

Phylogenetic Advances in Leotiomyces, an Understudied Clade of Taxonomically and Ecologically Diverse Fungi

C Alisha Quandt, University of Colorado, Boulder, CO, United States

Danny Haelewaters, Purdue University, West Lafayette, IN, United States; Ghent University, Ghent, Belgium; Universidad Autónoma de Chiriquí, David, Panama; and University of South Bohemia, České Budějovice, Czech Republic

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Introduction

The class Leotiomyces represents a large, diverse group of Pezizomycotina, Ascomycota (LoBuglio and Pfister, 2010; Johnston *et al.*, 2019) encompassing 6440 described species across 53 families and 630 genera (Table 1). Comprising, among other morphologies, the inoperculate discomycetes, Leotiomyces fungi represent an enormous amount of ecological diversity – including mutualists and pathogens of plants, saprotrophs, animal pathogens, et cetera. Owing at least partially to their small size or the absence of a fruiting body, Leotiomyces fungi are often overlooked in the field, in the mycology classroom, and in community ecology studies. The major challenges that currently exist in studying Leotiomyces include a lack of understanding about (1) the subclass-level relationships within this clade, (2) the diversity of taxa that are exclusively detected by environmental DNA studies, and (3) the functional roles of such undescribed taxa in the environment.

Morphological and Ecological Diversity of Leotiomyces

Morphology

Many Leotiomyces that form sexual fruiting bodies have various forms of small apothecia with exposed hymenia as well as inoperculate asci – hence the historical grouping to which many Leotiomyces belonged, the *inoperculate discomycetes* (Eriksson, 2005). The asci of Leotiomyces taxa do not possess a lid-like structure (operculum); ascospores are extruded through an amyloid apical ring or by the asci splitting open apically (in which case they are inamyloid). Variations of the typical apothecium morphology exist within the class including those that remain closed until maturity as seen in several orders including Lahmiales and Rhytismatales. Other types of sexual fruiting bodies include the permanently closed cleistothecia, produced by *Cleistothelobolus*, *Leptokalpion*, *Thelebolus* (Thelebolales); *Bicornispora* (Rutstroemiales), *Connersia*, *Pleuroascus* (Helotiaceae), and members of Amorphothecaceae, Erysiphaceae, and Myxotrichaceae (Helotiales).

Several lineages are amenable to growth in axenic culture. Asexual reproduction is commonly observed in Leotiomyces, with some taxa that are exclusively known from their asexual forms (e.g., Castañeda-Ruiz and Kendrick, 1990; Palmer *et al.*, 2014; Ashrafi *et al.*, 2018). Johnston *et al.* (2014) made nomenclatural recommendations to reconcile genera where both sexual and asexual morphs were independently named.

Ecology

Known ecologies from this clade are highly diverse, but Leotiomyces are most commonly known in association with plants either as saprotrophs on already dead material (Baral and Haelewaters, 2015; Hernández-Restrepo *et al.*, 2017; Haelewaters *et al.*, 2018b), endophytes of roots, leaves, bark (Griffith and Boddy 1990; Rodríguez *et al.*, 2009; Grünig *et al.*, 2011), mycorrhizae (Cairney, 2006), or pathogens of roots, shoots, and leaves (Glawe, 2008; Saharan and Mehta, 2008). The plant pathogens in this class are of considerable economic importance – including the powdery mildews of cucurbits and other food crops and the white mold, *Sclerotinia*, that can infect at least 408 species of plants at any stage of development and any tissue type. Bryophilous (or bryosymbiotic, moss-associated) taxa exist as well (Döbbeler, 1997; Stenroos *et al.*, 2010). Examples are members of *Bryoscyphus* and *Mniaecia*, which are biotrophic parasites. Many Leotiomyces are ecologically classified as aquatic hyphomycetes, which decay various plant material in freshwater ecosystems (Baschien *et al.*, 2013).

