

Annual Review of Entomology
**Laboulbeniomyces:
 Intimate Fungal
 Associates of Arthropods**

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Annu. Rev. Entomol. 2021. 66:257–76

First published as a Review in Advance on
 August 31, 2020

The *Annual Review of Entomology* is online at
ento.annualreviews.org

<https://doi.org/10.1146/annurev-ento-013020-013553>

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Keywords

biotrophs, fungal life history, Herpomycetales, insect dispersal, Laboulbeniales, Pyxidiophorales

Abstract

Arthropod–fungus interactions involving the Laboulbeniomyces have been pondered for several hundred years. Early studies of Laboulbeniomyces faced several uncertainties. Were they parasitic worms, red algal relatives, or fungi? If they were fungi, to which group did they belong? What was the nature of their interactions with their arthropod hosts? The historical misperceptions resulted from the extraordinary morphological features of these oddly constructed ectoparasitic fungi. More recently, molecular phylogenetic studies, in combination with a better understanding of life histories, have clearly placed these fungi among filamentous Ascomycota (subphylum Pezizomycotina). Species discovery and research on the classification of the group continue today as arthropods, and especially insects, are routinely collected and examined for the presence of Laboulbeniomyces. Newly armed with molecular methods, mycologists are poised

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to use Laboulbeniomyces–insect associations as models for the study of a variety of basic evolutionary and ecological questions involving host–parasite relationships, modes of nutrient intake, population biology, host specificity, biological control, and invasion biology. Collaboration between mycologists and entomologists is essential to successfully advance knowledge of Laboulbeniomyces and their intimate association with their hosts.

1. INTIMATE HABITATS

In this review, we examine the coexistence of Fungi and arthropods that have shared habitats for more than 300 million years (117). In this close proximity, parasitism evolved. In his book *Evolutionary Biology of Parasites*, Price (80, p. 3) wrote, “the most extraordinary adaptive radiations on the earth have been among parasitic organisms.” Windsor (133) argued that, instead of being considered a threat for conservation, parasites should have “equal rights,” initiating a shift in thinking to see them as an integral part of the earth’s biodiversity and recognize these often-hidden organisms as important to conservation (17). Indeed, interactions among trophic levels may be as important a driver of speciation as is competition among organisms within the same trophic level (125). The study of parasites, pathogens, and parasitoids together with their hosts in natural populations provides insight into the factors affecting historical biogeography and community structure. Studies of parasites of vertebrate and invertebrate fauna add new data regarding ecological interactions, patterns of distribution, and complex coevolutionary histories (62). Human transport of organisms and the current anthropogenic changes to the earth’s biosphere, atmosphere, and climate facilitate invasions, thus redistributing hosts and parasites, causing diseases of native hosts, and creating opportunities for host shifts and subsequent population divergences (35, 36, 79, 95). These circumstances provide an unprecedented challenge to biologists’ attempts to unravel the history of host–parasite interactions and relationships. Manipulation of these systems in the laboratory can help in this endeavor.

The number of extant fungi in general is vastly underestimated, and that of fungal parasites is even more so. With regard to insect-associated fungi, it is estimated that a meager 1.5% have been described (75). Since the publication of *Nova Plantarum Genera* by Pier Antonio Micheli in 1737, marking the start of modern mycology, researchers have described approximately 135,000 species of fungi (61). This is in stark contrast to the estimated total number of species: between 2.2–3.8 million (59) and 6 million (116). This disjunction between the known and the unknown in fungi is comparable to that between the known and unknown species of arthropods. Such gaps in knowledge of diversity often lead to problems in the interpretation of deep evolutionary relationships (14, 65) and biogeographical patterns (38). Knowledge gaps are especially evident for largely neglected groups, such as the large number of insect parasites in the Microsporidia—only recently classified as Fungi (10).

Our interest is in the Laboulbeniomyces, another group of incompletely known fungi that are obligate associates of arthropods as biotrophs or for dispersal. Study of these fungi has been difficult because they are microscopic, their infection prevalence is generally low, and they cannot be cultured in the laboratory without living hosts. In this review, we outline the current understanding of the group; discuss the historical context in which their study developed; and provide insight into evolutionary relationships, nutrition, and host relationships as we now understand them based on fungal and arthropod biology.

Fungal parasite: fungus living at the expense of another organism, usually for nutrition; fungi in the thallus-forming Laboulbeniomyces are biotrophic parasites

Biotrophs: parasites that use living hosts for nutrition, in contrast to necrotrophs, which kill their hosts (e.g., *Entomophthora*, *Ophiocordyceps*)

