its body disintegrates due to fungal activity. (B) Spore-producing cells (Conidiophores) in different stages of development. (C) Fly exhibiting a hole on the abdomen caused by the fungal infection. (D) Conidiophores exhibiting a terminal spore.

switching from a biotrophic phase (parasitism) to a saprophytic phase, growing on or in the host's body, even after its death (Roy et al., 2006).

The infections caused by *Strongwellsea castrans* in *Hylemya brassicae* and *Hylemya platura* (Diptera) are classic examples of these peculiar situations where the sporulation occurs while the host is still alive (Nair & McEwen, 1973) (Fig. 10C and D). In this case, the infected fly is characterized by the presence of a large circular hole on the lateral side of the abdomen. However, surprisingly the infected insects can be observed acting normally, despite the big hole in its body, filled with fungal tissue and conidiophores (spore-producing cells). Both, males and females were described infected by *S. castrans*, causing castration and premature death (Nair & McEwen, 1973). Another similar case occurs with *Massospora cicadina*, which attack cicadas (Fig. 10A and B). This fungus also initiates sporulation when the host is still alive (Goldstein, 1929; Speare, 1921). Due to the pressure caused by the swelling mass of fungus, the collapse of its whole abdomen is inevitable, exposing the fungal tissue. Since the fungus maintains its growth inside the insect, over time the abdomen falls apart until just the head and thorax of the living insect remain (Speare, 1921). The ability to fly is retained increasing dispersion of spores in the environment.

Although Mucoromycotina is the largest and morphologically most diverse group of "zygomycetes," the subphylum has just one single entomopathogenic species, *S. umbellata* found attacking the hemipteran genus *Umbonia* in Ecuador (Evans & Samson, 1977; Samson et al., 1988) and the lepidopteran genus *Acraea* in Taiwan (Chien & Hwang, 1997).

4.1.5 Basidiomycota

Although the phylum exhibits great diversity of species—over 1500 genera and 31,000 species described (Kirk et al., 2008); just three genera are known to infect insects. Those are (1) *Fibularhizoctonia* spp. (an undescribed species, see Yashiro and Matsuura (2007)) infecting termite eggs, (2) *Uredinella* (two species, *Uredinella coccidiophaga* and *Uredinella spinulosa*) infecting scale insects, and (3) *Septobasidium* (c. 240 species attacking scale insects, Hemiptera).

The order Septobasidiales Couch (*Uredinella* and *Septobasidium*) exhibits a peculiar and complex relationship with their hosts, the Diaspididae (Hemiptera). Diaspididae are small, sedentary phythophagous insects, which spend their whole lives in one spot on a plant, a consequence of their sucking mouthpart structure (Grimaldi & Engel, 2005). To protect themselves, since they are not able to fly away from enemies (Heimpel & Rosenheim, 1995) and do not survive unprotected, juveniles start to secrete fine threads of white wax, which within the first 24 h after their hatching will form a complete covering over the insect's body (Couch, 1938).

This waxy protection is fragile but does afford some degree of defense; however, they are still exposed to external factors. An additional defense structure can be provided when a colony of such plant-feeding insects are infected by the fungus *Septobasidium*. The fungus can grow up to 20 cm and creates an elaborate system of tunnels and chambers inside its "body," which provide the Diaspididae with life-long protection (Couch, 1938). However, not all insects are protected as this fungus infects some members of the colony often causing dwarfism and castration. The atrophy is due to penetrant haustoria that drain plant sap and nutrients from the insect's body, resulting in undernourishment (Couch, 1931). Even uninfected adult insects are surrounded and held by hyphal threads, and so are unable to escape: providing an example of a fungus farming an insect (Couch, 1931, 1938). Juveniles (crawlers) may become infected as they attempt to move out of the parental chamber to establish a new colony (Couch, 1938).

With respect to the less speciose genus among Septobasidiales, *Uredinella*, there are only two described species: *U. coccidiophaga* and *U. spinulosa* (see Couch (1937, 1941)). They can be divided based on spore shape and the substratum in which they infect insects; leaf and trunk for *U. spinulosa* and just trunk for *U. coccidiophaga*. As the genus infects the body of single insect (unlike *Septobasidium*), the death of the insect means the death of the fungus also. Spores are produced in the spring and reach 0.2–1.5 mm in diameter (Couch, 1937). In contrast, *Septobasidium* exhibits an undefined lifetime, since its body is "renewed" each season, by the infection of the newborn crawlers.