Taxa not associated with plants are also well represented throughout the class. These include the recently described *Polyphilus*, a genus associated with nematodes, truffle fungi, and plant roots (Ashrafi *et al.*, 2018). Other species associated with animals are species of *Pseudogymnoascus* such as *P. destructans* (the causal agent of white-nose syndrome in bats) (Gargas *et al.*, 2009) and *P. pannorum* (a pathogen of humans) (Gianni *et al.*, 2003). There are many so-called lichenicolous taxa (e.g., *Epicladonia*), which fruit epiphytically on lichens, and the newly described lichen-forming leotiomycete, *Lichinodium* (Prieto *et al.*, 2019). In addition, one species of *Trochila* is a potential mycoparasite on rusts (Gómez-Zapata *et al.*, 2021). Like many fungi in Ascomycota, Leotiomyces are important producers of secondary metabolites (Vaca and Chavez, 2019), including *Glarea*, which makes pneumocandin B – the precursor to one of the most potent antifungal drugs, Caspofungin B (Chen *et al.*, 2013). Yet many isolated Leotiomyces, such as *Glarea*, still have unknown ecological roles.

Table 1 Current classification of the class Leotiomycetes with numbers of genera and species per family^a

	<i>Order, Family</i>	<i>Genera</i>	<i>Species</i>
	<i>Order Chaetomellales</i>		
1	Family Chaetomellaceae	4	75
	<i>Order Cyttariales</i>		
2	Family Cyttariaceae	1	13
	<i>Order Helotiales</i>		
3	Family Amorphanthaceae	1	21
4	Family Arachnopezizaceae	4	65
5	Family Ascocorticaceae	3	4
6	Family Ascodichaenaceae	2	4
7	Family Bloxamiaceae	1	19
8	Family Bryoglossaceae	5	8
9	Family Calloriaceae	14	152
10	Family Cenangiaceae	11	156
11	Family Chlorociboriaceae	1	23
12	Family Chlorospleniaceae	1	17
13	Family Chrysodiscaceae	1	1
14	Family Cordieritidaceae	18	117
15	Family Dermateaceae	12	227
16	Family Discinellaceae	12	75
17	Family Drepanopezizaceae	8	48
18	Family Erysiphaceae	20	976
19	Family Gelatinodiscaceae	9	50
20	Family Godroniaceae	5	43
21	Family Helotiaceae (including Roesleriaceae)	31	483
22	Family Heterosphaeriaceae	1	7
23	Family Hyaloscyphaceae	38	219
24	Family Lachnaceae	17	237
25	Family Leptodontidiaceae	1	11
26	Family Loramycetaceae	2	4
27	Family Mitrulaceae	1	16
28	Family Mollisiaceae	19	382
29	Family Myxotrichaceae	4	45
30	Family Neocrinulaceae	1	2
31	Family Neolauriomycetaceae	3	8
32	Family Pezizellaceae	24	277
33	Family Ploettnerulaceae	12	245
34	Family Rutstroemiaceae	7	115
35	Family Sclerotiniaceae	31	278
36	Family Vibrissaceae	5	42
37	<i>Hysteropezizella</i> lineage	1	26
38	<i>Stamnia</i> lineage	8	119
39	<i>Strossmayeria</i> lineage	2	42
40	Helotiales genera <i>incertae sedis</i>	136	516
	<i>Order Lahmiales</i>		
41	Family Lahmiaceae	1	2
	<i>Order Lauriomycetales</i>		
42	Family Lauriomycetaceae	1	11
	<i>Order Leotiales</i>		
43	Family Cochlearomycetaceae	2	5
44	Family Leotiaceae	4	51
45	Family Mniaeciaceae	2	10
46	Family Tympanidaceae	7	123
47	Leotiales genera <i>incertae sedis</i>	4	12
	<i>Order Lichinodiales</i>		
48	Family Lichinodiaceae	1	4
	<i>Order Marthamycetales</i>		
49	Family Marthamycetaceae	9	60
	<i>Order Medeolariales</i>		
50	Family Medeolariaceae	1	1
	<i>Order Micraspidales</i>		
51	Family Micraspidaceae	1	3

(Continued)