2. LABOULBENIOMYCETES: THREE ORDERS AND SEVERAL OUTLIERS

Within the class Laboulbeniomyces, three orders have been described based on molecular data, life history, and morphology. Life history studies of the order Pyxidiophorales, which, among the three orders, is considered the most similar to other mycelium-forming ascomycetes, helped to develop a hypothesis of the evolution within the class from phoretic behavior to parasitic nutrition (13; but see 14). The best known genus in the Pyxidiophorales is *Pyxidiophora*. These fungi are contact mycoparasites feeding on fungal hyphae and tissues that grow in ephemeral substrates such as herbivore dung, decaying plants, and seaweed. The elongated meiospores of *Pyxidiophora* spp., equipped with an elaborate, sticky attachment structure at one end, are produced in a partially enclosed structure, the perithecium, in one such ephemeral substrate. The meiospores exit the perithecium, sticky end first, and attach to passing arthropods—often phoretic mites or insects directly. En route to an ephemeral substrate, the meiospores develop into distinct mitospore-producing forms. Once on the new substrate, the mitospores germinate and, in the vicinity of a suitable fungal host, produce hyphae, the elongated tubular filaments that spread to form a mycelium. The hyphae of *Pyxidiophora* and its host fungus are held in contact by pores, through which exchange of cytoplasm occurs. A third kind of spore, a second type of mitospore, is produced on the mycelium, and this spore type enhances local spread on the substrate. Study of the life history of *Pyxidiophora* spp. provided important clues to the link between these species and Laboulbeniales, and DNA analyses debunked earlier theories regarding the relationships of the Laboulbeniomyces that considered them to be derived from red algae or even related to animals. This scenario is discussed in detail elsewhere (14, 16).

The other two orders of the Laboulbeniomyces (Laboulbeniales and Herpomycetales) never form hyphae, and their entire life cycle occurs on an arthropod host, with meiospores dispersed to new arthropod hosts by direct contact or occasionally from a spore that survives a short time in the environment (25). The order Laboulbeniales (**Figure 1**) consists of obligate, microscopic ectoparasites of arthropods. Of the described species, 80% are known from beetles (Coleoptera), followed by 10% from flies (Diptera) (130). Around 2,200 species in 142 genera (81) are known; they can be found on all continents except Antarctica (100, 129, 130). Fungal growth on an arthropod host is observed as a thallus or multiple thalli on various body parts. A thallus consists of up to several thousand vegetative cells developed from the two-celled meiospore by mitotic division in three dimensions. The taxonomy and current classification of the group is based on the arrangement of cells of the thallus, and some of these arrangements seem to be phylogenetically informative (41, 44). There is no asexual reproduction known among Laboulbeniales, another unusual feature among these ascomycetes.

The third order, the recently described Herpomycetales (**Figure 1**), includes a single genus, *Herpomyces* (52). Its 27 known species are found only on cockroaches (Blattodea) (43). Recent molecular phylogenetic analysis supports the early divergence of the order within the class (14), and morphological, developmental, and host preferences confirm its separation from Laboulbeniales (52, 114, 115, 129).

In addition to the three orders Pyxidiophorales, Laboulbeniales, and Herpomycetales, several taxa of ambiguous taxonomic position have now been shown to belong in the Laboulbeniomyces based on sequence analysis: *Gliocephalis* (64), *Laboulbeniopsis* (60), *Chantransiopsis*, *Tetrameronycha* (41), and *Subbaromyces* (14). Some of these taxa are known to only reproduce asexually. One evolutionary model (15) suggests that shifts in reproductive strategies (meiosporic versus mitosporic) associated with arthropod dispersal have led to host shifts and given rise to phylogenetically and morphologically unique fungal lineages.

Hyphae: individual tubular filaments that absorb nutrients from the substrate; collectively, all hyphae of a fungus body are known as mycelium

Perithecium: an enclosed ascomycete structure with a single opening within which asci develop

State: condition in the life cycle of a fungus, typically defined by the type of nuclear division (meiosporic or mitosporic)

Thallus: a three-dimensional multicellular structure of up to several thousand cells, developed from two-celled meiospores by mitotic division in several planes; different from hyphal growth

Meiosporic: refers to the part of the fungal life cycle in which meiosis occurs and ascospores develop

Mitosporic: refers to the part of the fungal life cycle in which mitosis occurs and conidia may develop



(Caption appears on following page)

Figure 1 (Figure appears on preceding page)

