



# Laboulbeniomyces: Evolution, natural history, and Thaxter's final word

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## ABSTRACT

Historically, thallus-forming Laboulbeniomyces, including the orders Laboulbeniales and Herpomycetales, were set apart because of their distinctive morphology and ecology. Although some biologists correctly interpreted these arthropod ectoparasites as fungi, even ascomycetes, others thought they were worms, red algae, or members of taxa described especially for them. Speculation on the evolution of the group involving red algae, the morphological-based Floridean Hypothesis, persisted deep into the 20th century, in part because valid alternatives were not presented. Although the distinctive features of Laboulbeniales clearly set them apart from other fungi, the difficulty was in the absence of characters grouping them among the fungi. Thaxter considered the Laboulbeniales to be ascomycetes, but he avoided phylogenetic discussions involved in the Floridean Hypothesis all of his life. Eventually, developmental studies of the life history of *Pyxidiophora* species, hyphal perithecial ascomycetes with 2-celled ascospores, revealed characters connecting Laboulbeniales to other ascomycetes. The distinctive morphological features of Laboulbeniales (absence of mycelium, a thallus developed from 2-celled ascospores by cell divisions in several planes, arthropod parasitism) can be best understood by comparison with *Pyxidiophora*. The development of a 3-dimensional thallus composed of true parenchyma occurs not only in Laboulbeniales, but also in *Pyxidiophora* species. The life history of arthropod ectoparasitism of Laboulbeniales as well as mycoparasitism and phoretic dispersal by arthropods of *Pyxidiophora* species can be explained by Tranzschel's Law, originally applied to rust fungi. Molecular analyses including other arthropod-associated fungi have contributed to a better understanding of an enlarged class, Laboulbeniomyces, which now includes a clade comprising *Chantransiopsis*, *Tetrameronycha*, and *Subbaromyces*. A two-locus phylogenetic tree highlights evolutionary and life history questions with regard to the placement of Herpomycetales as the first diverging lineage of the Laboulbeniomyces. The sister group for all the Laboulbeniomyces remains to be discovered.

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## INTRODUCTION

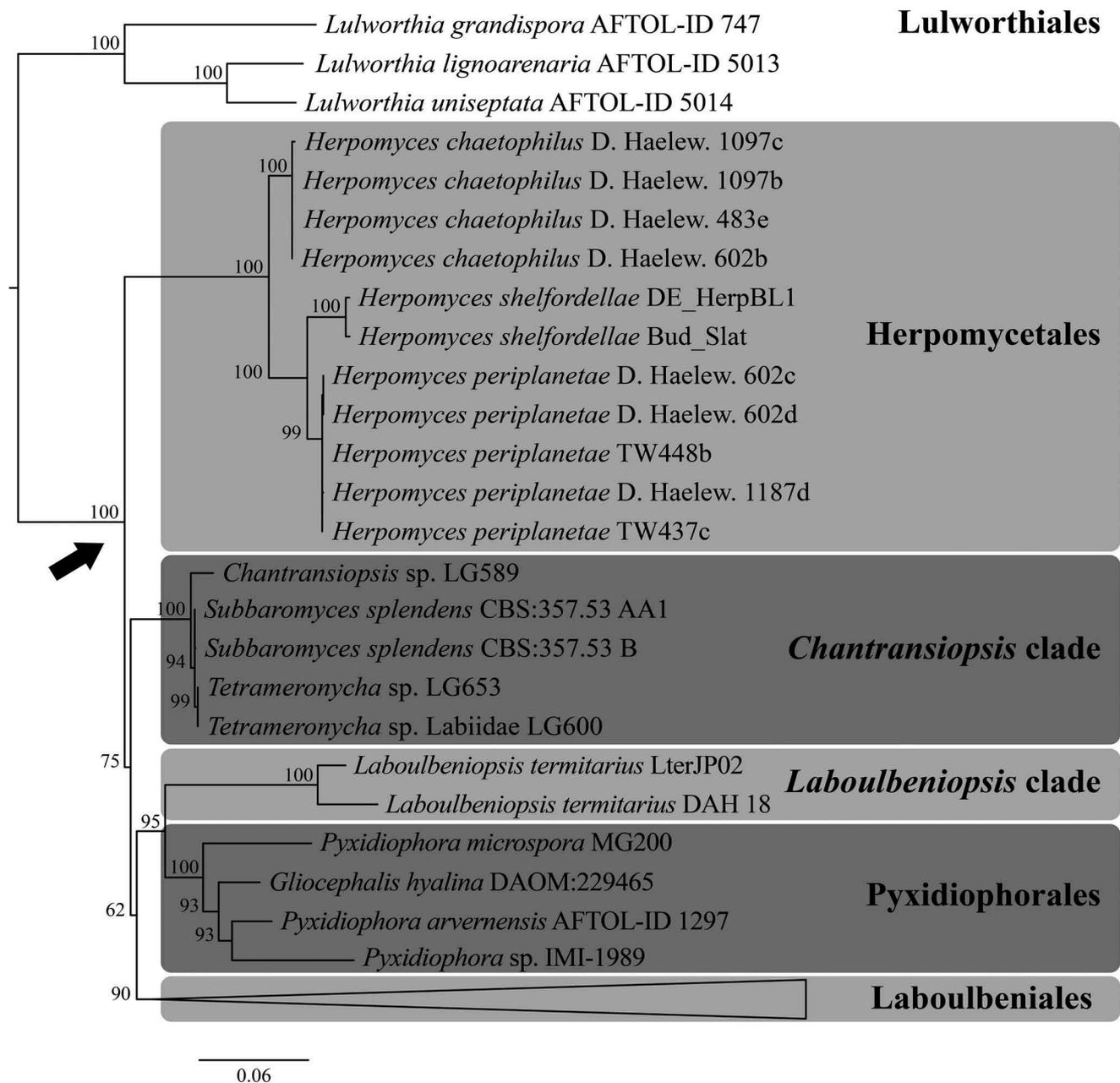
Since their discovery in the mid-19th century (Anonymous 1849; Rouget 1850; Robin 1852, 1853; Mayr 1853), debates have surrounded the placement of fungi currently classified in the orders Laboulbeniales and Herpomycetales (Ascomycota, Laboulbeniomyces; FIGS. 1–4). Their obligate ectoparasitic lifestyle on arthropods, absence of hyphae, unique direct thallus development from 2-celled ascospores, determinate growth, and deliquescent asci all combine to lead to questions of relationships with other organisms and within the fungi. Because of their distinctive development and morphology, speculation concerning their evolutionary origin has been a continuing focus.