Table 1 Continued

	Order, Family	Genera	Species
	<i>Order Phacidiales</i>		
52	Family Helicogoniaceae	7	33
53	Family Phacidiaceae	9	82
54	Phacidiales genera <i>incertae sedis</i>	1	1
	<i>Order Rhytismatales</i>		
55	Family Cudoniaceae	2	30
56	Family Rhytismataceae	52	607
57	Family Tribliaceae	2	15
58	Rhytismatales genera <i>incertae sedis</i>	9	12
	<i>Order Thelebolales</i>		
59	Family Pseudeurotiaceae	8	44
60	Family Thelebolaceae	10	90
	<i>Leotiomyces genera incertae sedis</i>	20	76

^aReferences: Minnis and Lindner (2013), Karakehian *et al.* (2014, 2019), Baral (2016), Crous and Groenewald (2016), Guatimosim *et al.* (2016), Prasher *et al.* (2016), Crous *et al.* (2017, 2018), Pärtel *et al.* (2017), Ashrafi *et al.* (2018), Marmolejo *et al.* (2018), Quijada *et al.* (2018, 2020), Baral (2019), Baral and Polhorský (2019), Ekanayaka *et al.* (2019), Fryar *et al.* (2019), Johnston and Park (2019), Johnston *et al.* (2019), Prieto *et al.* (2019), Wijayawardene *et al.* (2020), Species Fungorum (2020).

Current Understanding of Evolutionary Relationships

Challenges of Leotiomyces Systematics

Ascomycota is the largest phylum of fungi and among the best studied ones. As a consequence, one might assume that the diversity and systematics within this large and ubiquitous clade is well understood, and for most of its classes this is largely true. However, Leotiomyces have suffered from several issues that have impeded systematics of the class, such that its classification is one of the most poorly understood of any fungal clade. Marker loci designated for the large-scale Assembling Fungal Tree of Life project (AFToL) (Spatafora *et al.*, 2006), which have been successful for most fungal lineages, result in conflicting and unsupported relationships within Leotiomyces. These markers also suggest that traditional morphological characteristics are uninformative in discerning even family-level relationships (Wang *et al.*, 2006b; LoBuglio and Pfister, 2010; Zhang and Wang, 2015; Baral, 2016). Indeed, since molecular characters have become available, the classification of Leotiomyces has undergone multiple drastic updates. However, if there is something that has been consistent among all phylogenetic reconstructions of the class thus far, it is the presence of polytomies, polyphyletic higher taxa, and long branches. Even today, evolutionary hypotheses about family- and order-level relationships are being inferred using a single locus or few uninformative loci, and many taxa have no molecular data at all.

Whole-genome sequences for plant-pathogenic Leotiomyces such as *Botrytis cinerea* and *Sclerotinia sclerotiorum* were first published nearly a decade ago (Amselem *et al.*, 2011), but it was not until very recently that genomes were employed in Leotiomyces systematics. Johnston *et al.* (2019) provided the first evidence that genome-scale data have the potential to resolve relationships within the class, especially within the hyper-diverse order Helotiales. The authors also presented a 5–15 locus phylogeny, which still seems to conflict with the topology of genome-scale sampling (Fig. 1). Genome sampling for many clades was completely lacking, especially outside of Helotiales, which resulted in a lack of support at all deep nodes within the class (Johnston *et al.*, 2019). If genome-scale data are required for resolving the systematics of Leotiomyces, then much work is left to be done (Fig. 2).