Another case of Basidiomycota parasitic on insects can be found between *Fibularhizoctonia* spp. and some species of the subterranean termites *Reticulitermes*. These termite workers keep their eggs inside their nest in piles, taking care of them. Matsuura et al. (2000) found among these piles, some sclerotia, globose fungal structures, being cared for by the workers, as if they were eggs. The same study found that these sclerotia mimic the egg diameter and texture and because these traits are similar to those of the termite eggs themselves the worker termites mistake the sclerotium for a true egg and care for it. In nature it is rare to observe the fungus consuming the termite eggs, but there is the suggestion that the fungus becomes pathogenic and grows over the true termite eggs if the termites stop caring for the fungi (Matsuura, 2006).

4.1.6 Ascomycota

As mentioned, this diverse phylum comprises many entomopathogenic fungi: from the less speciose orders Pleosporales, Myriangiales, Ascosphaerales to hyperdiverse groups within the relatively well-known order Hypocreales. In each case the insect dies before the fungus begins its reproductive phase. Here, we describe each of these groups and their main characteristics.

Within the order Pleosporales, the entomopathogenic species belong to the genus *Podonectria* (Petch, 1921) that shares the unusual aspects of this group, such as bright coloration and fleshiness. All known species have been found infecting scale insects, covering the whole surface of the insects body with a cotton-like crust on which the perithecia is produced and later, multiseptated spores that do not disarticulate into part-spores (Kobayasi & Shimizu, 1977). The related anamorphs are the genus *Tetracrium* (Kobayasi & Shimizu, 1977; Petch, 1921) and the genus *Tetranacrium* (Roberts & Humber, 1981).

The Myriangiales includes a number of species associated with plants, resins, or scale insects on plants (Alexopoulos et al., 1996). The entomopathogenic species exhibit perennial growth for several years or at least until the scale-infested branch dies, probably because of decreased nourishment. The dead host insects can be found directly under each stroma, penetrated and covered by mycelium (Miller, 1938). The stroma are sometimes formed at the side of the insect (Petch, 1924). Growth commences when the rains begin with the fungus increasing in diameter, producing ascomata and then later, ascospores. Reproduction is entirely by ascospores, and no evidence of conidial (asexual spore) formation was found on stroma of any age or in culture (Miller, 1938). For taxonomic and additional discussions, see Miller (1938) and Petch (1924).

The order Ascosphaerales contains a unique group of bee pathogens within the genus *Ascosphaera*, which has approximately 30 species. These parasites are specialists that exploit the provisions of bees. Most species are exclusive saprophytes on honey, cocoons, larval feces, or nest materials such as leaf, mud, or wax of bees (Wynns, Jensen, Eilenberg, & James, 2012). However, some species are known as widespread fungal disease agents, attacking the brood of numerous species of solitary and social bees, causing a disease called "chalk-brood" (Klinger, James, Youssef, & Welker, 2013). The infection occurs when the larva ingests fungal spores. The fungus grows as hyphae within the body before killing the host and then developing spores on the cuticle of the dead larvae (Vojvodic, Boomsma, Eilenberg, &

Jensen, 2012). The morphology of *Ascosphaera* is very peculiar when compared to other fungal groups. The ascoma is a small brown to blackish brown spore cyst, which is a single enlarged cell containing ascospores (Wynns et al., 2012). Their spores also exhibit a curious similarity in appearance to pollen grains. For a detailed life cycle, see McManus and Youssef (1984).

The Hypocreales fungi encompass important genera of entomopathogenic fungi such as *Cordyceps, Tolypocladium, Hypocrella*, *Ophiocordyceps*, *Moelleriella*, *Samuelsia*, and *Torrubiella*. In addition to these species, there are many anamorphic species related to them, ie, *Hirsutella*, *Metarhizium*, *Hymenostilbe*, *Akanthomyces*, and many others (Roberts & Humber, 1981). In the past, these anamorphic species (which only produce asexual spores) were treated traditionally as a separate group within the now retired phylum Deuteromycota. However, with molecular techniques some of these species are now strongly supported or proven to be asexual stages of Ascomycota (Liu et al., 2001). Here we will address just the teleomorphic (ie, sexual stage) names since our goal is to provide the reader an overview of entomopathogenic groups and to avoid confusion between the same species, in which both teleomorphic and anamorphic (ie, asexual stage) phases are known.

Within the largest group of entomopathogens, the Hypocreales, we can highlight some genera that are notable due to their diversity and abundance in tropical forests worldwide. For example, *Hypocrella* (*Archersonia* is the anamorphic state) that was beautifully monographed by Chaverri et al. (2008). These fungi are known to infect whiteflies and scale insects in tropical forests, with few species recorded in the subtropics. They can cause epizootics in their host's population (but epizootics are by no means confined to this genus).