The literary history of the group is fraught with taxonomic confusion and phylogenetic misinterpretation. Although many mycologists considered members of the group to have evolved from the fungal lineage once known as Phycomycetes or lower fungi (zoosporic and zygosporic fungi), other biologists suggested that ascomycetes and basidiomycetes, sometimes with direct reference to the Laboulbeniales, were members of a lineage derived from floridean red algae, an idea known as the Floridean Hypothesis (see below). Moreover, two early described species were thought to be parasitic worms of their bat fly hosts (Kolenati 1857). The historical concept of Laboulbeniales usually includes the genus *Herpomycetes*, which is now placed in the recently recognized order Herpomycetales (Haelewaters

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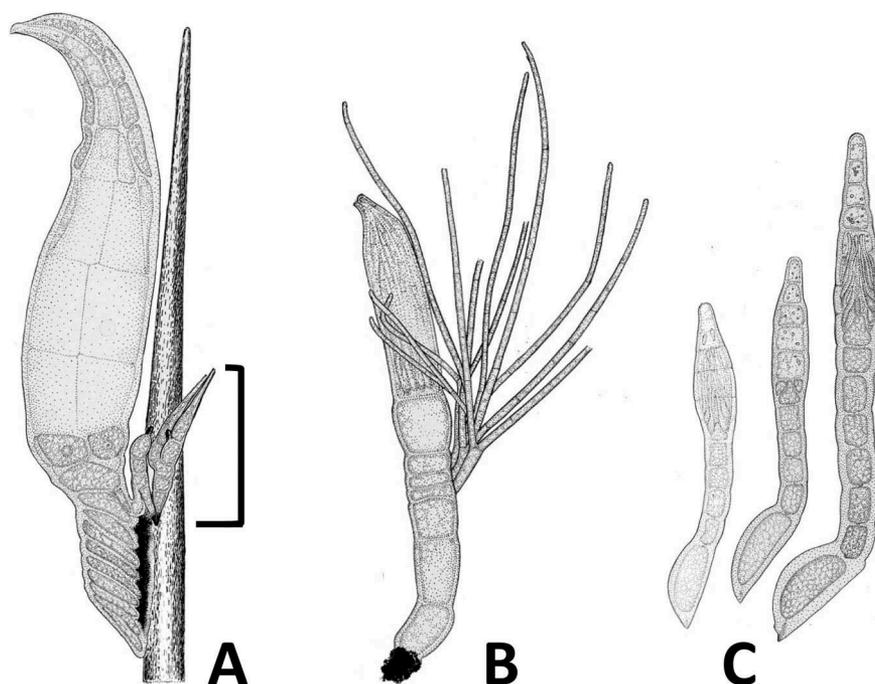


**Figure 1.** Phylogenetic reconstruction of the Laboulbeniomyces from a concatenated SSU+LSU rDNA data set of 75 isolates. The tree topology is the result of a maximum likelihood analysis performed in IQ-TREE (Nguyen et al. 2015). For each node, the ML bootstrap is presented next to the branch leading to that node. The class Laboulbeniomyces is denoted by the black arrow, and the order Laboulbeniales is shown as a collapsed branch. Several minute mitotic fungi described by Thaxter (1914, 1920) are found in multiple clades. As noted throughout this paper, the recently described order Herpomycetales does not form a monophyletic lineage with Laboulbeniales (Haelewaters et al. 2019). Other taxa are mapped on the tree, and some are discussed in the text. Taxa in the order Lulworthiales (Sordariomycetes) serve as outgroup. Methods and specimen information are detailed in SUPPLEMENTARY FILE 1.

et al. 2019) (FIGS. 1, 2A). We refer to these two orders as the thallus-forming Laboulbeniomyces.

Notwithstanding these debates, from his first observations of thallus-forming Laboulbeniomyces, Thaxter (1891, 1896, 1908, and elsewhere throughout his work) considered them to be fungi, an opinion he shared with Robin (1852, 1853) who first recognized

these organisms as fungi. Not only were they clearly recognized as fungi by Thaxter, but he considered them to be ascomycetes and the ascoma was considered a perithecium (Thaxter 1896). He further added that, because of the complexity of the reproductive structures, “they occupy a position among the highest members of their class.”



