

## Review

## Bats, Bat Flies, and Fungi: A Case of Hyperparasitism

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Bats are parasitized by numerous lineages of arthropods, of which bat flies (Diptera, Nycteribiidae and Streblidae) are the most conspicuous. Bat flies themselves can be parasitized by Laboulbeniales, fungal biotrophs of arthropods. This is known as hyperparasitism, a severely understudied phenomenon. Three genera of Laboulbeniales occur on bat flies: *Arthrorhynchus* on Nycteribiidae, *Gloeandromyces* and *Nycteromyces* on Streblidae. In this review we introduce the parasitic partners in this tripartite system and discuss their diversity, ecology, and specificity patterns, alongside some important life history traits. Furthermore, we cover recent advances in the study of the associations between bat flies and Laboulbeniales, which were neglected for decades. Among the most immediate needs for further studies are detailed tripartite field surveys.

*The vermin only teaze and pinch  
Their foes superior by an inch  
So, naturalists observe, a flea  
Has smaller fleas that on him prey;  
And these have smaller still to bite 'em,  
And so proceed ad infinitum.*  
Jonathan Swift (On Poetry: A Rhapsody, 1733)

**Parasites of Parasites: Associations among Fungi, Flies, and Bats**

Parasitism of parasites, or **hyperparasitism** (see [Glossary](#)) is thought to be a common phenomenon in nature [1]. Few examples of obligate fungal hyperparasites have been well studied. Within these systems, several questions are severely underexplored: How did such associations evolve? What population parameters are necessary to maintain these relationships? And how strict are the species-level relationships? The examples in this review involve bats, their blood-sucking dipteran ectoparasites, and fungal ectoparasites (hyperparasites) of the blood-sucking flies (Figure 1, Key Figure).

Bats are parasitized by numerous lineages of ectoparasitic insects (Table 1 and Figure 2), of which bat flies (Diptera, Hippoboscoidea, Nycteribiidae, and Streblidae) are the most conspicuous and relatively well studied. Current studies focus on specificity patterns, sex ratios and biases, population structure, and associations between functional traits of bats and parasitism by bat flies. The addition of a second level of parasitism to the bat **microhabitat** is a recent development (e.g., [2,3]). Here, we focus on the Laboulbeniales, microscopic fungi that parasitize a wide range of arthropods, including bat flies. The bulk of papers dealing with Laboulbeniales on bat flies date back almost 40 years [4,5]. Other topical papers date back to the time of Roland Thaxter (1858–1932). Some of his publications presented species

## Highlights

Bat flies have been studied in a variety of contexts, including host associations and specificity, how bat ecology and roosting biology affects parasitism, and how fly morphology functions to allow coexistence of species on bat individuals and populations. Many bat–bat fly parameters are applicable to understanding the dynamics of hyperparasites.

Laboulbeniales associated with bat flies have been recently rediscovered. Studies since 2015 have revealed many new hosts, host associations, and undescribed taxa. These trends hint at the true diversity of these unique fungi.

Morphological and molecular diversity of Laboulbeniales are not aligned. Several morphotypes of the same phylogenetic species as well as multiple phylogenetic species can occur on a single bat fly. Finally, phenotypic plasticity can be extreme, to the point that certain morphotypes of phylogenetic species overlap.

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descriptions and new records for *Arthrorhynchus*, a genus apparently restricted to Eastern Hemisphere bat flies, and two genera that thus far have been reported from Neotropical bat flies only, *Gloeandromyces* and *Nycteromyces* (Figure 2). Until recently [6–8], five species were only known from the type collections. This demonstrates the need for more exploratory studies on Laboulbeniales hyperparasites (Box 1). In addition to reports of parasitism by Laboulbeniales [4–10], only two other groups of bat fly hyperparasites are known – parasitoid wasps [11,12] and mites [13].

### Parasitism, an Exploitative Way of Life

Natural populations of organisms are often strongly affected by biotic factors of their environment. Most striking among these is the effect of predators on population size and individual fitness of prey [14]. Competition molds populations by pushing them to adapt or perish, whereas the most outstanding arms race, produced by millions of years of coevolution, is visible between **parasites** and their hosts [15].

Despite a low fondness for them, a large fraction of the world's biodiversity consists of parasites; parasitic species may even equal free-living species in number [16,17]. However, richness and diversity of parasites have been impossible to estimate because many parasite groups are only scarcely studied [18]. Generally, species richness of parasites and hosts correlates strongly and positively [19]. Despite parasites being a key component of biodiversity [20], studies on species diversity that include parasites are rare [21]. However, parasites are thought to maintain the stability and integrity of an ecosystem and contribute to ecosystem functioning [22–24] by controlling population sizes of their hosts [25]. Parasites may reduce reproduction, increase predation risk, and even alter host morphology and mating behavior [24]. Depending on the dominance of the host species in an ecosystem, the presence of a parasite may even influence the entire food-web and ecosystem structure [26,27]. Given this impact, parasite communities may be indicative of the general health of an ecosystem [28,29] or, for parasites with high **host specificity** and direct development, of particular host populations [30].

The **dilution effect** predicts that diverse and healthy ecosystems should reduce the average risk of disease. Habitat loss causes a direct loss of biodiversity [31], which leads to an elevated risk of wildlife diseases [32]. The reaction of parasites to habitat alterations may be specific to the level of species, higher taxonomic levels, or even ecological assemblages, with endoparasites often decreasing and ectoparasites thriving in fish [27]. Similarly, tick species richness on a small rodent was higher in fragmented areas than in pristine forest [33]. Moreover, Piloosof and colleagues [34] showed that the intensity of parasitism of bats was, depending on host species, positively or negatively correlated with anthropogenic disturbance. How any given parasite reacts to habitat alteration is dependent on both the particular parasite species and its associated host species.