The classification by Kirk *et al.* (2008) in the *Dictionary of Fungi* included six orders: Cyttariales, Erysiphales, Helotiales, Leotiales, Rhytismatales, and Thelebolales (with uncertainty). Baral (2016) accepted ten orders of Leotiomyces: Cyttariales, Erysiphales, Helotiales, Lahmiales, Leotiales, Medeolariales, Phacidiales, Rhytismatales, Thelebolales, Tribliidiales. Since that time, several papers were published describing new orders within Leotiomyces (Crous *et al.*, 2017; Hernández-Restrepo *et al.*, 2017; Prieto *et al.*, 2019; Johnston *et al.*, 2019; Quijada *et al.*, 2020). On the other hand, Karakehian *et al.* (2019) synonymized Tribliidiales under Rhytismatales, and Johnston *et al.* (2019) found support for the powdery mildews (Erysiphaceae) to be part of Helotiales. In addition, several leotiomycetous families have shifted in time from one place to another. For example, Tympanidaceae was placed in Phacidiales (Baral, 2016) but the 5–15 locus tree from Johnston *et al.* (2019) recovered the family in Leotiales with high statistical support. Both families Amorophthecaceae and Myxotrichaceae were previously considered Leotiomyces *familiae incertae sedis* but multilocus and genomic-scale phylogenetic analyses have shown that they are both placed in Helotiales (Johnston *et al.*, 2019). Myxotrichaceae is paraphyletic based on the ITS region (Seifert *et al.*, 2007). Ekanayaka *et al.* (2019) synonymized Myxotrichaceae under Amorophthecaceae but only had a single isolate of *Amorophtheca* available, which was placed sister to their Myxotrichaceae clade – highlighting the need for improved taxon sampling in addition to increased sequencing efforts.

Chaetomellales

Well-defined and distinct from other families (Baral, 2016), this group was formerly treated as family Chaetomellaceae within Helotiales. The elevation to ordinal level was based on a nuclear ribosomal RNA large subunit (LSU) phylogeny (Crous *et al.*, 2017)

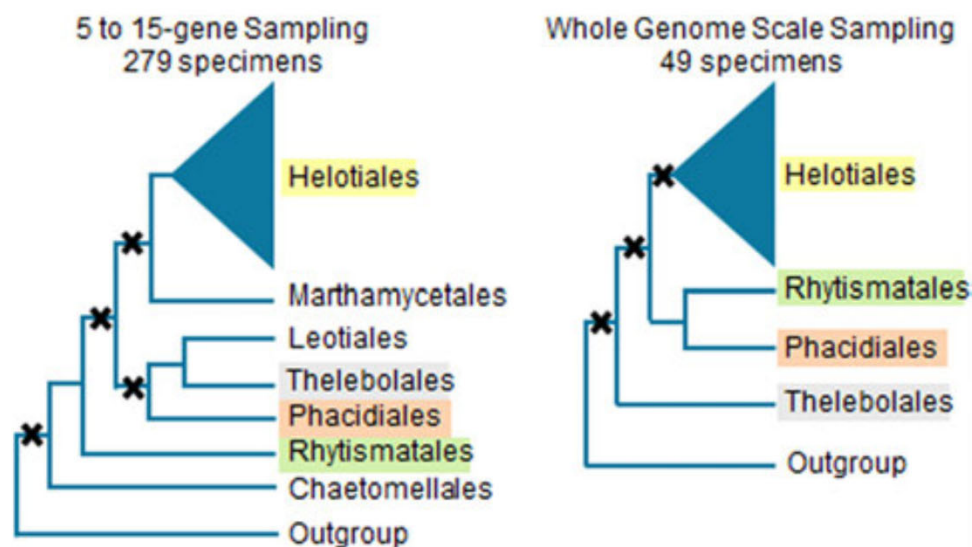


Fig. 1 Current evolutionary hypotheses about interordinal relationships within the class Leotiomyces, based on Johnston *et al.* (2019). *Left*, 5–15 locus phylogeny based on 279 isolates; *right*, whole-genome scale phylogeny based on 49 isolates. Nodes where support is lacking are marked with a black “x”. Orders that are represented in *both* analyses are highlighted in color (Helotiales in yellow, Phacidiales in peach, Rhytismatales in green, Thelebolales in gray), showing major topological disagreement between the two analyses. Other orders have thus far not been considered in Leotiomyces-wide multilocus or genome-scale analyses. Modified from Johnston, P.R., Quijada, L., Smith, C.A., *et al.*, 2019. A multigene phylogeny toward a new phylogenetic classification of Leotiomyces. IMA Fungus 10, 1.