**Figure 2.** Laboulbeniomycetes. A. Female and small male (bracket) thalli of dioecious *Herpomyces chaetophilus* on antennal spine of *Periplaneta* sp. (Blattodea, Blattidae) from Zanzibar, Africa; perithecia 125–185  $\mu\text{m}$  (Thaxter 1908). B. *Coreomyces corixae* [as *corisae*] thallus with long, slender appendages (200–250  $\mu\text{m}$  long) from abdomen of *Corixa* sp. (Hemiptera, Corixidae); locality not clear but perhaps Arlington, Massachusetts; perithecia 100–110  $\mu\text{m}$  (Thaxter 1908). C. *Coreomycetopsis oedipus* from tips of legs of *Nasutitermes corniger* [as *Eutermes morio*] (Blattodea, Termitidae) (Scheffrahn et al. 2005), Grand Etang, Grenada; Thaxter noted the similarity with *Coreomyces* sp., but classified it among “genera incertae sedis” because it did not produce asci and ascospores (Thaxter 1920). From the original illustrations by Roland Thaxter at the Farlow Reference Library of Cryptogamic Botany, Harvard University.

Karsten (1869) was perhaps the first to mention that the thallus-forming Laboulbeniomycetes were “rightly compared to the similar conditions [of the sexual apparatus] to the Florideae.” Not only have the thallus-forming Laboulbeniomycetes been placed outside the fungi among floridean red algae (Sachs 1874), but at various times the group has been placed among Ascomycota, Basidiomycota, between Ustilaginaceae and Pyrenomycetes (Karsten 1895) and far outside ascomycetes in a truly heterogeneous group (Cavalier-Smith 1998, 2000). Much of the early work was hindered by researchers not having observed asci because of their early deliquescence (but see Peyritsch 1871, 1873). Thaxter (1896) was the first to study and illustrate the development of Laboulbeniales from ascospore to mature thallus using *Stigmatomyces baeri*, including perithecium and ascus development. Faull (1911) improved observations of early developmental stages by using paraffin sections and cytological stains.

### THE FLORIDEAN HYPOTHESIS

Of the various ideas regarding the phylogenetic placement of thallus-forming Laboulbeniomycetes, perhaps the most

instructive regarding morphological convergence in the reproductive apparatus is the Floridean Hypothesis. The hypothesis centers on a group of red algae, class Florideophyceae (previously Florideae)—known for complexity in life cycle and reproductive and vegetative structures, characteristics that were interpreted as being similar to certain features of ascomycetes, including nonmotile male gametes, a female gametangium with a trichogyne, nuclear migration through multiple carpogenic cells, development of a multicellular thallus, morphological similarity of outgrowths from the female gametangia, similarities of the ascocarp and cystocarp, pores (pit connections in red algae) at cell junctions, and life cycles with a triphasic alternation of generations (Freshwater 2000).

The Floridean Hypothesis was first espoused by Karsten (1869) for the thallus-forming Laboulbeniomycetes and further developed by Cohn (1872) and Sachs (1874), who derived all the fungi from various algal groups, arguing that multiple losses of photosynthetic mechanisms had occurred in the fungi and that all fungi could be tied to particular algal groups. These workers were followed by many prominent botanists and mycologists who accepted some form of the hypothesis (Cohn 1872; Sachs 1874; Dodge 1914; Orton 1927; Bessey 1942, 1950; Jackson

1944; Chadevaud 1960; Corner 1964; Denison and Carroll 1966). That this line of thought persisted beyond the mid-20th century can be attributed to the limits of morphological characters in divining phylogenetic relationships. Other mycologists (de Bary 1884, 1987; Faull 1911; Atkinson 1915; Gäumann 1926, 1964; Heim 1952; Martin 1955; von Arx 1967; Savile 1968) rejected the Floridean Hypothesis in favor of an origin of the ascomycetes—including the thallus-forming Laboulbeniomyces—from zoosporic and zygosporic groups.

Denison and Carroll (1966) interpreted new evidence based on ultrastructural features as supportive of the Floridian Hypothesis. They presented five propositions as a basis for a close relationship between ascomycetes and red algae. Their argument was that, although thallus-forming Laboulbeniomyces were not in the “main evolutionary line from the red algae to the Ascomycetes,” both groups exhibited similar morphological characters. The characters included formation of a holdfast, a multicellular thallus with determinate growth and—in common with primitive ascomycetes—similar wall composition, a dikaryotic phase, deliquescent asci, and 2-celled ascospores. Later, Demoulin (1985) expanded his previous views (Demoulin 1974) and continued to espouse a close relationship of ascomycetes with red algae. In addition to ultrastructural features, he advanced new biochemical information supporting the hypothesis; at the same time, he called for “urgently” needed molecular data to be brought to the argument (Demoulin 1985).

In the pre-molecular phylogeny era, the discovery of *Spathulospora* species (Spathulosporales), marine algal parasites that were considered living fossils by Kohlmeyer (1973, 1975), reinvigorated the debate. Kohlmeyer based his argument largely on perceived morphological similarities of *Spathulospora* spp. and red algae. Nakagiri's (1993) beautifully illustrated developmental study of a related red algal parasite, *Hispidicarpomyces galaxauricola*, however, refuted any similarity to red algae. For example, he found that infection of the red algal host was systemic rather than superficial. Eventually, Inderbitzin et al. (2004) used developmental and DNA evidence to suggest the placement of *Spathulospora* in the ascomycete order Lulworthiales, a group of marine fungi unrelated to thallus-forming Laboulbeniomyces.