### My Host Is My Castle – Bats as Hosts

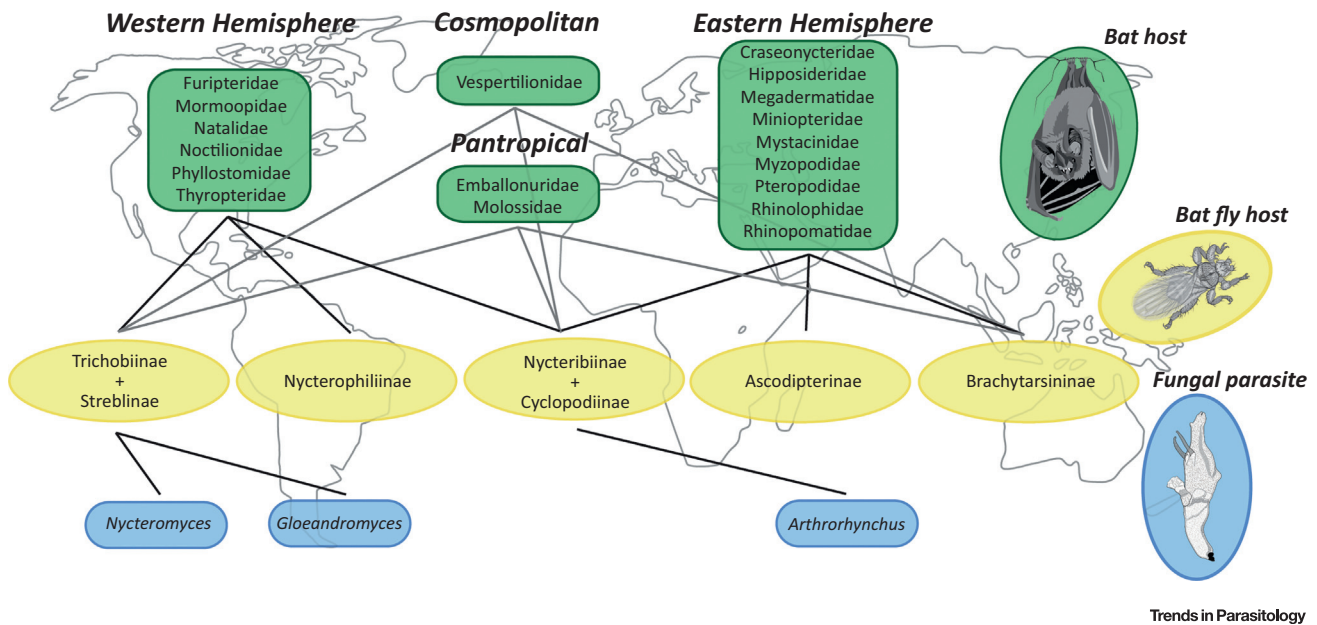
Each host individual forms the living environment for its associated parasites. Within this microhabitat, parasites interact on multiple levels with their surrounding ecosystem. While host body condition in part limits parasite burden, competition for nutrients as well as the host's immune response have led to the evolution of highly diverse parasite communities. Within parasite communities morphological adaptations have often led to **resource partitioning**, allowing the coexistence of various parasite species in and on a single host individual [35]. Whereas endoparasites are more strictly bound to a single host individual, ectoparasite species are mobile and may inhabit the entire host population as habitat even while occupying their specific niche.

### Glossary

- Adenotrophic viviparity:** a fertilized egg hatches inside the female, and the three larval stages are carried inside the female, nourished by an intrauterine accessory or 'milk' gland.
- Ctenidium:** a comb of stout spines on the ventral side of the head (some Streblidae) or thorax and abdomen (Nycteribiidae), generally allowing forward movement but preventing rearward dislodgement from the host fur.
- Dilution effect:** a phenomenon that exists when increased species diversity reduces the risk of disease by 'diluting' the host populations and therefore reducing possible transmission events between individuals.
- Host specificity:** the degree to which a parasite (or hyperparasite) species is limited to one host species or population.
- Hyperparasitism:** a condition in which a parasite develops in or on an organism that is itself a parasite.
- Microhabitat:** a small, localized environment within a larger ecosystem.
- Parasites:** organisms that live at the expense of a single host, are multicellular (in contrast to pathogenic microorganisms), and do not directly cause death of the host (in contrast to parasitoids).
- Resource partitioning:** division of a resource (e.g., host individual) into niches, which are occupied by specific consumers (e.g., parasites) showing morphological and/or behavioral adaptations and therefore allowing the coexistence of various species.
- Thallus:** a multicellular unit of determinate growth, resulting from subsequent divisions of a single ascospore.