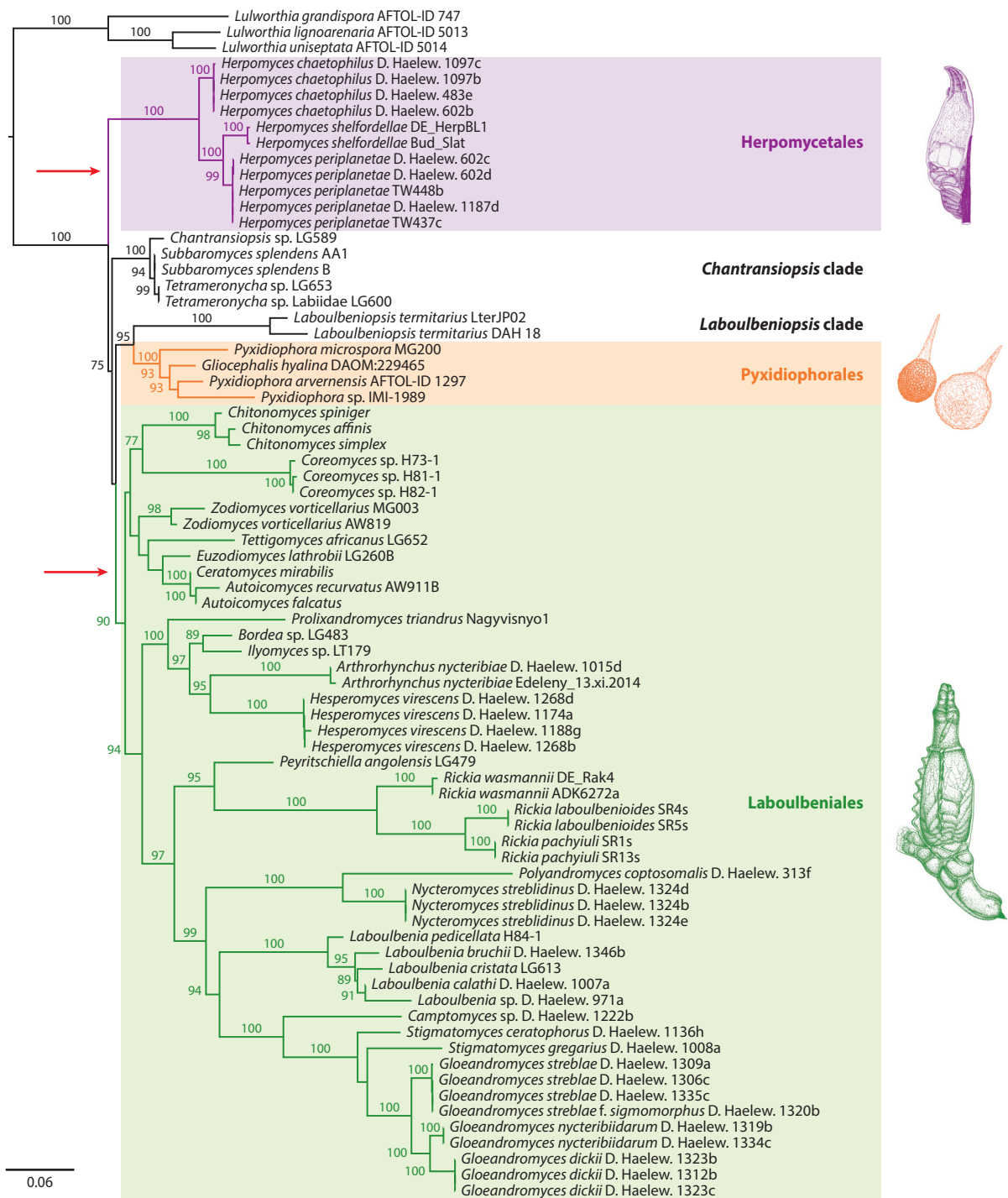
Laboulbeniomycetes fungi, (a,b) Herpomycetales and (c–l) Laboulbeniales. (a,b) Two species of *Herpomyces* growing on the same individual of a *Periplaneta americana* cockroach, (a) *Herpomyces periplanetae* on the antenna and (b) *Herpomyces chaetophilus* on the hairs of a mouthpart. (c) *Laboulbenia calathi* thalli on a leg of *Calathus melanocephalus*. Photos in panels a–c courtesy of André De Kesel. (d) *Laboulbenia femica* on a *Gyrinus marinus*; two individual thalli are annotated with arrowheads. Photo courtesy of Bart Horvers. (e) A dense population of *Arthrorynchus nycteribiae* thalli on the abdomen of a *Penicillidia conspicua* bat fly, collected from a *Myotis daubentonii* bat. Photo courtesy of Walter P. Pfliegler. (f) A worker of *Myrmica sabuleti* infected with *Rickia wasmannii* on all body parts; thalli are whitish-yellow, similar in size to the setae on the cuticle. Photo courtesy of Theodoor Heijerman. (g) *Laboulbenia separata* on *Pericalus guttatus*. Photo from the original illustrations by Roland Thaxter, courtesy of the Farlow Reference Library of Cryptogamic Botany, Harvard University. (h) A thallus of *Gloeandromyces nycteribiidarum* from a *Trichobius yunkerii* bat fly, collected from a *Pteronotus parnellii* bat; the arrow points to the filamentous haustorium, which has become tightly coiled during mounting, rather than a simple foot. (i) *Neobaplomyces medonalis* from a Paederinae sp.; the arrowhead shows the compound antheridium with antheridial cells discharging spermatia into a common chamber. Panels b and i adapted with permission from Reference 55, figure 1. (j) Detail of a *Laboulbenia disenoebi* thallus, removed from the elytron of *Platynus purpurellus*; the arrow denotes two cells each carrying 2–3 simple antheridia. (k) A thallus of *Teratomyces* sp. 1 with elaborate appendages, from an unidentified *Heterothops* collected in Panama. (l) One of the largest and strangest species of Laboulbeniales, *Zodiomyces rhizophorus*, from the legs of a hydrophilid beetle; the base of the thallus is a many-celled thallus of pseudoparenchyma bearing up to 30 lateral outgrowths with unknown function. Photos in panels j–l courtesy of Danny Haelewaters and the Farlow Herbarium, Harvard University.

The evolutionary relationships among clades of the class Laboulbeniomycetes, based on the latest work (14), are summarized in **Figure 2**. We focus on the order Laboulbeniales, which has undergone a remarkable radiation—perhaps driven by the diversification of its primary host clade, the beetles (Coleoptera). The colloquial name of the order, beetle hangers, is derived from the principal hosts (21).