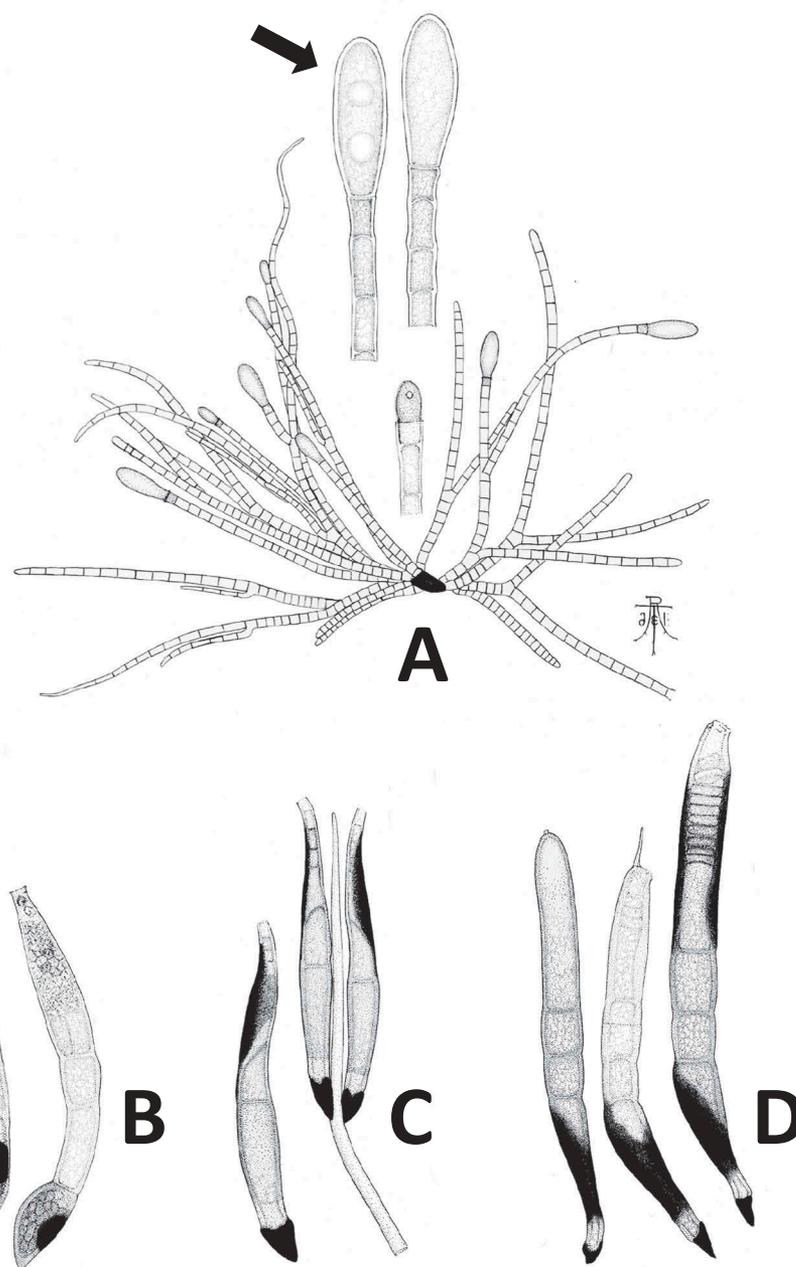
Yet another proposed affiliation placed the thallus-forming Laboulbeniomyces outside the ascomycetes in a new fungal class, Zoomycetes (see below). Other evidence, such as the ultrastructural study of ascosporeogenesis in which free cell formation was shown in *Herpomyces* (Hill 1977), and the first molecular data enlightened the debate. Kwok et al. (1986) obtained DNA-DNA

hybridization data to suggest that red algae and fungi were not closely related. Bailey (1996) reviewed morphological and DNA data that strongly refuted the Floridean Hypothesis. This combined evidence eroded any remaining support for a red algal origin for ascomycetes and basidiomycetes, and small subunit (SSU) rDNA analysis revealed the unity, as true fungi, of chytrids, zygomycetes, ascomycetes, and basidiomycetes (Bowman et al. 1992).

## THALLUS-FORMING LABOULBENIOMYCETES AS ASCOMYCOTA

The thallus-forming Laboulbeniomyces (FIG. 2A, B) moved from being central to the argument of an algal ancestry for ascomycetes to being an orphaned taxon. The question became: if thallus-forming Laboulbeniomyces are ascomycetes, then where do they belong among the ascomycetes? In other words, how are the unique laboulbenialean characters derived from among the known ascomycetes? A breakthrough came from Thelma J. Perry (US Department of Agriculture [USDA], Forest Service, Southern Research Station, Pineville, Louisiana), an extremely observant, self-taught mycologist. She had “discovered the ascomycete that gave rise to *Thaxteriola*” (T. J. Perry, pers. comm., 1985). Perry was adept at finding fungi in galleries of the southern pine beetle and recognized that *Thaxteriola* (FIG. 3C), a minute arthropod-associated fungus with a few linearly superposed cells, was derived from the ascospore of *Pyxidiophora*, a perithecial ascomycete with 2-celled ascospores. The ascospores of *Pyxidiophora* have a dark foot (FIG. 4B) that develops an attachment region similar to that of the spores of the Laboulbeniales (Blackwell et al. 1986a, 1986b, 1989). This breakthrough discovery provided a target for new research directed toward elucidating laboulbenialean relationships and led Blackwell and Malloch (1989a) to propose that *Pyxidiophora* was the “missing link” between the Laboulbeniales and the rest of the ascomycetes. Eriksson and Hawksworth (1993) accepted the morphological and life history evidence and placed *Pyxidiophora* in the Laboulbeniales. Phylogenetic evidence from SSU rDNA analysis confirmed the close relationship of *Pyxidiophora* and *Rickia*, a member of the Laboulbeniales (Blackwell 1994).