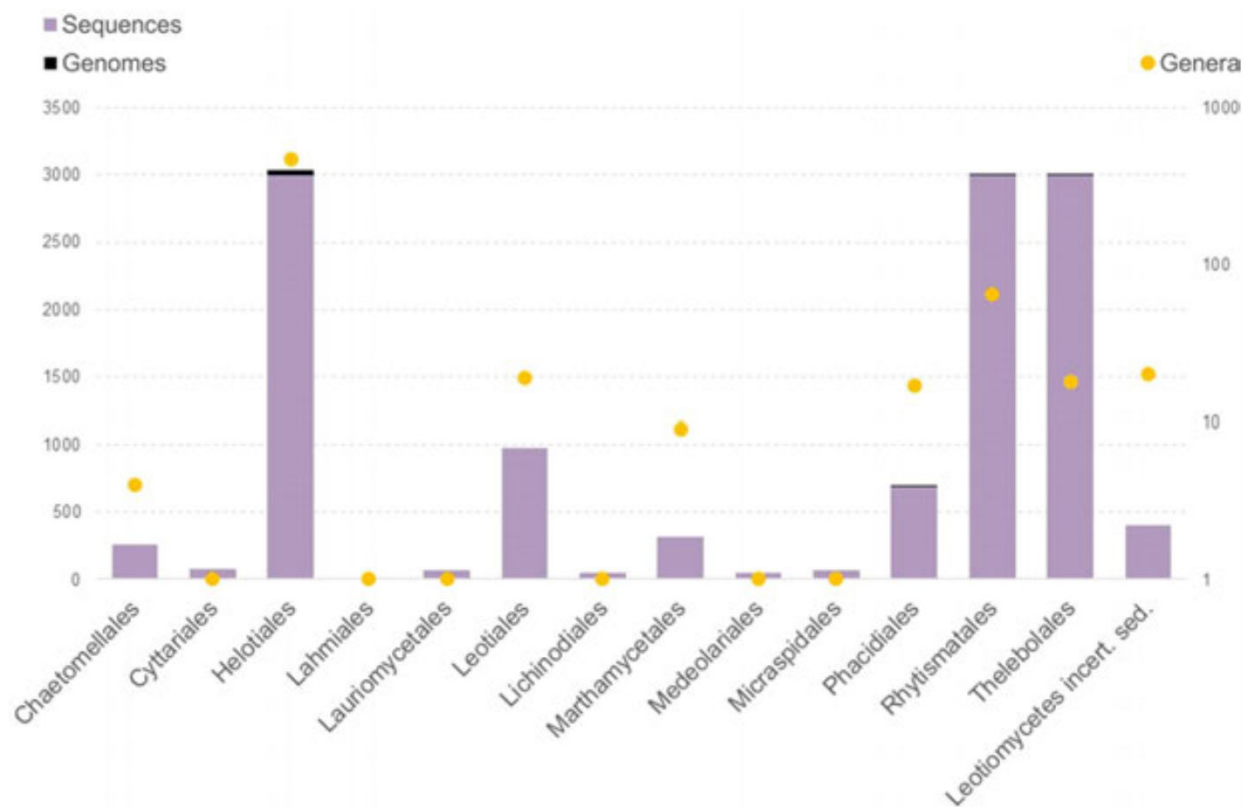


Fig. 2 Current status of sequences submitted to NCBI GenBank and published and/or publically available genomes of class Leotiomyces. Numbers of sequences were capped at 3000. A logarithmic scale is used for numbers of genera (Y axis, *right*). Note that whole-genome sampling has only been done in four orders: Helotiales (35 genomes), Phacidiales (1), Rhytismatales (6), and Thelebolales (4). Sequences are available for every order with the exception of Lahmiales.

and confirmed by the phylogenetic reconstruction of a 5–15 locus dataset (Johnston *et al.*, 2019). Apothecia of Chaetomellales are 0.2–1.0 mm in diameter, develop beneath the host epidermis, and are hairless (*Pilidium*) or covered with long setae (*Chaetomella*). The asci are 8-spored and have a thick-walled inamyloid apex. There are two distinct anamorphs (synanamorphs): sessile pycnidia that open by fissures in *Sphaerographium* and sessile or long-stalked sporodochia in *Synchaetomella*. These anamorphs can be hairless or have scattered setae. Members of Chaetomellales are parasitic or saprotrophic on leaves, herbaceous stems, and dicot fruits. Some taxa are host-specific, whereas others may have multiple hosts.