3. WORMS, RED ALGAE, OR ASCOMYCETES: FOUNDATIONAL STUDIES

Species of Laboulbeniales and Herpomycetales—collectively referred to as thallus-forming Laboulbeniomycetes—are obligate biotrophic parasites of arthropods. They are associated primarily with beetles and flies (130); other hosts include ants, mites, harvestmen, and millipedes (see sidebar titled Distribution of Hosts of the Phylum Arthropoda Parasitized by Laboulbeniales). The hosts often live in close quarters (e.g., termites, carabid beetles) with overlapping, overwintering generations (e.g., passalid beetles, coccinellids). Usually, only adults become infected; thalli are known to develop on hosts within 2–3 weeks (7, 22, 77). The ectoparasitic thalli of Laboulbeniales and Herpomycetales have been described as bizarre and otherworldly. Their distinctive morphological features and ecology led to taxonomic uncertainty when they were first discovered (14 and references therein). Although these fungi were considered ascomycetes by many mycologists, others placed them among several different phyla. Relying on reproductive traits, several prominent mycologists suggested they evolved directly from Floridean red algae (the Floridean hypothesis) or secondarily from other ascomycetes derived from red algae. One early observer described a bat fly–associated species of Laboulbeniales as a parasitic worm (68). A few earlier workers, such as Robin (84, 85), investigated these unusual organisms, but Roland Thaxter’s (1858–1932) work on what were then called Laboulbeniaceae (at that time including *Herpomyces*) dramatically outshined all earlier and most more recent efforts (118, 120, 122–124) and remains a standard in the field. Thaxter’s intended overview of genus *Laboulbenia*, the largest and eponymous genus of the order (47, 53, 55), was unfinished when he died. Thaxter, an accomplished entomologist, described 103 genera and approximately 1,260 species (8) of these fungi. In the 44 years of his active research on this group, and building on detailed microscopic observations and illustrations in combination with fieldwork, he described, on average, one new species every other week.

Floridean hypothesis: a discredited evolutionary hypothesis, resulting from morphological similarities between red algae of class Florideophyceae and thallus-forming Laboulbeniomycetes



(Caption appears on following page)

Figure 2 (Figure appears on preceding page)

Phylogeny of the Laboulbeniomyces, reconstructed from a two-locus ribosomal DNA data set including 75 taxa (14). Bootstrap values are shown at every node (when ≥ 70). Orders Herpomycetales, Laboulbeniales, and Pyxidiophorales are highlighted in color. Minute mitosporic fungi are placed in two informally named clades, the *Chantransiopsis* clade and the *Laboulbeniopsis* clade. Arrows in red denote two independent origins for thallus formation within the class under this evolutionary hypothesis. Drawings, from top to bottom, are of *Herpomyces chaetophilus*, an undescribed species of *Pyxidiophora*, and *Gloeandromyces hilleri*. Drawings by Jingyu Liu.

The Laboulbeniales were briefly discussed in this journal by Madelin (71) in a survey of many diverse groups of insect-associated fungi. The first major review was a book-length treatment (8) written to accompany the reprint of Thaxter's five-volume monograph. We revisit some topics discussed by Benjamin (8) to provide context, but the emphasis in this review is on work conducted since the late 1980s. During that time frame, major advances enabled the study of these unculturable fungi; these advances included not only the development of molecular methods, especially the polymerase chain reaction (PCR), but also the discovery of outlying taxa that filled in some of the morphological mysteries discussed above.

Phylogenetic advances have led to understanding of the unusual morphological forms and interpretation of how these fungi develop (see **Figure 2**). Continued molecular and field studies have

DISTRIBUTION OF HOSTS OF THE PHYLUM ARTHROPODA PARASITIZED BY LABOULBENIALES

This list is annotated with common names where applicable; the information is updated from Weir & Hammond (130).

- Class Insecta
 - Subclass Pterygota (winged insects)
 - Order Blattodea (cockroaches and termites)
 - Order Coleoptera (beetles)
 - Order Dermaptera (earwigs)
 - Order Diptera (flies)
 - Order Hemiptera (true bugs)
 - Order Hymenoptera
 - Family Formicidae (ants)
 - Order Orthoptera (crickets and allies)
 - Order Psocodea (lice)
 - Order Thysanoptera (thrips)
- Subphylum Chelicerata
 - Class Arachnida
 - Acari (mites)
 - Order Opiliones (harvestmen)
- Subphylum Myriapoda
 - Class Diplopoda (millipedes)
 - Subclass Chilognatha
 - Order Callipodida
 - Order Julida
 - Order Sphaerotheriida
 - Order Spirostreptida

also (a) broadened taxon sampling in phylogenies, (b) suggested relationships at lower taxonomic levels, and (c) tested morphological stability of species. Species-level information is critical to the study of host–parasite interactions. Intriguing questions about host specificity among Laboulbeniales are being answered through DNA analyses. Early molecular phylogenetic studies relied on the small subunit of the nuclear ribosomal RNA gene because of its many copies and the difficulty in priming other regions (13, 128). The development of specific primers and improved methods for amplifying minute quantities of DNA enable species-level resolution based on multiple markers (48, 52). Long-standing questions of species variability and host specificity are intriguing for both mycologists and entomologists. Molecular methods and examination of ascomycete life histories have allowed for acquisition of new data to address these questions. Today, with a firm foundation of higher-level classification and the development of improved molecular methods, more extensive studies on fungus–host relationships involving the Laboulbeniomyces can be undertaken in collaboration with entomologists.