The genus *Ophiocordyceps*—especially species attacking ants—are known to cause huge infestations in small areas, called graveyards (Evans & Samson, 1982, 1984; Pontoppidan, Himaman, Hywel-Jones, Boomsma, & Hughes, 2009). Indeed, one of the most fascinating phenomena regarding entomopathogenic fungi is the zombie-ant behavior caused by *Ophiocordyceps unilateralis s.l.* (Andersen et al., 2009; Hughes et al., 2011). This species was originally described by Tulasne and Tulasne (1865) as *Torrubia unilateralis*. Species within this complex adaptively manipulate the behavior of worker ants, causing the insect to leave the colony to find an optimum microclimate site, which is required by the fungus to grow and produce ascospores (Fig. 11D). The ants die biting firmly on the underside or edge of a leaf, twig, branch, etc. (the death position is related to each species or group of species of the fungus).

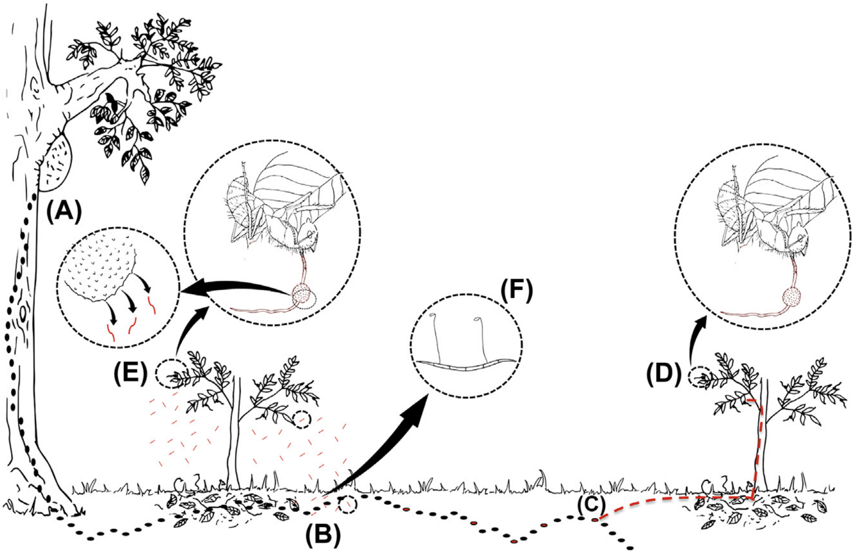


Figure 11 ***Ascomycota*—Ophiocordyceps unilateralis *s.l.*** (A) Ants leave the nest to forage on the forest floor; (B) Eventually they get infected with *Ophiocordyceps* ascospores that were previously shot on the forest floor; (C and D) About 10 days after infection (depending on the species and the geographical location) the infected ant leaves the nest to die on an elevated position, biting the edge or the main vein of a leaf. The fungus places the ant on a precise location, which is optimal for fungal development and further dispersion of the spores; (E) Two to eight weeks after the ant's death, depending on the weather conditions, the fungus starts to produce spores and shoot them into the environment; (F) From 24 to 72 h after being shot, the spores will germinate and form a secondary spore, the capilliconidiospore.

Following the ant's death, the fungus grows a fruiting body from the back of the ant's head, which will spread the ascospores on the forest floor (Fig. 11E).

5. DISCUSSION

5.1 Factors Promoting Diversity Within Entomopathogenic Fungi and Oomycetes

5.1.1 Hemipterans as a Host Group Promoting Hyperdiversity of Entomopathogens

The host is the ecological niche for the fungus. Some ecological niches, ie, host groups, are notable because the abundance and diversity of entomopathogenic species infecting them are very high. For instance, the broad diversity found among entomopathogenic fungal species attacking sap-sucking

Hemiptera. Based on teleomorphic species (ie, species identified by their sexual stage) within Hypocreales, which is the most diverse group of entomopathogens in Ascomycota, we found 180 species of fungi exclusively parasitizing hemipteran insects (Fig. 3 and Supplementary table 5). Most cases are infections on adult stages (n = 165 records). The *Hypocrella*–*Aschersonia* species are responsible for the majority of infections, with 92 species infecting scale insects (Coccidae and Lecaniidae, Hemiptera) and whiteflies (Aleyrodidae, Hemiptera) (*Aschersonia* is the anamorphic stage and some species have no described teleomorphic stage). The other Hypocreales species attacking hemipterans are spread among the six other genera: *Moelleriella* (25 species), *Ophiocordyceps* (19 species), *Torrubiella* (18 species), *Cordyceps* (17 species), *Samuelsia* (6 species), and *Regiocrella* (2 species). These are all from the order Hypocreales. Other orders such as Myriangiales (3 species: *Myriangium asterinosporum*, *Myriangium curtisii*, and *Myriangium duriaei*, Ascomycota), Septobasidiales (approximately 240 species, Basidiomycota) and even the chytrid fungi *M. ucrainicus* (Chytridiomycota) in the order Chytridiales are known to infect exclusively hemipteran sap-sucking insects. No other group of insects is attacked either by so many different groups of fungi or so many distinct species of entomopathogens.