## Key Figure

## Interaction Diagram



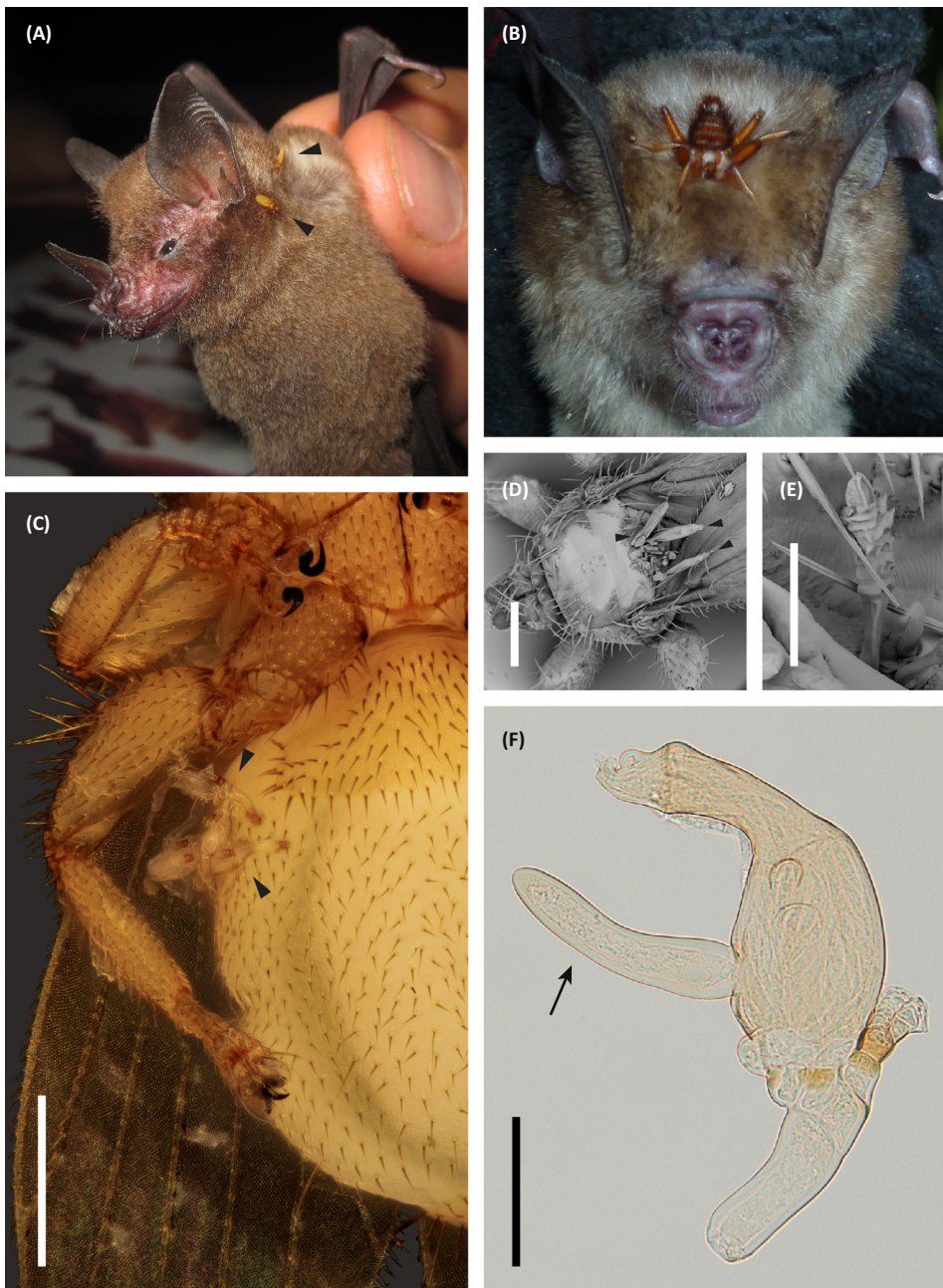
Trends in Parasitology

**Figure 1.** An overview of associations between families of bats, monophyletic groups of bat flies (subfamilies), and genera of Laboulbeniales fungi, presenting the geographic distributions of the taxonomic groups as currently understood (Eastern Hemisphere, Western Hemisphere or cosmopolitan). In this tripartite system, bats are hosts, the fungi are parasites, and the bat flies are both parasites (on bats) and hosts (to Laboulbeniales fungi).

**Table 1.** Ectoparasitic Insects Associated with Bats (Chiroptera)

Family	Order (common name)	Genera	Species	Distribution	Source
Nycteribiidae	Diptera (flies)	11	275	Cosmopolitan	[95]
Streblidae	Diptera (flies)	33	239	Cosmopolitan	[96]
Cimicidae	Hemiptera (true bugs)	13	66	Cosmopolitan	[97,98]
Polyctenidae	Hemiptera (true bugs)	5	32	Cosmopolitan	[99]
Ischnopsyllidae	Siphonaptera (fleas)	20	125	Cosmopolitan	[100–102]
Arixeniidae	Dermaptera (earwigs)	2	5	Southeastern Asia	[103]

Parasite diversity is positively correlated with host longevity, home range, and colony/population size [36]. These characteristics are all met by bats (order Chiroptera). Bats are the second most diverse mammal order worldwide, capable of active flight, with nightly commuting flight distances up to 100 km [37] and total flight distances in migrating species up to 3000 km [38]. Compared to similar-sized mammals, their life expectancy is very high [39] and they spend half of this time in roosts, some species in small conspecific groups and others in huge multispecies assemblages of thousands of individuals [40]. Bats utilize a variety of roost structures, including foliage, tree cavities, abandoned buildings, and large cave systems [41]. These traits most likely contribute to the great diversity of bat-associated parasites.



## Trends in Parasitology

**Figure 2. Illustrating the Bat-Bat Fly-Laboulbeniales Tripartite System.** (A) A *Carollia perspicillata* bat with two *Speiseria ambigua* bat flies (arrowheads), captured at La Virgen de Sarapiquí, Costa Rica. (B) A bat of the *Hipposideros caffer/ruber* complex with a single *Penicillidia* sp. on its head, from Forikrom, Ghana. Image provided by Julian Schmid. (C) *Trichobius joblingi* (from *Carollia perspicillata*), with thalli of an undescribed species of *Gloeandromyces* on its abdomen (arrowheads). Image provided by André De Kesel. (D, E) *Trichobius costalimai* (from *Phyllostomus discolor*), with thalli of *Nycteromyces streblidinus* on its thorax, from Peña Blanca Peninsula, Panama. Scanning electron microscopy images provided by Alena Maidel. *Nycteromyces* is dioecious, which means that (male) antheridia and (female) perithecia are housed on separate individuals. (D) Three female thalli are indicated (Figure legend continued on the bottom of the next page.)

## Box 1. Bats, Bat Flies and Laboulbeniales Fungi in a Panamanian Cloud Forest

In June 2017 a targeted survey was carried out to investigate the tripartite host–parasite–parasite network (Figure 1) of a remote cloud forest in the Darién Province in Panama [8]. A total of 227 bat individuals representing 17 species were examined for the presence of bat flies. One bat (*Micronycteris schmidtorum*) represented a new report in Darién, and for another bat (*Platyrrhinus dorsalis*) the known geographic range was expanded. A total of 148 bats were parasitized (65%), and 437 bat flies were collected, representing 16 species (one in Nycteribiidae, 15 in Streblidae). One bat fly (*Trichobius anducei*) was previously unknown in Panama, and five bat fly species represented new reports for the province. Thirty bat flies (6.9%) presented an infection with Laboulbeniales. Six bat flies carried two species of Laboulbeniales at the same time. The bat–bat fly interactions were highly specific, with 97.5% of all collected individuals being associated with their reported primary bat host, which is comparable to previous studies [92–94]. Of the seven species of Laboulbeniales collected in this study, only three were known to occur in Panama [6], whereas one was a new country record and the remaining three taxa were undescribed. Despite the rather small sample size from a geographically restricted area, this study offers a first insight into the diversity of Laboulbeniales on Neotropical bat flies and emphasizes the importance of multitrophic surveys. Furthermore, to understand infection patterns and host associations, it is inevitable to include not only the direct bat fly hosts of the Laboulbeniales, but also their associated bat hosts. Bats vary highly in mobility and especially roosting behavior; these ecological features may shed light on the evolution and phylogeny of these unique fungi in this tripartite host–parasite system.