4. ASSOCIATIONS OF LABOULBENIALES WITH THEIR HOSTS

4.1. A Wide Distribution of Arthropod Hosts

The host spectrum of the Laboulbeniales includes three subphyla of Arthropoda (see sidebar titled Distribution of Hosts of the Phylum Arthropoda Parasitized by Laboulbeniales). Approximately 80% of all described Laboulbeniales occur on beetles, followed by flies, true bugs, and earwigs as the most frequent host groups (130). Most Laboulbeniales exhibit some level of host specificity. Some of these fungi are univorous; that is, they occur on one or two congeneric hosts. For example, *Laboulbenia hyalopoda* has only been reported from *Dromius linearis* (Coleoptera, Carabidae) (28, 49), and *Triainomyces hollowayanus* only associates with the pill millipede *Procyliosoma tuberculatum* (Sphaerotheriida, Procyliosomatidae) (see below) (94). Plurivorous taxa, in contrast, occur on phylogenetically distant host species. For example, *Euzodiomyces lathrobii* has been found on beetles in two families, Carabidae (*Patrobus*, *Pterostichus*) and Staphylinidae (*Achenium*, *Homeotarsus*, *Lathrobium*) (11, 29, 92, 131). Scheloske (103) specified main hosts, occasional hosts, and accidental hosts. The occurrence of thalli on occasional and accidental hosts (e.g., 56, 78) may be explained by sharing of niches among the main host species and other arthropods. For example, the army ant *Eciton hamalis* (Hymenoptera, Formicidae) and some of its associates (ant-mimic staphylinids, histerid beetles, and mites) are all hosts of *Laboulbenia ecitonis* (18). A fungus may not persist on these alternative hosts, but accidental transmission probably has played an important role in speciation processes in Laboulbeniales (31, 87).

4.2. Host-Segregated Species Delimitation

One of the most commonly observed species of Laboulbeniales is *Hesperomyces virescens*, which is only found on ladybird hosts (Coleoptera, Coccinellidae). Following the traditional morphological species concept, *H. virescens* is discerned as a single species with near-global distribution occurring on a wide range of ladybird hosts, including approximately 30 species in 20 genera (46). Using DNA data of *Hesperomyces* isolates from nine ladybird hosts and applying sequence-based species delimitation methods, Haelewaters et al. (48) showed that *H. virescens* is a complex of multiple species, which are segregated by host. A morphometric approach considered variation in thallus morphology of different host species; statistical processing indicated that variation in certain structures is important in species delimitation (48). Such integrative taxonomic approaches (33, 34) have been adopted in many groups of fungi (1, 2, 66, 69, 106–108, 110) and were recently discussed in this journal in relation to arthropod studies (105).

Following contributions by Goldmann & Weir (40), Goldmann et al. (42), and Haelewaters et al. (48), more studies of Laboulbeniomyces have used multisource data to delimit species in the genera *Gloeandromyces* (51), *Laboulbenia* (47), and *Rickia* (45) in Laboulbeniales and *Herpomycetes* (43) in Herpomycetales. Not surprisingly, these studies have shown multiple patterns of speciation. For example, two species of *Gloeandromyces* show host specialization that does not lead to speciation (ephemeral speciation model) (86). However, position-induced morphological adaptations of the same phylogenetic species can occur, leading to multiple morphotypes of a single species (51). In addition, in species of *Chitonomyces* (Laboulbeniales), position-specific morphotypes of the same phylogenetic species exist—a result of limited direct ascospore transmission during host mating in aquatic habitats (40). In *Rickia wasmannii*, unlike in *Gloeandromyces* spp. and *H. virescens*, no segregation by host is observed. *Laboulbenia flagellata*, once presumed to be one of the most cosmopolitan species of Laboulbeniales (98), represents yet another species complex. Recent work by Haelewaters et al. (47) found evidence for ecological speciation in *L. flagellata* owing to differential habitat choices of the host species *Limodromus assimilis* and *Loricera pilicornis* (Coleoptera, Carabidae).

4.3. Attachment and Nutrition

Haustoria are penetrating structures that are continuous with the cytoplasm of the basal-most cell of the thallus (often called the foot cell). They have neither nuclei nor septa, range in complexity from simple to elaborately branched, and may function to increase surface area for nutrient uptake (8, 37, 74, 115, 118, 120, 127). Although they have only been observed in some taxa, many authors believe that all Laboulbeniales produce haustoria (8, 103, 132). Haustoria have been observed in many genera: *Arthrorhynchus* (12), *Gloeandromyces* (121, 124) (**Figure 1b**), *Herpomycetes* (82), *Hesperomyces* (67, 127), *Laboulbenia* (90, 119), *Microsomyces* (124), *Rhizomyces* (118, 120), and *Trenomyces* (74). Interestingly, the presence of a haustorium is not a consistent characteristic of all species within some genera; most species in *Gloeandromyces*, *Laboulbenia*, and *Rhizomyces* form a simple foot. The haustorium of *Arthrorhynchus nycteribiae* is branched and extends into the host skeletal muscles (12). Host cells of lice invaded by haustoria of *Trenomyces histophthorus* undergo degenerative ultrastructural changes (74).

Currently, the most widely accepted feeding hypothesis is that Laboulbeniales obtain their nutrition from their host either by absorption via a haustorium in contact with the fluids in the body cavity (haemocoel) or by absorption through the pore canals in the host cuticle (8, 103, 115). Evidence comes from culture attempts that failed to produce mature individuals (132). Additional evidence in support of haustorial feeding was provided by Scheloske (103), who observed that Nile blue sulfate dye injected into the insect flowed from elytral tissues to the attached thalli of a *Laboulbenia* species. Spegazzini (109), however, given the absence of visible damage on the host cuticle, stated that species of Laboulbeniales can take up nutrients from the environment.