Why are hemipterans such common hosts? According to Spatafora, Sung, Sung, Hywel-Jones, and White (2007) the order Hypocreales evolved from an ancestor with a plant-based nutritional mode. They made a horizontal host-jump from plants to the insects feeding on plants. There are estimated to have been around five to eight interkingdom host-jumping events between Plant, Animal and Fungi within Hypocreales (Spatafora et al., 2007). In general terms the shifts between different host groups is suggested to be important for expanding the host range of the Hypocreales (Kepler et al., 2012; Nikoh & Fukatsu, 2000). But how did this happen?

Although the insects arose in the Devonian (earliest fossils from around 407 mya) their major diversification occurred after the seed plant (spermatophytes) radiation (Permian, 299–251 mya), when most of the modern orders of insects emerged, including small basal groups of Hemiptera (Grimaldi & Engel, 2005). Afterward, a new episode transformed all the ecosystems on Earth as the flowering plants (angiosperms) evolved and radiated (Lower Cretaceous, 130 mya) along with the diversification of insects (Gaunt & Miles, 2002; Grimaldi & Engel, 2005). A result of the codiversification of insects and flowering plants was the expansion of the different insect mouthparts (Labandeira, 1997). In the case of the hemipterans, the mouthparts evolved into two pairs of long and fine stylets, which are able to create strong suction in order to draw fluids from plant tissues (Grimaldi & Engel, 2005). This derived feature was essential for this group of insects, since it allowed them to exploit a new niche: living on the plants and feeding from their sap.

On the other hand, in the same way that hemipterans adapted their mouthparts into stylets, the fungi, in order to exploit another food source, switched from plant-based to insect-based ecology about 170–150 mya. This event would have been facilitated by the ecological proximity between hemipterans and a Hypocreales ancestors; one feeding from plant exudates, the other living inside the plant as endophytes, at least 190 mya (Sung, Poinar, & Spatafora, 2008). After the fungi adapted to their new ecological niche in insects they necessarily would have had to optimize horizontal transmission between insects. For instance, the hyperdiverse *Hypocrella–Aschersonia* group exhibit mitotic slime spores, adapted for short distance dispersal by rain-splash on leaf surfaces, which are a hot spot to find their hosts (Chaverri et al., 2008; Evans, 1989).

Afterward, the transition from growing within plants to infecting sap sucking insects would have provided a route to infect other insects that were not phytophagous. As a result, all these changes have given rise to three of the most important entomopathogenic families Clavicipitaceae (eg, *Hypocrella*, *Moelleriella*, *Samuelsia, Metacordyceps*), Cordycipitaceae (eg, *Cordyceps*, *Torrubiella*), and Ophiocordycipitaceae (*Ophiocordyceps, Tolypocladium*, formerly *Elaphocordyceps* (Quandt et al., 2014), *Polycephalomyces*) (Kepler et al., 2013; Sung et al., 2007, 2008). Those families have undoubtedly achieved great success with this host shifting from plant-based ecology to other groups, as illustrated by their species richness, ecological abundance, and worldwide distribution, especially in tropical forests.

5.1.2 Broad Range of Ecologies, High Diversity of Pathogens

Another interesting case of unusual diversity among fungal–insect infections can be found within the insect order Diptera (flies). Although the number of species is comparatively low (Fig. 3 and Supplementary table 5) it is notable that flies are the only order of insect with records of entomopathogens infecting all stages of development: eggs, larvae, pupae, and adults. Only the phylum—Basidiomycota—of the six phyla we recorded infecting insects do not infect dipterans. Why are flies such magnets for fungal infections? One reason is probably because they are the most ecologically diverse group of insects, found all over the world, occupying a broad range of niches: blood feeders, endoparasites and ectoparasites of vertebrates and invertebrates, gall makers, larval and adult predators, leaf miners, parasitoids, pollinators, saprophages, and wood borers (Grimaldi & Engel, 2005). In addition, their larvae can be found in many different breeding sites such as aquatic, semiaquatic (wet soils, stones on stream edges), or terrestrial (mushrooms, rotten wood, trunk). We suggest that by occupying such diverse niches, the flies have increased the opportunity for infection by fungal and oomycete pathogens, which like the insects, occupy diverse environments. It is still notable, however, that while flies are infected by five of the six different groups of entomopathogens and all developmental stages of flies are attacked, the overall species diversity of entomopathogens attacking them is still low given that flies are a very specious order (over 150,000 species). Flies would appear to be magnets for entomopathogens, but they are not cradles of diversity in the same way hemipterans have apparently been.