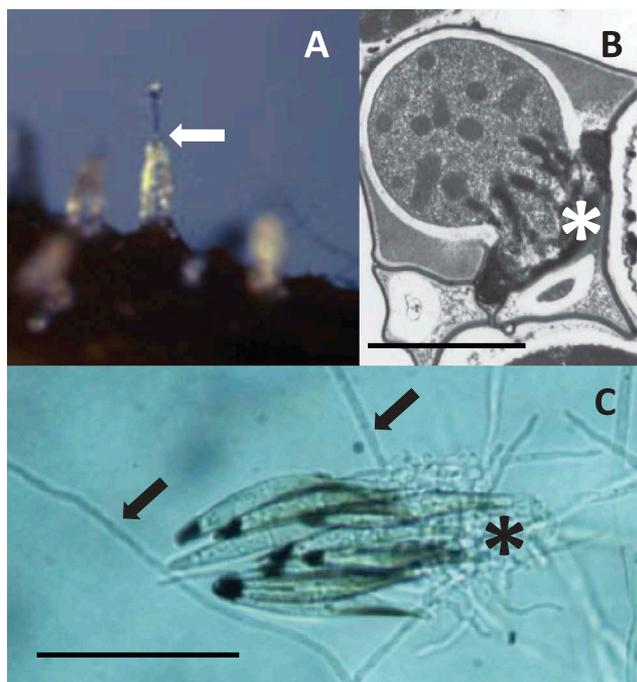
Despite strong evidence of their placement among the Ascomycota, Cavalier-Smith (1998, 2000) classified the orders Laboulbeniales and Pyxidiophorales in his class Zoomycetes. This group, composed of Entomophthorales, fungi formerly placed in the Trichomycetes and Zoopagales, was based on superficial morphology of the attachment region to their animal hosts. This assignment was proposed by Cavalier-Smith (1998) even after he had accepted chytrids, zygomycetes, ascomycetes, and



**Figure 3.** Mitosporic members of the Laboulbeniomycetes. A. *Chantransiopsis decumbens* showing tuft of filaments attached at blackened base to undetermined staphylinid beetle (Coleoptera, Staphylinidae); Malang, Java, Indonesia; enlarged tips of sporiferous branchlets are also shown (arrow); longest branch 350  $\mu\text{m}$  (Thaxter 1914). B–D. "Thaxteriolae." B. *Laboulbeniopsis termitarius*, thalli described as 100–130  $\mu\text{m}$  in length, from legs of *Nasutitermes corniger* [as *Eutermes morio*], Grand Etang, Grenada; Thaxter noted its similarity to *Endosporella* and *Thaxteriola* and placed them all in the "Thaxteriolae" (Thaxter 1920). C. *Thaxteriola nigromarginata*, now known as an ascospore-derived conidial state of *Pyxidiophora* sp.; described as 62–68  $\mu\text{m}$  in length, from hairs of a minute staphylinid (Coleoptera, Staphylinidae); Samarang, Java, Indonesia (Thaxter 1920). D. *Endosporella diopsisidis*, thalli 100–150  $\mu\text{m}$ ; note terminal spine and spores in the most mature thallus (right); on legs of *Diopsis* sp. (Diptera, Diopsidae), Cameroon, Africa (Thaxter 1920). From the original illustrations by Roland Thaxter at the Farlow Reference Library of Cyptogamic Botany, Harvard University.

basidiomycetes as true fungi based on the evidence of Bowman et al. (1992) mentioned above. Taxon sampling for the phylogenetic analysis of Weir and Blackwell (2001b) was designed to test the Cavalier-Smith (1998) hypothesis; their SSU rDNA topology gave strong support for establishing the class Laboulbeniomycetes including

*Pyxidiophora* and Laboulbeniales (*Hesperomyces*, *Stigmatomyces*, *Zodiomyces*) and showed the polyphyletic nature of the Zoomycetes. The Herpomyceales (Haelewaters et al. 2019) was later segregated from the Laboulbeniales to accommodate the genus *Herpomyces*, forming the third described order in the class (FIGS. 1, 2A).



**Figure 4.** *Pyxidiophora* spp. A. Habit on moose dung; a group of ascospores of *Pyxidiophora* sp. released at the perithecial tip (arrow) awaiting an arthropod disperser, often a mite phoretic on a dung beetle. From Blackwell (1994, fig. 11). Reprinted with permission from Mycologia. © The Mycological Society of America. B. Distal end of recently released ascospore showing foot cell with channels of electron dense material (\*) extruded to the foot surface and presumed to be adhesive for spore attachment to an arthropod disperser; it is similar to foot cells of *Laboulbeniopsis termitarius* (Blackwell and Kimbrough 1976a) and *Coreomyetopsis oedipus* (Blackwell and Kimbrough 1976b); serial section adjacent to one shown in Blackwell (1994, fig. 13). C. Ascospores of *Pyxidiophora* sp. removed from tip of perithecium to agar where they germinated by producing conidia; conidia produce several rounds of yeast cells, which in turn produce short, thin hyphae (\*); hyphae grow toward and attach to mycelium of a fungal host (arrows). Bars: B = 5  $\mu$ m; C = 60  $\mu$ m.