Other research (19, 46) has suggested that Laboulbeniales species receive nutrients from the environment by uptake through appendages. In his description of *R. wasmannii* growing on *Myrmica* ants, Cavara (19) contended that the sole purpose of the foot is for attachment, and the sterile appendages or (ephemeral) trichogyne are the absorbing structures of the thallus. Thaxter (120) rejected this claim; he noted that many species of Laboulbeniales lack appendages. Almost 100 years later, the work of Tragust et al. (126) renewed debate on the mode of nutritional uptake. Observations of the host cuticle after removal of thalli using light and electron microscopy, both in situ and on sections of parasitized hosts, revealed the absence of haustorial penetration in four ant-associated species (126). Another suggested source of nutrients might be waxy lipids, produced by the epidermal cells of the host (70); these are not static, but instead move to the

Ascospore:

a spore within a typical sac-like structure (ascus); formed after nuclear fusion (karyogamy), meiosis, and often an additional mitotic division

Trichogyne:

elongated terminal cell that is part of the female reproductive apparatus; site of fusion of spermatium and female cell (plasmogamy)

cuticle surface, presumably because of surface tension. The wax is visible at the pore canals, which provides further support for the idea that species of Laboulbeniales without visible haustoria may take up nutrients by the absorption of waxes through pore canals. It is possible that there are different modes of nutrient acquisition across the Laboulbeniales.

4.4. Position Specificity

Laboulbeniales are unique in that they exhibit different types of specificity. In addition to host specificity, the types are position specificity, sex-of-host specificity, and habitat specificity. Traditionally, researchers had opposing views about naming fungal taxa based on host species, sex of the host, and location on the same host specimen. Some have described species with disparate morphologies and restricted to a certain portion on the host integument (position specificity) or to a given host sex (sex-of-host specificity) (9), whereas others consider these forms as morphotypes of a single species (91, 102). These taxonomic problems are best evaluated by applying an integrative taxonomic approach. Indeed, in recent years, the combination of detailed morphological study with molecular phylogenetic analysis has revealed morphotypes of single phylogenetic species in *Gloeandromyces* (51) and *Hesperomyces* (42).

Thaxter and others reported incidences of several morphologically distinct taxa distinguished by the specific positions that they occupy on the host. For example, Thaxter (118) described 12 such position-specific species of *Chitonomyces* on a single individual of the water beetle *Laccophilus maculosus* (Coleoptera, Dytiscidae). In a study that aimed to resolve the debate regarding position specificity, Goldmann & Weir (40) examined 308 beetles, approximately half of which were infected with at least one species of *Chitonomyces*. Based on ribosomal DNA, thirteen morphotypes of *Chitonomyces* were found to represent five sister pairs and a triplet of phylogenetic species. The corresponding position of thalli on infected male and female beetles—for example, right metatarsal claws in males and right epipleuron in females—strongly suggested that limited spore dispersal during host mating allows for and maintains the distinctive species through reproductive isolation. A study of *Coreomyces* from corixids (Hemiptera) showed correlation among four *Coreomyces* species (referred to as clades) and position on the host, but species and host sex were only weakly correlated, leaving questions about dispersal and maintenance of the associations (113). Sundberg et al. (113) stated that each of the four species is limited to 2–3 positions on the host's integument, with one position preferred over the others. In an extreme example of position specificity, *T. hollowayanus* is restricted to the second pair of legs of mature females of its host, *P. tuberculatum*. The infection is often on the coxae of the second leg, where the genital openings of both male and female millipedes are located; remarkably, though, the fungus has never been found on males (94).

4.5. Habitat Specificity

Although some Laboulbeniales are assumed to have broad host and geographical ranges (but see 48), many species appear to have a single host or a few closely related hosts (host specificity). Few premolecular studies addressed specificity on related hosts, but evidence from experimental studies has supplied ample data. The experimental work of De Kesel (26) stands out for its large scale. He tested the influence of environment, more specifically, soil composition, on the potential transmission of a presumed univorous species, *Laboulbenia slackensis*. Infection of 26 atypical carabid species and 5 species belonging to other families was attempted. The experiments were done in different environments, that is, sand–salt marsh gradients with salt marsh clay from the preferred microhabitat of *Pogonus chalceus* (Coleoptera, Carabidae), the main host of *L. slackensis*. Successful infections occurred on *P. chalceus* and on most carabid species when reared on 100% salt marsh clay.

Furthermore, successful infection of atypical hosts was significantly affected by the environment. In this experiment, the environmental factor was soil type. De Kesel's (25) pivotal work highlighted the fact that altering the environment can drive a univorous species to become potentially plurivorous.

Ecologists have emphasized the role of habitat as a factor in driving speciation of Laboulbeniales. The observation that the habitat, as imposed by the host, determines the success of these ectoparasites (25) is an important element in considering the specificity of Laboulbeniales. It helps to explain why, in certain habitats, a parasite can be found on an atypical host. The recently described *Laboulbenia littoralis* (31) is a species parasitizing the salt-tolerant *Cafius xantholoma* (Coleoptera, Staphylinidae) in Belgium, France, Italy, and the Netherlands. Of taxa reported on Staphylinidae and Carabidae, *L. littoralis* is morphologically most closely related to *L. slackensis*. In Belgium, *L. littoralis* was collected fewer than 50 m from coastal marshlands with vast populations of *P. chaldeus* (Carabidae) infected by *L. slackensis*. *C. xantholoma* and *P. chaldeus* are both salt tolerant, locally abundant, and mobile. De Kesel & Haelewaters (31) suggested that, in this case, opportunities for host shifting exist, which may have resulted in reproductive isolation and subsequent speciation. Whether *L. littoralis* and *L. slackensis* are indeed sister species needs to be tested by a molecular approach.