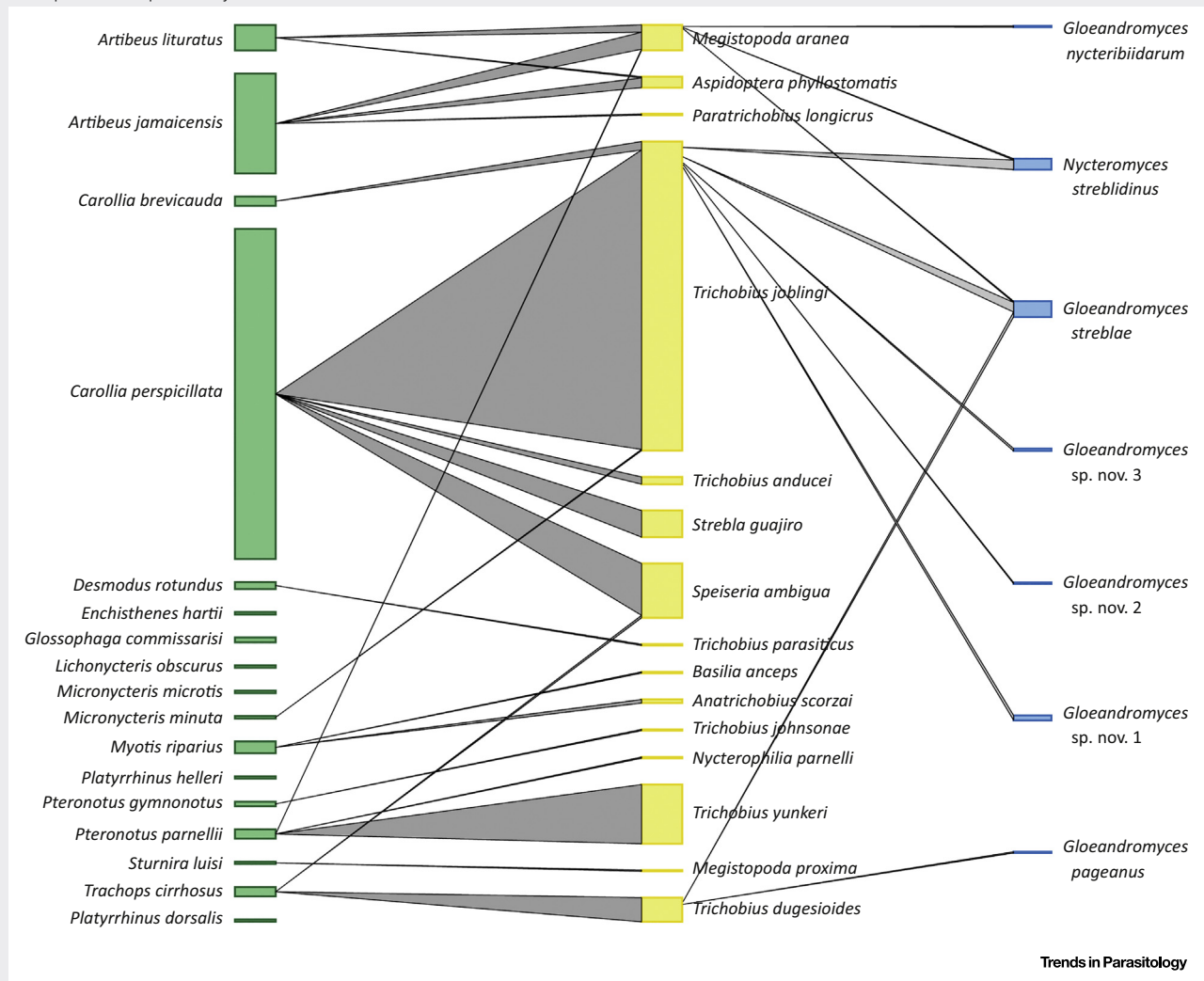


Figure 1. Host–Parasite–Parasite Network. Web of interactions between bat species (left), bat fly species (middle), and Laboulbeniales species (right) from Chucantí Nature Reserve in Darién, Panama (revised from [8]). The width of bars in each network level is proportional to the number of individuals.

by arrowheads. (E) Close-up of a single male thallus, forming antheridia that produce spermatia. (F) *Gloeandromyces* sp. nov., recognized by its finger-like projection at the perithecium (the spore-producing organ) and supported by sequence data (referred to as *Gloeandromyces* sp. nov. 3 in [8]). Scale bars: (C,D) = 250  $\mu$ m, (E,F) = 50  $\mu$ m.

### Runners, Swimmers, and Crawlers – Ectoparasitic Flies on Bats

Among the most conspicuous bat ectoparasites are two families of true flies (Diptera). These are the Nycteribiidae and Streblidae. Together with two other families (Hippoboscidae and Glossinidae) they form the superfamily Hippoboscoidea within the calyptrate Diptera. Whereas all hippoboscooid flies are blood-feeding parasites of a wide diversity of vertebrates (mammals, birds, reptiles), bat flies are exclusively associated with bats. They are obligate and highly specialized ectoparasites, exhibiting many adaptations understood to be advantageous to parasitism [42]. These include a body form often compressed laterally or dorsoventrally, setae modified into heavy spines or combs, the occasional loss of eyes and wings and corresponding thoracic musculature, movement behavior to escape host grooming efforts and prevent dislodgement, and life cycles attenuated to tie the parasites more closely to the host.

Relative to other insect groups, the bat flies are relatively few in number of described species. Nycteribiids comprise around 280 species in 11 genera; streblids comprise around 230 species in 33 genera. Both families are generally cosmopolitan, with a dearth of species at high latitudes and increasing richness toward the tropics, a pattern that largely corresponds to species richness patterns in bats [43]. However, species richness within each bat fly family is not equally distributed among the tropical regions of the globe. Whereas nearly 70% of streblid species are found in the American tropics and subtropics, the reverse is true for nycteribiids, with just over 80% of the species occurring in the Eastern Hemisphere tropics and subtropics, mostly in the Indo-Malayan and Australasian regions.

Insight into aspects of host associations of bat flies, including associations and coevolution of hyperparasites like Laboulbeniales, has been hampered by an immature understanding of evolutionary origins and phylogenetic histories. Although Diptera had their origin at least as early as late Permian (260 mya) [44], a date for the origin of bat flies has been more elusive. They are likely not older than bats themselves; the earliest fossil evidence for bats is early Eocene, just over 50 mya [45]. Early hypotheses regarding evolutionary relationships of bat flies and close relatives were based on morphology, yet rampant convergence due to selection for parasitism has confounded morphology-based pursuits. The monophyly of the Hippoboscoidea is generally accepted, but a stable arrangement of the constituent four families had been elusive. Regarding bat flies, one hypothesis suggested that the two families were monophyletic [46,47], whereas an early molecular phylogeny [48] suggested two independent origins (polyphyly) for bat flies. Later molecular phylogenies generally corroborated, although not always strongly, the hypothesis of monophyletic bat flies (Streblidae + Nycteribiidae) [49,50].