Cyttariales

Species of *Cyttaria*, the single genus in this order, are obligate biotrophic associates of *Nothofagus* trees in southern South America and southeastern Australasia (Peterson and Pfister, 2010). They produce trunk and branch cankers on their host trees. During his voyage on the *HMS Beagle*, Charles Darwin collected in Chile the golf ball-shaped fruiting bodies that would serve as type material for the first two described *Cyttaria* species (Berkeley, 1842). *Cyttaria* species are distinct from other leotiomycetous taxa by their spherical fruiting bodies of sterile stroma with numerous apothecial cavities in a honeycomb-like arrangement. Asci have an amyloid apical ring and the anamorph stage is pycnidial. Peterson *et al.* (2010) found high co-phylogenetic structure between *Cyttaria* and *Nothofagus*, even though they did not report simple one-to-one relationships. Cyttariales is apparently closely related to Helotiales. Using a four-locus phylogenetic reconstruction of a Leotiomyces-wide dataset, Peterson and Pfister (2010) retrieved Cyttariales as sister to Cordieritidaceae, leaving the order Helotiales paraphyletic. In the concept of Helotiales sensu Johnston *et al.* (2019), Cyttariales would be a family-level clade in this mega-order, but multilocus data for *Cyttaria* is still lacking.

Helotiales

The most speciose and best studied order in the class (Table 1), initial phylogenetic work discerned that this expansive order was polyphyletic (Wang *et al.*, 2006a,b; Schoch *et al.*, 2009). Currently, a broad concept is maintained based on multilocus and genome-scale phylogenetic analyses; Helotiales sensu Johnston *et al.* (2019) also includes Cyttariaceae (pending multilocus data) and the Erysiphaceae family of powdery mildews (see Section “Helotiales, a Mega-Order in Disarray”). Helotiales fungi are mostly apothecial, with apothecia that are usually < 2 mm in diameter, sessile to long-stalked, dark to bright-colored, superficial or erumpent through the plant tissue. Some representatives form non-apothecial ascomata; *Amorphotheca* (Amorphothecaceae), *Bicornispora* (Rutstroemiaceae), *Connersia*, *Pleuroascus* (Helotiaceae), and members of Erysiphaceae and Myxotrichaceae sensu Johnston *et al.* (2019) are cleistothecial, whereas members of Loramyetaceae and *Unguicularia* (Hyaloscyphaceae) are perithecial. Most members of Helotiales are saprotrophs, decaying dead organic material, but some are associated with living organisms as either parasites, pathogens, or mutualists (Stenroos *et al.*, 2010; Baral, 2016; Haelewaters *et al.*, 2018b; Tanney and Seifert, 2020).

Lahmiales

The order Lahmiales was introduced by Eriksson (1986) to accommodate a single genus with currently two species (Species Fungorum, 2020). In 2007, the order was placed as *Pezizomycotina incertae sedis* by Hibbett *et al.* (2007), along with orders Medeolariales and Triblidiales, which we now know all belong to Leotiomyces. Raitviir and Spooner (1994) suggested several placements for Lahmiales, in Dothideomycetes and Lecanoromycetes. Currently, still, no sequences exist but Baral (2016) suggested a placement of the order within Leotiomyces, noting the resemblance of ascomata of *Lahmia* with Rhytismatales except the bitunicate asci and the absence of a clypeate stroma. Ascomata of *Lahmia* measure 0.13–0.30 mm in diameter, they are black, erumpent, and open at maturity by irregular radial splits. These fungi occur on the bark of *Populus* trees in boreal North America and northern Europe. The Lahmiales order is probably the least studied of leotiomycetous orders; the last described species, *Lahmia waghornii*, dates from 1900. (Note that *Lahmia plumbina* was described in 1930, but has since been recombined in the genus *Toninia*, Ramalinaceae, Lecanoromycetes.) Recent collections of Lahmiales are scarce and ambiguous, and no sequences are currently available (Fig. 2).