4.6. Harm Caused by Biotrophic Parasites

Gäumann & Dodge (37, p. 364) wrote that the “very existence of [Laboulbeniales] parasites seems to depend on the fact that the host is not destroyed, since their own life ends with that of the insect.” There is no development, growth, or survival of thallus-forming Laboulbeniomycetes without a living host. Yet Laboulbeniales and Herpomycetales species can cause observable injuries to host appendages, cuticles, and body tissues via attachment to the host or penetration of the host by haustoria (8, 39, 120). Some researchers have suggested that heavily infected hosts with large numbers of thalli on the head, eyes, antennae, mouthparts, legs, and/or elytra could demonstrate reduced ability to detect food, predators, prey, and mates (76, 103). Apparently, infections by Laboulbeniales can alter reproductive behaviors of hosts, such as oviposition patterns (111). Several recent studies have obtained experimental data revealing interesting details with regard to the presumed pathogenicity of Laboulbeniales. For example, experiments with *Myrmica* ants (Hymenoptera, Formicidae) deprived of food and water revealed that *R. wasmannii*-infected ants survive at a lower rate than do uninfected ones. In addition, infected ants show reduced boldness and reduced aggressive behavior (4, 6, 23, 24).

Several studies have evaluated the effects of *H. virescens* on its ladybird hosts (Coleoptera, Coccinellidae). This species is known to parasitize *Harmonia axyridis*, which is a globally invasive pest species occurring in a rapidly increasing number of countries (57, 96). Parasite prevalences of *H. virescens* on *H. axyridis* can be locally very high, an observation that has prompted consideration of this fungus as a biological control agent. A report of decreased mating frequency of infected female ladybirds has attracted recent interest (76). Another experimental study with simulated winter conditions found decreased survival in infected *H. axyridis*, especially males (83). A report of an epizootic linking *H. virescens* infection to premature mortality of *Chilocorus bipustulatus* ladybird populations (67) was later dismissed based on additional data from controlled experiments (3).

A recent study examined mortality of a North American-native ladybird, *Olla v-nigrum*, and the invasive *H. axyridis* (50). This study attempted to assess the biocontrol potential of *H. virescens* while embracing the idea that organisms are exploited by multiple natural enemies; mortality of uninfected ladybirds and ladybirds heavily infected with *H. virescens* was compared with that of ladybirds coinfecting with *H. virescens* and an entomopathogenic fungus (*Beauveria bassiana*

Enemy release

hypothesis: explains the success of invasive alien species due to reduced regulatory effects from natural enemies compared to the local native species

or *Metarhizium brunneum*) (50). The laboratory assays involving 1,289 ladybirds showed that *H. virescens*-infected ladybirds of both species showed increased mortality after 18 days compared to uninfected individuals. In addition, *H. virescens*-infected *O. v-nigrum* ladybirds were more susceptible to entomopathogenic fungi, leading to an increased mortality of coinfecting individuals. In contrast, no increase in *H. axyridis* mortality was seen when coinfecting with *H. virescens* and either *B. bassiana* or *M. brunneum*. These results provide direct support for the enemy release hypothesis (20, 97). Answering the question of whether *H. virescens* can be used as a biological control agent against *H. axyridis* will require further experiments, especially considering the new concept that each ladybird species hosts its own *Hesperomyces* species (48).

5. SINCE THAXTER: CONTINUING DISCOVERY OF NEW TAXA AND NEW HOSTS

After Thaxter's death in 1932, the majority of contributions concerning thallus-forming Laboulbeniomyces were regional studies reporting on specific geographic areas. Many of these have been short notes, but when considered collectively, they provide extensive data on the geographic distribution and host associations of Laboulbeniales and Herpomycetales. On a larger scale, detailed surveys have been published, with descriptions and illustrations of all taxa, for Belgium (27, 30, 32), Finland (63), Germany (103), Poland (72, 73), the Iberian Peninsula (98, 99), and Japan (112).

Even today, the kind of work that Thaxter did, with his own collections of insects, visits to museum insect collections (see sidebar titled A Model to Study Fungal–Host Relationships), and specimens supplied by correspondents, results in critical discoveries. For example, based on insect specimens preserved at the Natural History Museum in Denmark, nine new species of *Rickia* were recently described, and a new genus was erected for a species on *Dicranolasma* harvestmen (Arachnida, Opiliones), the first time any member of Laboulbeniales was found on a member of this host group (100, 101). Likewise, screening the insect collection at the American Museum of Natural History (New York) brought to light 10 undescribed species in the genera *Corethromyces*, *Diphymyces*, and *Rodaucea* (54).

In addition, collaborative, long-term fieldwork efforts have resulted in outstanding taxonomic contributions—e.g., those of Rossi and colleagues in Ecuador (88, 89, 93), and Haelewaters and colleagues in Panama (51, 55). Today, approximately 2,200 species of Laboulbeniales are formally described, indicating that, since Thaxter's work, only 940 species have been described. Compare this number to the 1,260 species of Laboulbeniales (and *Herpomyces*) described by a single worker.