Relationships within the two bat fly families remain understudied and unclear. The current understanding of these relationships was most recently summarized as follows [51]: (i) there is strong support for 'bat flies' as a monophyletic group; (ii) the family Nycteribiidae has support as monophyletic, and appears derived relative to other bat flies; and (iii) as currently classified, the Streblidae are not monophyletic. Overall, and based on what were referred to as 'preliminary results', there was support for three distinct clades with respect to the monophyletic bat flies: (A) the Brachytarsiniinae (previously known as Nycteriboscinae, Old World 'streblids'), (B) the Trichobiinae + Streblinae (New World 'streblids'), and (C) the Ascodipterinae (Old World 'streblids'), Nycterophiliinae (New World 'streblids') and Nycteribiinae + Cyclopodiinae ('nycteribiids') (Figure 1). Within the last clade, Ascodipterinae + Nycterophiliinae and Nycteribiinae + Cyclopodiinae each formed reciprocally monophyletic groups. Further insights into the phylogenetic history of bat flies, and how that might inform natural classification schemes, await publication of phylogenies based on more molecular markers and denser taxon sampling than has heretofore been available.

### Habitat and Ecomorphology

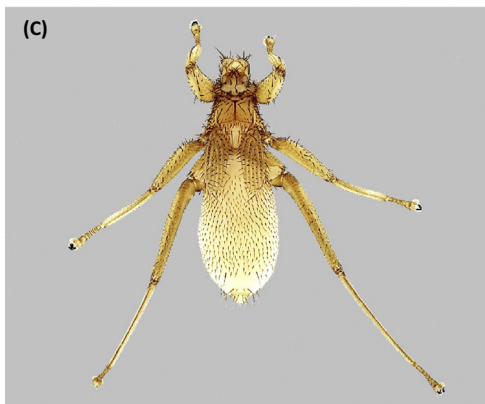
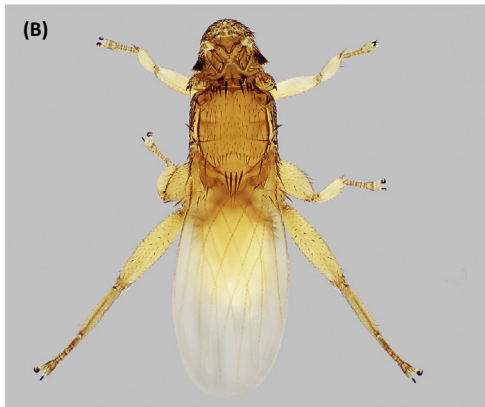
Generally, adult bat flies specialize on two main host habitats, the flight membranes and the furred body and head. Likewise, they appear to have morphological and behavioral characteristics corresponding to these habitats [52,53]. In terms of ecomorphology, species of the 11 genera of nycteribiid flies are all roughly similar in overall morphological appearance and vary mainly on the basis of size. However, among the 33 genera of Streblidae we see a bewildering variety of overall body plan, varying mostly along the lines of body shape, leg size and shape, and degree of **ctenidial** development. Species of one genus, *Ascodipteron*, even become endoparasitic in that reproductively mature females lose their wings and legs, and burrow beneath the skin of bats (often along finger bones). Ecomorphology of neotropical Streblidae has been examined based on position specificity, avoidance behavior, and hind leg structure [52,53]. There are three primary eco-groups (Figure 3); they are defined by body plan, behavior, and position specificity on bat hosts. The first, termed 'wing crawlers', is the most generalized (and probably the ancestral form) and specializes on the membranous areas of the bats. The second and third groups specialize on the furred habitat. The 'fur runners' tend to have broad thoracic sterna and long legs (particularly the hind legs), and when disturbed they run quickly over the surface of the fur. The 'fur swimmers' tend to have compressed (flattened) bodies and relatively more ctenidia, and when disturbed they swim into and through the fur (much like Siphonaptera).

### Bat Roosts and Life History

Although some studies of parasites have focused conceptually on 'host as habitat' [54], for bat flies the host itself is only the direct habitat for the adult stage. Bat flies reproduce by **adenotrophic viviparity** [55]. Upon maturation of the third instar larva, the gravid female fly leaves the host to locate a suitable substrate within the bat roosting area, and deposits the single larva by adhering it to the substrate. The pupal case then hardens, and this pupal stage typically lasts 3–4 weeks. During this time, the pupa is separated from the host and is subject to 'roost as habitat' dynamics. Following pupal development, the teneral adult emerges and must seek, find, and colonize a bat in order to feed and find mates [56]. Few studies have investigated the pupal stage of bat flies [57]. However, it has become apparent that the roost environment plays crucial roles in maintaining associations in the bat–bat fly system. Populations of certain streblid species are segregated at different stages of development, with adult flies largely associated with bats in a 'main roost' and pupal stages located exclusively in 'pupal fields' in flyway passages at considerable distances from the main roost [58]. In fact, the estimated population size of the pupal stage in a single roost cave may far exceed that of the adult stage associated directly with bats. Teneral flies indeed must often travel relatively great distances to re-establish association with their hosts, which suggests the importance of the roost environment, namely spatial and microclimatic dynamics, on maintaining bat fly reproductive cycles, population sizes, and recolonization and association with host bats. Moreover, in a rather unique 'hot cave' roost in Puerto Rico, Dittmar and colleagues [59] corroborated earlier findings separating pupae from adults, and in this case determined that the main bat roosting chamber of the cave maintained a temperature of 34°C, too hot for pupal development. Rather, the pupal field was located in a shallower and cooler chamber with a temperature of around 28°C.

### Bat Roosts and Parasitism Rates

Owing to the unique life cycle of bat flies and the decoupling with hosts during the pupal stage, the type and duration of the roosting structures themselves are related to measures of parasitism. Bats are known to utilize myriad structures as day-roosting sites, including foliage, leaf-tents, tree bark, hollow cavities in trees and other structures, buildings, mines, and caves [41]. These structures vary greatly in their duration (longevity) and the protection they afford to roosting bats. Based on a large and carefully sampled dataset of bats and bat flies from



**Figure 3. Ecomorphology of Streblid Bat Flies.** (A) *Trichobius galei* from a *Natalus stramineus* bat (Michoacan, Mexico). This bat fly is a ‘wing crawler’, specialized to the membranous areas of its bat host. (B) *Strebba guajiro* (from *Carollia perspicillata*, Rio de Janeiro, Brazil), a ‘fur swimmer’ showing more ctenidia and a dorsoventrally compressed body plan. (C) *Megistapophysis mordax* (from *Sturnira mordax*, Cartago, Costa Rica), a ‘fur runner’ with elongated posterior legs. Fly species demonstrate ecomorphological diversity and are not to scale.