5.1.3 Susceptibility of Lepidoptera and Coleoptera Larval Stages to Fungal Infections

Beyond the insect orders discussed earlier (Hemiptera and Diptera), it is worthwhile to mention the prevalence of infections in larval stages of Lepidoptera and Coleoptera (Fig. 3) by hypocrealean fungi (Ascomycota). The larvae of both insect orders are the preferred host for two of the most diverse and ecologically abundant genera of entomopathogenic fungi, *Cordyceps* and *Ophiocordyceps* (Supplementary table 1). We found 80 and 87 species of teleomorphic Hypocreales infecting Lepidoptera and Coleoptera larvae, respectively. In contrast, we found just 10 and 11 records of teleomorphic Hypocreales species attacking adults of Lepidoptera and Coleoptera, respectively (Fig. 3). What factors promote such a predominance of infections on larval stages?

A number of biological traits that are different between the larvae and adults may be crucial to understanding this pattern: (1) partition of niches, (2) predictability in time–space scales, (3) feeding rate, and (4) protection (cuticle). (1) As holometabolous insects (which exhibit complete metamorphosis), the larval and adult stages are ecologically separated, occupying completely different microenvironments, thus avoiding competition between juveniles and adults (Gullan & Cranston, 2009). (2) Both coleopteran and lepidopteran larvae generally exhibit modest mobility compared to the wandering adults, tending to be closer to the breeding site and eating ferociously, hence being more predictable in time–space scales. (3) The larva needs to eat massive amounts of food and store that food, in order to grow as quickly as possible, making them a huge reservoir of energy. (4) Furthermore, larvae need to grow at a high rate and this would be impossible if they had the hard exoskeleton that adult coleopterans have. However, on the other hand, having such soft and thin skin would make these organisms much easier to be invaded by fungal spores equipped with their enzymatic and physical tools for infection. It is important to emphasize that the usual defenses that larvae exhibit—mimicry, aposematism, gregarious behavior, stinging hairs—that are very useful against predators are completely useless against the effective entomopathogenic fungi. These four ecological traits that distinguish larvae and adults from each other may explain why entomopathogenic fungi exhibit such a greater prevalence for infecting larvae rather than adults.

The other major holometabolous order infected by Hypocreales is Hymenoptera (wasps, bees, termites, and ants). Here, however, most infections are of the adult stages. There are few records ($n = 47$) so it is harder to contrast with Lepidoptera and Coleoptera. But it is noticeable that the hymenopterans build nests for their larvae, and in the case of ants, some wasps, and some bees these larvae are nursed and cleaned by their siblings, which is known to reduce fungal infections (Cremer, Armitage, & Schmid-Hempel, 2007).

6. CONCLUSION

This is the first time that an extensive review encompassing all entomopathogenic fungal phyla and oomycetes explored entomopathogenic fungi with a fungal–host approach. Despite the importance of insect–fungal associations, they have been overlooked and their diversity is poorly studied. The lack of interaction between mycologists and entomologists might play an important role in this gap of knowledge, and efforts to address this issue are crucial to better understand the parasitic relationship between insects and the multiple lineages of entomopathogenic fungi.

Fungi that are able to infect insects are not just comprised by a single monophyletic group. Different groups have arisen independently and repeatedly in many different lineages through fungal evolution (Humber, 2008). As presented here, they are spread from more basal to more complex Dikaria members. The basal groups, such as aquatic chytrids, infect mostly Diptera, while Microsporidia and Entomophthoromycota infect a wide range of hosts. Basidiomycota infects mostly Hemiptera, while Ascomycota, the most speciose group, infects a vast number of insect groups.

Insect pathologists, entomologists, and life scientists in general have traditionally seen entomopathogenic fungi as having a single role: to kill insect pests (Vega, 2008). But the coevolution of fungi and insects across hundreds of millions of years has resulted in a wide range of complex and intricate interactions. The purpose of this work is to provide a wide overview of these relationships by focusing on the impressive diversity of morphologies, ecologies, and interactions between insects and fungi. Our work also highlights the ways that biological and ecological aspects of the hosts likely played an important role to explain why and how some groups of insects are more susceptible to fungal infection than others.

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SUPPLEMENTARY DATA

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