### WHAT IS THE SISTER GROUP OF THE LABOULBENIOMYCETES?

*Pyxidiophora* was firmly established as a sister group to the Laboulbeniales within the taxa sampled, and the Laboulbeniomyces were sister to all the Pezizomycotina (Weir and Blackwell 2001b). This relationship was the basis for an evolutionary scenario involving phoretic dispersal, mycoparasitism, and life cycle reduction (Blackwell 1994). However, a closer relative of the Laboulbeniomyces was still needed to help understand the evolution of the distinctive characters of the class members. With the sister group of the Laboulbeniomyces still in question, a broad sampling of representatives of Pezizomycotina was included in subsequent phylogenetic analyses. A variety of ascomycetes with known insect associations or characters

associated with insect-dispersed ascomycetes with evanescent asci, long perithecial necks, and exuded sticky spore masses (ophiostomatoid ascomycetes; Malloch and Blackwell 1993) were selected for DNA sequencing. The taxa were species of *Ophiostoma*, *Ceratocystis*, and related fungi, as well as less well-known ascomycete species of genera such as *Kathistes*, *Subbaromyces*, and *Pyxidiophora* and mitosporic arthropod-dispersed fungi (FIG. 3A–C) (Blackwell and Jones 1997; Blackwell et al. 2003; Goldmann and Weir 2018). The analyses of minimal sequences (only SSU rDNA) acquired by the Sanger method usually placed species of *Kathistes* and *Subbaromyces* as close relatives of *Pyxidiophora* depending on taxon sampling.

Species of *Kathistes* occur in dung and are arthropod-dispersed. Perithecia have a single layer of wall cells, an unusual feature among Pezizomycotina, but one shared with *Pyxidiophora*. The ascospores of *Kathistes* spp. are aseptate at first, becoming 2-, 4-, or more septate at maturity. Mature ascospores produce multiple buds after their release. Conidia are not produced, but small long-necked structures are associated with the perithecia in culture; these sporidiomata produce sporidiolae of unknown function. These structures were not connected to hyphae when they are observed in culture (Malloch and Blackwell 1990).

Another fungus included in some analyses was *Subbaromyces splendens*, often still classified as the member of Ophiostomataceae (Blackwell and Jones 1997; Blackwell et al. 2003), the biology of which is not well understood. *Subbaromyces* spp. have been isolated from human-made habitats—from trickling filters used in sewage purification and from an open drain. Natural habitats are unknown, but these species are common to abundant in these human-influenced habitats. Species grow better in mixed culture than in pure culture where they produce large 1-celled conidia and perithecia with 2-celled ascospores surrounded by a gelatinous envelope or covered by mucilage. Arthropod dispersal has not been observed, but long-necked perithecia, evanescent asci, and the accumulation of ascospores at the tip of the perithecium neck are all suggestive of such dispersal (Hesseltine 1953; Chary and Ramarao 1974). In the past, some analyses have placed *S. splendens* (Blackwell and Jones 1997) or *Kathistes* spp. (Blackwell et al. 2003; Goldmann and Weir 2018) as sister to Laboulbeniomyces.

### ADDITIONAL SEQUENCES OF SUBBAROMYCES SPLENDENS

We reconstructed a two-locus phylogenetic tree using SSU and large subunit (LSU) rDNA sequences of *S. splendens*, generated from a Hesseltine (1953)

culture, CBS 357.53 (SUPPLEMENTARY FILE 1). An older SSU rDNA sequence (GenBank U63552) does not agree with the newly generated sequences, a finding we have observed with some of our other early sequences. These discrepancies are due in part to the use of non-optimized primers and images on X-ray film that were difficult to read (e.g., Jones and Blackwell 1996). The phylogenetic tree (FIG. 1) reveals that Laboulbeniomyces is monophyletic and includes a number of asexual insect-associated fungi. *Subbaromyces splendens* is placed in a clade with the insect-associated *Chantransiopsis* sp. (FIG. 3A) and *Tetrameronycha* spp. with maximum support. *Chantransiopsis* and *Tetrameronycha* are mitosporic forms, producing hyaline filaments from a darkened basal structure (Thaxter 1914; Spegazzini 1918; Rossi and Blackwell 1990). The Pyxidiophorales clade comprises *Pyxidiophora* spp. and *Gliocephalis hyalina* with maximum support (Jacobs et al. 2005). Sister to Pyxidiophorales is *Laboulbeniopsis termitarius* (FIG. 3B), a result that was also found by Henk et al. (2003). This *Laboulbeniopsis* + Pyxidiophorales clade is in turn the sister group to Laboulbeniales with weak statistical support. All these clades together are sister to Herpomycetales, validating the recent separation of this order from the Laboulbeniales (Haelewaters et al. 2019). Previous class-level classifications that recognized the Laboulbeniales including *Herpomyces* (Thaxter 1896, 1908; Tavares 1985) would no longer be monophyletic. In this evolutionary hypothesis, the thallus has independently evolved in the Herpomycetales and Laboulbeniales (FIG. 2A, B). We recognize that some deeper nodes of the tree remain unresolved, and the question remains: what are the sister taxa to the entire group?

The addition of new taxa has helped to clarify relationships within Laboulbeniomyces, to discover new relatives in the lineage, and to identify new clades such as the one comprising *Chantransiopsis*, *Tetrameronycha*, and *Subbaromyces*, which is strongly supported as a monophyletic lineage separate from the three existing orders (FIG. 1). In addition to meiosporic taxa, the inclusion of mitosporic taxa is essential to understanding evolutionary trends in the class. For example, the tree in FIG. 1 indicates that *Laboulbeniopsis* may have arisen by simplification of the *Pyxidiophora* life history (see below, discussion of Tranzschel's Law). *Termitaria snyderi*, another minute mitosporic fungus associated with termites, was previously shown to be related to *Kathistes* spp. based on SSU rDNA (Blackwell et al. 2003). Neither *Termitaria* nor *Kathistes* is included here for want of additional sequences. The ability to adequately sample small, rarely encountered fungi, many of which cannot be grown apart from their host, is a continuing problem (Weir and Blackwell 2001a; Haelewaters et al. 2015, 2019;

Sundberg et al. 2018) and may obscure estimates of biodiversity as well as evolutionary hypotheses (sensu Federman et al. 2018).