Venezuela, bat species utilizing more durable and protective roosts were found to have more species of bat flies, a higher prevalence of parasitism, and a higher mean intensity of parasites [56]. These patterns were understood to be driven by the probability of host bats being present for colonization by emerging teneral adult flies, where the linkage between bats and colonizing bat flies was more sporadic and less probable in exposed and short-lived roosting structures. In contrast, large, long-lasting, and protective structures such as caves, experiencing high fidelity of bats returning to the same roosts, increase the probability of the colonization linkage, encouraging higher numbers of bat flies parasitizing a larger proportion of the host population. A less studied aspect of this system regards bat social structure. Some bat species are rather solitary, living in small groups consisting only of a few close relatives [60]. Other bat species form huge colonies, forming some of the largest localized congregations of any mammalian species (e.g., *Tadarida brasiliensis* with colonies of 20–40 million individuals). In addition to breaking the fly–bat linkage, bat species that form only small groups also represent less usable host-as-habitat, including fewer bat bodies to colonize and feed upon. Certain bat flies can effectively transfer to any conspecific host in a given roost area [61] (and occasionally to noncongeners [62]); so the number of bats and their spatial arrangement within large colonies should provide a much higher carrying capacity for bat flies than do less numerous and diffusely arranged groups of bats.

#### Host Specificity

One of the properties of any host–parasite system is the phenomenon of host specificity, which should also greatly inform our understanding of hyperparasitism by Laboulbeniales [63]. As a general rule, the more permanently associated a given parasite species is with a host, the higher the specificity. Historically, bat flies were thought to be relatively unspecific to their hosts, in part because multiple bat species are known to routinely cohabit the same roosting structures, providing ample opportunity for host switches. However, a series of large-scale and carefully collected field studies focused on bats and bat flies [52,63–68] have demonstrated that high host specificity in bat flies is the rule rather than the exception. Specificity is high despite numerous ecological and behavioral characteristics (e.g., motility of flies and hosts, multiple species roosts, and an obligate decoupling of flies from hosts) that should discourage specificity [66]. Generally, when nonspecific or ‘accidental’ host associations are reported, they are the result of human error during sampling rather than naturally occurring ecological phenomena [65]. Still poorly understood are the proximate mechanisms (e.g., sensory cues) used by bat flies to locate and colonize their specific host species.

#### Laboulbeniales, Morphologically and Developmentally Unique among Fungi

Laboulbeniales, or *beetle hangers* [69], are one of two orders in the class Laboulbeniomyces, the other being Pyxidiophorales. All members of the class are obligate associates of arthropods for dispersal (Pyxidiophorales) or as biotrophs (Laboulbeniales). What sets Laboulbeniales apart is its wide variety of arthropod hosts and its species diversity (>2100 described species and many more awaiting discovery). Representatives of three subphyla serve as host to Laboulbeniales: Chelicerata, with harvestmen (Opiliones) and mites (Acari); Myriapoda, with millipedes (Diplopoda); and Hexapoda, with cockroaches and termites (Blattodea), beetles (Coleoptera), earwigs (Dermaptera), flies (Diptera), true bugs (Hemiptera), ants (Hymenoptera, Formicidae), crickets and allies (Orthoptera), lice (Psocodea), and thrips (Thysanoptera). Laboulbeniales are ectoparasites; they are attached at the host exoskeleton where they form a **thallus** (plural: thalli). Upon maturity, the thallus produces structures that form spermatia (antheridia) and ascospores (perithecia). Laboulbeniales are developmentally unique among the fungi in that they do not produce mycelia of unlimited growth. Their two-celled ascospores are predominantly transmitted directly from infected to uninfected hosts [70], primarily during

sexual contacts, but also as a result of allogrooming behavior of social insects or random physical contacts (e.g., in overwintering aggregations of ladybirds).

Studying Laboulbeniales is extremely difficult: the average size of thalli is around 200–300  $\mu\text{m}$ , with extremes ranging from 35  $\mu\text{m}$  (*Rickia depauperata* on mites of the genus *Celaenopsis*) to 4 mm (*Laboulbenia kunkelii* on *Mormolyce phyllodes* beetles). Because thalli are externally attached to a host, study requires micromanipulation with sterile techniques; hosts may bear a large number of thalli, but often only a few thalli are available for study. In some cases, thalli of a given (phylogenetic) species or morphotype may be restricted to a particular position on the host body [71,72]. Laboulbeniales have not been grown in axenic culture to more than a few cells (never reaching maturity) [73]. Isolation of DNA has often been unsuccessful because of the often heavily pigmented cell walls [74]. This pigment, melanin, interferes during the PCR step by binding to the polymerase enzyme [75]. In addition, the cells are resilient to absorb impacts and friction on the host's integument. The combination of melanized cell walls and resilient cells means that the thalli are hard to break open. Recent advances in extraction of DNA from thalli of Laboulbeniales include mechanical disruption of single thalli combined with minimized risk of losing tissue [76] and the incorporation of a whole-genome amplification step prior to PCR (D. Haelewaters, PhD thesis, Harvard University, 2018).

Many species of Laboulbeniales are host specific to the genus or even species level. For example, *Rickia wasmannii* is specific to *Myrmica* ants and has been reported on ten species in this genus thus far [77]. Based on experimental work, De Kesel [78] showed that this specificity is driven by characteristics of the integument, living conditions of the arthropod host, and the habitat chosen by that host. For a number of species, such as *Euzodiomyces lathrobii*, *Hesperomyces virescens*, *Laboulbenia flagellata*, and *Rhachomyces lasiophorus*, many host species are known, often in more than one host family. Recent studies have demonstrated that it is impossible to make accurate species-level delimitations for Laboulbeniales without molecular data [8,72]. It could be that parasite taxa with multiple hosts are species complexes, whether or not cryptic, segregated by host species. A different scenario is posed when hosts co-occur in a single microhabitat. In this situation, opportunities exist for ascospores to transmit from a 'typical' host to an 'atypical' one. Microhabitats can be ant nests [79], subterranean caves [80], and seaweed and plant debris on beaches [81]. Moreover, position specificity is displayed when a given fungus shows 'a remarkable tendency to grow on very restricted portions of the host integument' [82]. For example, 13 'species' of *Chitonomyces* are observed on restricted positions of the aquatic diving beetle *Laccophilus maculosus*. Based on the combination of molecular and ecological data, Goldmann and Weir [71] found that these taxa represented pairs (and one triplet) of morphotypes of six phylogenetic species and confirmed that sexual transmission was the mechanism behind the observed position specificity patterns (*sensu* [82]).