Lauriomycetales

This recently described order (Hernández-Restrepo *et al.*, 2017) consists of a single family with a single asexual genus, *Lauriomyces* (Castañeda-Ruiz and Kendrick, 1990), and includes 11 species (Somrithipol *et al.*, 2017). Characterized by brown conidiophores with acropetal chains of hyaline conidia, these species are only known from leaf litter and have a cosmopolitan distribution. Phylogenetic placement of Lauriomycetales suggests this is an early diverging lineage within Leotiomyces, possibly sister to Chaetomellales (Hernández-Restrepo *et al.*, 2017; Somrithipol *et al.*, 2017) although so far this relationship is based solely on ribosomal DNA data.

Leotiales

Carpenter (1988) erected Leotiales with genus *Leotia* as its type to accommodate taxa in Helotiales. The most recent conception of Leotiales is based on Johnston *et al.*, 2019, and includes large, stipitate to clavate apothecial members of Leotiaceae (including

Leotia and *Microglossum*), stipitate to sessile apothecial taxa in Tympanidaceae s.s. and *Mniaecia*, and several aquatic hyphomycete genera. Ecological niches in this order vary from plant-pathogenic taxa such as *Tympanis* (Ouellette and Pirozynski, 1974), to the liverwort-parasitic or possibly lichenized *Mniaecia* (Raspé and de Sloover, 1998; McCune and Stone, 2020), and potentially arbutoid mycorrhizal taxa such as *Leotia* (Kühndorf et al., 2015), although some consider this genus to be saprobic.

Lichinodiales

Formerly classified in Lichinomycetes, the lichenized genus *Lichinodium* is now recognized as a member of Leotiomycetes (Prieto et al., 2019). Lichinodiales is possibly allied with Leotiales, although more sampling is needed, especially given the recent report that *Mniaecia* could be lichenized (McCune and Stone, 2020). The four recognized species of *Lichinodium* are filamentous lichens (so called because of the lack of a stratified lichen thallus) with cyanobacterial photobionts in the genus *Rhizonema* (Prieto et al., 2019). They produce gelatinous brown apothecia with indistinct margins, 0.5–1 mm in diameter, and prototunicate asci without amyloid staining. They are found on various substrates in cool, humid environments.

Marthamycetales

Erected by Johnston et al. (2019) to accommodate the single family Marthamycetaceae, these taxa are all saprobic on plant material and produce ascomata erumpent through host tissue (Minter, 2003). Asci are thin-walled and amyloid with variation in ascospore septation among the different taxa. Notable genera include *Propolis* (see Minter, 2003 for a review of species traditionally placed in this genus) and *Marthamyces* in which there has been considerable recent work describing new species (Johnston, 2006; Johnston and Park, 2019; Crous et al., 2019).

Medeolariales

This monotypic order is represented by *Medeolaria farlowii*, a pathogen of *Medeola virginiana*, a small tuber-bearing plant found in eastern North America (Thaxter, 1922). Korf (1973), in Eriksson (1982), placed this taxon in its own family and order but with uncertain placement within Ascomycota presumably due to lack of characters (including ascomatal and ascus types) that unite it with other clades. Based on the phylogenetic reconstruction of a nuclear ribosomal DNA dataset, LoBuglio and Pfister (2010) transferred Medeolariales into Leotiomycetes. This enigmatic taxon makes a loosely organized hymenium directly below the leaf whorls of its host. The fungus is present in multiple parts of infected plants, including in seemingly uninfected leaves (Pfister and LoBuglio, 2013). *Medeolaria farlowii* has an inamyloid ascus apex and a thus far unknown method of ascospore release from the ascus (Korf, 1973; LoBuglio and Pfister, 2010). Its occurrence is reported as widespread in the northeastern US (Pfister, 1984; Pfister and LoBuglio, 2013).