Minute fungi from insects, including *Chantransiopsis* (FIG. 3A) and *Tetrameronycha* (Thaxter 1914, 1920; Spegazzini 1918), have few morphological characters that are useful to posit relationships (Blackwell and Kimbrough 1976a, 1976b). Based on the *Thaxteriola* model (FIGS. 2C, 4A–C), others such as *Endosporella* (FIG. 3D) may be dispersal forms developed from ascospores (Blackwell 1994). Thaxter considered these forms variously to be fungi imperfecti or Hyphomycetes, but he made clear that he did not connect them with either red algae or thallus-forming Laboulbeniomyces (Thaxter 1914, 1920; Gäumann and Dodge 1928). He placed these forms in genera incertae sedis to await the “discovery of further types” that might help to place them (Thaxter 1920). Additional taxa have been essential to resolve phylogenetic relationships, but only after the discovery of molecular methods—something never envisioned by Thaxter (see discussion below).

## LABOULBENIOMYCETES THROUGH THE LENS OF PYXIDIOPHORA

***Plectenchyma but rarely true parenchyma in fungi.***—Study of thallus development in the thallus-forming Laboulbeniomyces has been critical to the recognition of genera and in phylogenetic speculation. Development involves the derivation of a 3-dimensional thallus from a 2-celled ascospore through successive divisions in multiple planes, a distinguishing feature of the thallus-forming Laboulbeniomyces. Developmental studies were used by Thaxter (1896) to set generic limits and to interpret life cycles. In fungi, there are few examples of cell divisions in multiple planes. On the other hand, members of the green plant lineage, including Charophyceae, as well as Chlorophyceae and some other “algae,” produce true parenchyma, a 3-dimensional tissue formed by cell divisions in two or more planes with various means of cytokinesis (Bold and Wynne 1985). With few exceptions, fungi produce plectenchyma, tissue derived from apical cell divisions occurring in only one plane. Fungal fruiting bodies (mushrooms, bracket fungi, cup fungi, and lichen thalli) are formed in this way (Alexopoulos et al. 1996; Kirk et al. 2008).

Two types of fungal plectenchymata are recognized: prosenchyma, which maintains its hyphal configuration at maturity, and pseudoparenchyma, which becomes compacted to form isodiametric cells at maturity. The

ascospores, conidia, and stromatic tissues of some of the Pleosporales have cell divisions in several planes (in Pleosporales [as Pseudosphaeriales]: Vuillemin 1912; Orton 1924; Chesters 1938; in lichens: Sanders and de los Ríos 2017, 2018). Laboulbeniomyces have never been considered in such discussions, but they provide the best example of the exceptional pattern of parenchymatic tissue development in fungi. Although developmentally and morphologically different (Tavares 1966, 1980, 1985), the 3-dimensional multicellular thallus of Laboulbeniales and Herpomycetales develops from a 2-celled ascospore through multiple divisions in several planes. The details of cell division are not known, but the process is presumed to be similar to divisions in other fungi, visualized by centripetal deposition of new wall as in divisions in Pleosporales (Mims et al. 1997; Mims and Richardson 2005).

Thalli of Laboulbeniales and Herpomycetales may reach up to several thousand cells (Thaxter 1896; Benjamin 1971; Tavares 1985). The *Thaxteriola* anamorph of *Pyxidiophora* (FIGS. 3C, 4A–C) is usually composed of a few linearly superposed cells, but 3-dimensionality is also found in older thalli on mite hosts held in moist chambers: the ascospores develop into 3-dimensional forms by divisions in more than one plane (Blackwell and Malloch 1989b). This mode of development may have been present in the ancestor of the Laboulbeniomyces and evolved toward the elaborate condition of the thallus in some members of the group (Laboulbeniales, Herpomycetales).

**A three-morph life cycle in the Laboulbeniomyces lineage.**—Phylogeny has been critical to understanding the life history changes among the species of Laboulbeniomyces. Species of *Pyxidiophora* with known life histories have been interpreted as having three distinct morphs or forms (an independent meiosporic state and two mitosporic states). One mitosporic state produces conidia on the mycelium (there are several names for these morphs depending on *Pyxidiophora* species, i.e., *Gliocephalis*), and the other mitosporic morph (*Thaxteriola*) is derived directly from the ascospore. Although synanamorphs (two or more conidial forms produced on the same mycelium) are not uncommon among ascomycetes, ascospore-derived morphs are not common. Coincidentally, the ascospore-derived morph recalls arguments in support of the Floridean Hypothesis—some red algae also have a third morph used in dispersal. *Pyxidiophora* species, however, were not part of the argument for the Floridian Hypothesis. The life histories of well-studied species of *Pyxidiophora* involve

two hosts—fungi (Lundqvist 1980; Kirschner 2003; Jacobs et al. 2005) and arthropods (Blackwell et al. 1986a, 1986b; Blackwell and Malloch 1989b)—and three morphs (Malloch 1995). Ascospores observed on agar produced conidia; conidia gave rise to several rounds of yeast cells, which then produced hyphae that infect a fungal host (FIG. 4C). Although we have focused on the *Thaxteriola* dispersal state (FIGS. 3C, 4A–B), the mycoparasitic mycelium produces the other type of conidium and, eventually, perithecia and ascospores (FIG. 4A) (Blackwell 1994; Weir and Blackwell 2005).