### Laboulbeniales of Bat Flies: First They Were Acanthocephalans

About 10% of Laboulbeniales species parasitize flies. Species of Laboulbeniales on flies belong to eight genera: *Arthrorhynchus*, *Dimeromyces*, *Gloeandromyces*, *Ilytheomyces*, *Laboulbenia*, *Nycteromyces*, *Rhizomyces*, and *Stigmatomyces*. The genus *Laboulbenia* is by far the largest genus with hundreds of species, of which only 24 species are on flies [83]. *Stigmatomyces* is the second-largest genus in the order, with 144 described species, all on flies [84]. The genera *Arthrorhynchus*, *Gloeandromyces*, and *Nycteromyces* are specific to bat flies (Table 2), whereas none of the other genera have been recorded from bat flies.

Species in the genus *Arthrorhynchus* are found on Eastern Hemisphere species of Nycteribiidae. Kolenati [85] was the first to report Laboulbeniales from bat flies; he described two species,

Table 2. Laboulbeniales Fungi Associated with Bat Flies: An Overview of Associations

Laboulbeniales <sup>a</sup>	Bat fly family <sup>b</sup>	Bat fly host	Bat host
<i>Arthrorhynchus acrandros</i>	N	<i>Phthiridium biarticulatum</i>	?
<i>Arthrorhynchus cyclopodiae</i>	N	<i>Cyclopodia macrura</i>	?
<i>Arthrorhynchus eucampsipodae</i>	N	<i>Basilia pumila</i>	<i>Pipistrellus javanicus</i>
	N	<i>Cyclopodia ferrarii</i>	<i>Chironax melanocephalus</i>
	N	<i>Eucampsipoda africana</i>	<i>Rousettus aegyptiacus</i>
	N	<i>Eucampsipoda hyrtli</i>	<i>Rousettus aegyptiacus</i>
	N	<i>Eucampsipoda inermis</i>	?
	N	<i>Nycteribia kolenatii</i>	<i>Myotis daubentonii</i>
	N	<i>Nycteribia parvula</i>	<i>Miniopterus schreibersii</i>
	N	<i>Nycteribia pedicularia</i>	<i>Myotis capaccinii</i>
			<i>Rhinolophus hipposideros</i>
	N	<i>Nycteribia progressa</i>	?
	N	<i>Nycteribia schmidlii</i>	<i>Miniopterus schreibersii</i>
			<i>Miniopterus natalensis</i>
	N	<i>Nycteribia vexata</i>	<i>Myotis oxygnathus</i>
	N	<i>Penicillidia dufourii</i>	<i>Myotis myotis</i>
<i>Arthrorhynchus nycteribiae</i>	N	<i>Penicillidia conspicua</i>	<i>Miniopterus schreibersii</i>
			<i>Myotis capaccinii</i>
			<i>Myotis daubentonii</i>
			<i>Myotis myotis</i>
			<i>Rhinolophus euryale</i>
			<i>Rhinolophus ferrumequinum</i>
	N	<i>Penicillidia dufourii</i>	<i>Miniopterus schreibersii</i>
			<i>Myotis murinus</i>
			<i>Myotis myotis</i>
	N	<i>Penicillidia fulvida</i>	<i>Miniopterus natalensis</i>
			<i>Rhinolophus ferrumequinum</i>
	N	<i>Penicillidia indica</i>	<i>Miniopterus schreibersii</i>
	N	<i>Penicillidia jenyensii</i>	<i>Miniopterus schreibersii</i>
	N	<i>Penicillidia oceanica</i>	<i>Miniopterus schreibersii</i>
	N	<i>Penicillidia pachymela</i>	<i>Hipposideros caffer</i>
	N	<i>Phthiridium biarticulatum</i>	<i>Miniopterus</i> sp.
			<i>Rhinolophus ferrumequinum</i>
	N	<i>Phthiridium ceylonicum</i>	<i>Hipposideros lankadiva</i>
	N	<i>Phthiridium phillipsi</i>	<i>Rhinolophus rouxii</i>
	N	<i>Nycteribia blasii</i>	?
	N	<i>Nycteribia kolenatii</i>	<i>Myotis daubentonii</i>
			<i>Myotis nattereri</i>
	N	<i>Nycteribia latreillei</i>	<i>Myotis emarginatus</i>
	N	<i>Nycteribia pedicularia</i>	?

Table 2. (continued)

Laboulbeniales <sup>a</sup>	Bat fly family <sup>b</sup>	Bat fly host	Bat host
	N	<i>Nycteribia schmidlii</i>	<i>Miniopterus schreibersii</i>
	N	<i>Nycteribia vexata</i>	<i>Myotis murinus</i>
	N	<i>Nycteribia</i> sp.	<i>Myotis murinus</i>
<i>Arthrorhynchus</i> sp.	N	<i>Nycteribia parvula</i>	<i>Miniopterus schreibersii</i>
<i>Gloeandromyces nycteribiidarum</i>	S	<i>Megistopoda aranea</i>	<i>Artibeus jamaicensis</i>
	S	<i>Trichobius yunker</i>	<i>Pteronotus parnellii</i>
<i>Gloeandromyces pageanus</i>	S	<i>Trichobius dugesioides</i>	<i>Trachops cirrhosus</i>
<i>Gloeandromyces streblae</i>	S	<i>Strebla wiedemanni</i>	<i>Desmodus rotundus</i>
	S	<i>Trichobius dugesioides</i>	<i>Trachops cirrhosus</i>
	S	<i>Trichobius joblingi</i>	<i>Carollia castanea</i>
			<i>Carollia perspicillata</i>
	S	<i>Trichobius yunker</i>	<i>Pteronotus parnellii</i>
<i>Gloeandromyces</i> sp. nov. 1 (sensu [8])	S	<i>Trichobius joblingi</i>	<i>Carollia breviceuda</i>
			<i>Carollia perspicillata</i>
<i>Gloeandromyces</i> sp. nov. 2 (sensu [8])	S	<i>Trichobius joblingi</i>	<i>Carollia perspicillata</i>
<i>Gloeandromyces</i> sp. nov. 3 (sensu [8])	S	<i>Trichobius joblingi</i>	<i>Carollia perspicillata</i>
<i>Gloeandromyces</i> sp.	S	<i>Speiseria ambigua</i>	<i>Carollia perspicillata</i>
<i>Nycteromyces streblidinus</i>	S	<i>Megistopoda aranea</i>	<i>Artibeus jamaicensis</i>
	S	<i>Strebla wiedemanni</i>	<i>Desmodus rotundus</i>
	S	<i>Trichobius costalimai</i>	<i>Phyllostomus discolor</i>
	S	<i>Trichobius joblingi</i>	<i>Carollia castanea</i>
			<i>Carollia perspicillata</i>

<sup>a</sup>For all data sources see Table S1 in [7], and [8,104].