A MODEL TO STUDY FUNGAL–HOST RELATIONSHIPS

The Laboulbeniales are currently understudied, but they have several characteristics that make them suitable as models for the study of parasite and invasion biology. These microscopic (40 μm –4 mm in length) ectoparasites have a rapid life cycle and exhibit different types of specificity (host, position, sex-of-host, and habitat specificity). Laboulbeniales remain firmly attached to their dead hosts, where they can be easily observed with a dissecting microscope by trained mycologists and entomologists in museum specimens that may span several hundred years. Insect collections can reveal previously undescribed species (54, 101), but also geographic records (5, 58) and geographic distributions over time (57). An additional advantage of using curated insect collections to study fungal parasites is that the host species are usually already identified, which solves the huge problem of host identification encountered by mycologists in many studies of Laboulbeniales.

In addition to the general life history information included in descriptions, several researchers have contributed to our current understanding of the biology of these organisms. Scheloske (103, 104) was interested in the question of whether the morphology of thalli could be shaped by host activities or their position on the host body. In addition to describing *Stigmatomyces ceratophorus* on flies, Whisler (132) cultured a species of Laboulbeniales on brain–heart infusion and tryptone agar containing fly wings, but the thalli he grew failed to reach maturity. Finally, De Kesel (25) focused experiments on the effects of environmental conditions on the growth of *L. slackensis* on a wide range of hosts (see Section 4.5).

SUMMARY POINTS

1. The class Laboulbeniomycetes is comprised of three orders of obligately arthropod-associated fungi (Laboulbeniales, Herpomycetales, and Pyxidiophorales) and two informal clades of unusual mitosporic fungi. Of these, the Laboulbeniales represents the most broadly diversified group.
2. Molecular phylogenetic approaches often conflict with morphology-based classification systems proposed for the Laboulbeniales but allow more precise correlation of structural characters. Many currently accepted higher taxa are polyphyletic.
3. Several factors may explain the morphological and phylogenetic diversity of thallus-forming Laboulbeniomycetes. Specialization to arthropod hosts drives diversification, leading in some cases to speciation. The habitat as selected by the host has also been proposed to drive speciation, but molecular data are lacking. Finally, host behavior during mating, grooming, and other activities may be involved in phenotypic modifications of these fungi.
4. At this time, it is uncertain how Laboulbeniales obtain their nutrition from their arthropod hosts. Several hypotheses have been proposed, including penetrating haustoria that make contact with the host's body cavity or absorption of waxes at the cuticle surface. Absorption of nutrients indirectly from the environment through the appendage system has been proposed but lacks evidence.
5. Laboulbeniales and Herpomycetales are biotrophic parasites, but some moderate adverse effects on their hosts have been suggested. A recent paper points to increased mortality of ladybirds by heavy infection of *H. virescens* and differential susceptibility of *H. virescens*-infected ladybirds to entomopathogenic fungi, suggesting some potential for biocontrol.
6. Synergy between mycologists and entomologists is long-standing. International collaborations have led to an upsurge of Laboulbeniomycetes research over the past decade, which is reflected in increased taxon sampling—including rare taxa and mitosporic states.

FUTURE ISSUES

1. Phylogenetic analyses of multilocus and genome-scale data sets will improve our understanding of the evolutionary relationships in the class Laboulbeniomycetes.
2. How are the different Laboulbeniomycetes clades related to one another, and what morphological characters are phylogenetically informative?

3. The generation of the first genome sequences of Laboulbeniomyces, coupled with functional genomics, will help to understand their ecological functions and the molecular basis of thallus development.
4. A trait-based framework is needed to study the effects of life history traits of hosts and human-mediated habitat on parasitism. This effort may be facilitated by using historical insect collections.
5. While parasite prevalences of some species of Laboulbeniales are very low, certain population thresholds of host species may be needed to sustain viable populations of fungal parasites. To date, no study has addressed this issue.
6. More remains to be discovered about nutritional modes in Laboulbeniales, and future work should include nutrition studies. Stable or radioactive isotope analyses may give more insights into the nutritional relationships between Laboulbeniales and their hosts.
7. Population-level fungal studies correlated with host information will allow comparison of genetic variation among individuals and inform studies of invasion biology. Again, large systematic collections of pinned insects could be helpful.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

We gratefully acknowledge postgraduate research assistant Jingyu Liu (Purdue University, Department of Botany and Plant Pathology, M. Catherine Aime Lab), who provided the drawings of Laboulbeniomyces used in **Figure 2**. We thank André De Kesel (Meise Botanic Garden), Theodoor Heijerman (EIS-Netherlands), Bart Horvers (The Netherlands), and Walter P. Pfliegler (University of Debrecen) for permission to use their photographs. M.B. thanks the Radcliffe Institute for Advanced Study at Harvard University for an intellectual environment. D.H. has been financially supported for his Laboulbeniomyces research by the Mycological Society of America, American Museum of Natural History, Smithsonian Tropical Research Institute, David Rockefeller Center for Latin American Studies, and Friends of the Farlow. M.B. acknowledges previous funding from the National Science Foundation, grants NSF BSR-8604656, NSF BSR-8918157, NSF DEB-9208027, and NSF DEB-9615520, and from the Louisiana State University Boyd Professor Research Fund. We acknowledge curatorial support and the use of unsurpassed resources at the Farlow Reference Library and Herbarium of Cryptogamic Botany, including specimens and original drawings of Roland Thaxter. Finally, we would like to give a special shout-out to the Emergency Temporary Access Service developed by HathiTrust in response to the COVID-19 pandemic and subsequent lockdown, which made it possible to access necessary literature.

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