Malloch (1995) presented an enlightening comparison of the life histories of parasites with multiple serial hosts, a condition he termed *heteroxenous* (the zoological term instead of *heteroecious* used by most mycologists for plant parasitic rust fungi). His discussion included not only fungi but also parasitic animals, which he used to elucidate the evolutionary and ecological aspects of the various lifestyles. He juxtaposed the evolutionary transition of the *Pyxidiophora* life history with two hosts, with that of the thallus-forming Laboulbeniomyces with a single host. He explained the evolution of this life cycle by comparing *Pyxidiophora* with rust fungi (Pucciniales), evoking Tranzschel's Law. The rust fungi manifest complicated life cycles that may involve several spore forms produced exclusively on two different hosts. These so-called full cycle or heteroecious rusts have five spore stages occurring on two hosts. But some rusts, related to heteroecious species, occur on a single host and have a reduced number of spore stages. These reductions occur in a fixed pattern: the host on which the basidiospore stage (meiosporic stage) occurs will be the host of the aecial stage (mitosporic stage). In other words, the host in the reduced cycle that carries the meiosporic stage will be that which previously carried a mitosporic stage. If this scenario of an abbreviation of a two-host system is applied to *Pyxidiophora*-Laboulbeniales-Herpomycetales evolution, then the occurrence of the ascus-bearing stage (meiosporic stage) on the insect host that previously carried the mitosporic stage would be the expectation. In other words, asci are produced on the insect host that had carried the mitosporic stage. This pattern, however, is complicated by our current findings that Laboulbeniales and Herpomycetales are not monophyletic (FIG. 1), and inference of the life style of ancestors of the Laboulbeniomyces becomes more complicated.

## ROLAND THAXTER'S FINAL WORD

Thaxter's (1931) last volume of his *Contribution towards a monograph of the Laboulbeniaceae* is devoted to the description and illustration of many new species. Indeed, the undertaking “has become so unwieldy, however that, in

order to publish it at all, it has been necessary to shorten the text ... reduce the number of figures” (Thaxter 1931). There is a single-page introductory note without any statement to culminate his lifetime of work on the group. One is thrust back to his first and second volumes for his general comments on the group (Thaxter 1896, 1908). He did communicate some of his later ideas to Carroll W. Dodge for Dodge’s translation of Gäumann’s book. At the time Gäumann and Dodge’s (1928) book was published, Thaxter (1858–1932) was 70 years old and focused on his last volume of the monograph, which would be published in 1931. Gäumann (1893–1963) and Dodge (1895–1988) were near-contemporaries, but Gäumann was apparently too ill at the time of the translation to participate in updating the text. Dodge, curator of the newly established Farlow Library and Herbarium of Cryptogamic Botany, was fortunate to be at Harvard where he could call upon Roland Thaxter to read and discuss the chapter on the Laboulbeniales in the translation.

Because of the divergence of views between Gäumann and Thaxter, Dodge appended a discussion to the chapter, albeit in small print. This section closes with Thaxter’s refutation of some of Gäumann’s ideas as conveyed by Dodge. At the same time, it represents Thaxter’s final published ideas on the general subject of the thallus-forming Laboulbeniomycetes—their evolution and biology. Of note is the somewhat outdated discussion on evolution, including commentary on the Floridean Hypothesis. Of particular interest is the discussion on the homology of laboulbenialean spermatia with conidia, the difference being the ability of conidia to germinate by a germ tube. Several intriguing ideas, suggested by Gäumann, foreshadow results now gained from DNA analyses. *Thaxeriola* and *Endosporella* were suggested to be mitosporic relatives of thallus-forming Laboulbeniomycetes based on their morphological similarity to male thalli of the dioecious species, and antheridia were homologized with conidiophores of *Thielavia* and *Pyxidiphora* (Gäumann and Dodge 1928). Thaxter refused to be drawn into discussions of the Floridean Hypothesis and other such speculation, and he continued to reject suggestions that the asexual forms, many of which he had discovered (Thaxter 1914, 1920), had anything to do with the thallus-forming Laboulbeniomycetes (Gäumann and Dodge 1928).

Mycologists did develop evolutionary hypotheses before DNA technology came to the forefront, as evidenced by a wealth of literature. Numerous hypotheses based on development, morphology, and life history studies have been tested with DNA analyses and further advanced methods. Sometimes hypotheses based on nonmolecular characters differ only in the polarity of an evolutionary lineage. Thaxter (1891,

1896, 1908) did not dwell on the origin of the Ascomycota but supported placement of the thallus-forming Laboulbeniomycetes within pyrenomycetes in a group at the same rank as Hypocreales, an opinion he shared with Robin as mentioned above (Thaxter 1908). In his studies of the minute fungi (Thaxter 1914, 1920; Gäumann’s “asexual Laboulbeniales”; FIGS. 2C, 3A–D) from the exoskeletons of insects, he recommended placing these novel fungi in “pigeon-holes” until their “fellows” be found that these might help determine relationships (Thaxter 1914, 1920). His views were prophetic and can be extended to all the Laboulbeniomycetes. As alluded to above, the discovery of new fungi, studies of life histories of insect-associated fungi including the minute fungi he had discovered, and the use of new methods involving DNA were all required to bring together fungi with such disparate morphology—but not until almost a century after Thaxter’s monumental studies (Thaxter 1896, 1908, 1924, 1926, 1931).

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