<sup>b</sup>N, Nycteribiidae; S, Streblidae.

*Arthrorhynchus diesingii* from *Nycteribia vexata* [as *Acrocholidia montguei* (*vexata*)] and *A. westrumbii* from *Penicillidia conspicua* (as *Megistopoda westwoodii*). Interestingly, Kolenati described these species as acanthocephalan worms! Peyritsch [86] described *Laboulbenia nycteribiae* and suggested that Kolenati's species were synonyms of his newly described taxon. He later erected a new genus to accommodate his species: *Helminthophana nycteribiae* [87]. Thaxter [88] followed Peyritsch's opinion but later retained *Arthrorhynchus* and described two additional species, *A. cyclopodiae* and *A. eucampsipodae* [89]. A fourth species, *A. acrandros*, was described by Merola [90] from the bat fly *Phthiridium biarticulatum* [as *Nycteribia* (*Celepries*) *biarticulata*]. The taxonomic status of all these species is unclear because no sequence data exist for any of them, except for four ribosomal sequences for *A. nycteribiae* from a single bat fly species [7]. *Arthrorhynchus nycteribiae* has been reported from several host genera: *Nycteribia*, *Penicillidia*, *Phthiridium* [5]. Consequently, this taxon could be a complex of species each specialized to a single bat fly host or several hosts in a single genus – as is the situation in *Hesperomyces virescens* [91].

Thus far, the only reports of species in the genera *Gloeandromyces* and *Nycteromyces* (Figure 2) have been made on streblid flies in North and South America [6,9,10]. The diversity of both genera is thus far limited, as is knowledge of their distribution and biology. After their original description [9], *G. nycteribiidarum*, *G. streblae* (both described as *Stigmatomyces*), and *Nycteromyces*

*streblidinus* were only reported again a century later by Haelewaters and colleagues [6]. *Gloeandromyces nycteribiidarum* was described on *Megistopoda aranea* (as *Pterellipsis aranea*) from Grenada, and *G. streblae* on *Strebla wiedemanni* (as *S. vespertilionis*) from Venezuela. A third species of *Gloeandromyces*, *G. pageanus*, was recently discovered from *Trichobius dugesioides* flies collected in Gamboa, Panama [6]. Finally, *Nycteromyces streblidinus*, the only species known in the genus, was described on the same individual of *S. wiedemanni* from which *G. streblae* was described [9] and has only recently been recollected on bat flies from Honduras and Panama [105].

Except for a few disparate records of bat-fly-associated Laboulbeniales, virtually nothing is known about this tripartite system. Bat flies are dependent on their bat hosts [62], and it has been shown that habitat disturbance affects parasitism of bats by bat flies [34]. The direction of the correlation (positive or negative) was reliant on the bat host species. Similarly, life history traits of both bats and bat flies may affect the ecology of Laboulbeniales. If bat flies are affected by habitat disturbance, then Laboulbeniales could be affected as well. For example, elevated population densities of bat flies would potentially increase transmission success of ascospores, if they co-occur on the same bat hosts or in the same roosts. However, for these sorts of data, hundreds to thousands of bat flies need to be collected and screened for parasitic fungi. How life history traits and environmental factors such as habitat modification can shape species responses remains poorly understood and requires a large, nonbiased dataset.

### Concluding Remarks and Future Perspectives

This tripartite system involving bats, bat flies, and Laboulbeniales fungi is intriguing and ripe for future study. To expand our understanding of these associations, we require (i) phylogenetic reconstructions of both the bat flies and Laboulbeniales fungi, and (ii) statistical analyses correlating ecological and life history traits of hosts with parasitism by Laboulbeniales (see Outstanding Questions). Among the most immediate needs for any future work are large, carefully designed field studies including the three levels of associates. Continued development of molecular protocols for effective isolation and amplification of fungal DNA will go far to allow the detection of cryptic species and further our understanding of Laboulbeniales phylogeny and host associations. The production of robust phylogenetic reconstructions for both bat flies and Laboulbeniales on a global scale is critical to future studies of the ecology, evolution, and co-evolution of this tripartite system. Finally, large field surveys and the study of well-curated museum collections will result in a database of tens of thousands of bat flies, which can be used for finding associations between ecological and life history traits of bats and bat flies and parasitism by Laboulbeniales. Such comprehensive datasets will enable us to provide insight into broader-ranging questions such as how habitat disturbances can shape symbiotic relationships.

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### Outstanding Questions

Which ecological and life history traits of both levels of hosts – bats and bat flies – affect parasitism of bat flies by Laboulbeniales (e.g., roosting behavior of bats, ecomorphology of bat flies, life cycles)?

Does habitat alteration have an effect on parasitism by either bat flies or Laboulbeniales? Is there a population threshold of the host species to sustain viable parasite populations? How does parasitism change in bat species thriving in altered habitats relative to those species critically threatened or endangered?

What is the position of bat-fly-associated Laboulbeniales in the phylogenetic tree of the order? Do the three lineages – *Arthrorhynchus*, *Gloeandromyces*, and *Nycteromyces* – represent multiple independent colonization events?

Do bats with similar roosting preferences, or phylogenetically close bat species, share more closely related bat fly species and subsequently, does this pattern reflect also in Laboulbeniales? In other words, is there evidence for coevolution on multiple layers?

Is morphological diversity in both bat flies and fungi consistent with molecular phylogenetic diversity?

Do abiotic factors such as temperature, humidity, and precipitation, contribute to shaping geographic distribution and parasite prevalence of Laboulbeniales on bat flies? Are any contributions less profound when bats roost in cave systems?

Is the geographical distribution of bats, and subsequently their bat flies, reflected in the geographical distribution of Laboulbeniales?

If parasitism by bat flies is higher on bat species that form large colonies, will parasitism by Laboulbeniales of bat flies on those bats be increased as well?

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