Micraspidales

The genus *Micraspis* was described by Darker (1963) to accommodate a fungus that caused a foliar disease resembling *Phacidium* snow-blight of *Picea mariana* in Canada. Ascomata (apothecia) and conidiomata are macroscopically indistinct; they are immersed and become erumpent from the host tissue. The genus was historically placed in either Helotiales (Eriksson, 1999; Lumbsch and Huhndorf, 2009) or Phacidiales (Darker, 1963; Korf, 1973; Baral, 2016). However, based on the combination of unique morphological features and a multilocus phylogenetic reconstruction, Quijada et al. (2020) proposed a new family (Micraspidaceae) and order (Micraspidales). Synapomorphic morphological characteristics of *Micraspis* are: the ectal excipulum and covering layers of both ascomata and conidiomata are composed of *textura epidermoidea*, ectal excipulum is covered on the outside by a thick refractive yellowish gel, ascospores germinate at the poles, and conidia are produced directly from germ tubes or ascospore walls (Quijada et al., 2020).

Phacidiales

Quijada et al. (2018) referred to Phacidiales as “a good example of the chaotic situation within the class.” Since Bessey (1907) described the order, different genera and families have been placed in it. Baral (2016) and Quijada et al. (2018) considered four major lineages: Helicogoniaceae, Phacidiaceae, Tympanidaceae, the *Mniaecia* lineage, and *Coma* as Phacidiales *incertae sedis*. However, the 5–15 locus phylogenetic analysis of Johnston et al. (2019) retrieved the *Mniaecia* lineage (as family Mniaeciaceae) and Tympanidaceae as highly supported clades within Leotiales. Phacidiales includes both saprobic and parasitic species of plants, fungi, and lichens. Whereas intrahymenial parasitic *Helicogonium* species only form ascogenous hyphae (no ascomata), other members of Phacidiales produce apothecia that open in the prothymenial to mesohymenial phase (fide Kimbrough, 1981b), with paraphyses lacking vacuolar bodies, asci with or without amyloid ring, and ascospores with variable lipid content (Baral, 2016).

Rhytismatales

Fungi in Rhytismatales are plant-associated either as pathogens, endophytes, or saprotrophs with a near-global distribution. Fruiting bodies vary from the earth-tongue to club-shaped members of Cudoniaceae to the host-immersed fruiting structures of Rhytismataceae (the largest family in the order), which may or may not include a stromatic layer that splits open to reveal the hymenium at maturity. Some recognize Cudoniaceae as included within Rhytismataceae (Lantz *et al.*, 2011) while others maintain both families. In the 5–15 locus phylogenetic reconstruction of Johnston *et al.* (2019), Cudoniaceae was retrieved as a well-supported clade within a paraphyletic Rhytismataceae. Although no synapomorphy exists for the modern concept of Rhytismatales, filiform ascospores with a gelatinous sheath are characters present in many taxa. Many genera formerly included in Rhytismatales such as *Propolis*, *Marthamyces*, *Pseudophacidium*, and *Ascodichaena* were recognized as belonging to different clades (Lantz *et al.*, 2011) and subsequently reclassified into other orders (Johnston *et al.*, 2019; Karakehian *et al.*, 2019).

Thelebolales

Historically, most coprophilic discomycetes were placed in Pezizales (Pezizomycetes), however as now conceived that order is composed mainly of taxa with operculate asci. Based on morphology and more recently molecular phylogenetic studies, *Thelebolus* and other allied coprophilic taxa with inoperculate asci were recognized as an independent lineage from Pezizales (Kimbrough and Korf, 1967; Landvik *et al.*, 1998). Species of *Thelebolus* have cleistothecoid ascomata with variable numbers of ascospores per ascus, ranging from 8 to more than 1000 spores (Kimbrough, 1981a). An expansive definition of the order now includes Pseudeurotiaceae in addition to Thelebolaceae (Johnston *et al.*, 2019; Batista *et al.*, 2020). Coprophilic and psychrophilic species are found in both of these families (Robinson, 2001; de Hoog *et al.*, 2005) although morphologically they differ significantly, hence their previously unrecognized relationship (Baral, 2016). Species of Pseudeurotiaceae form immersed or superficial cleistothecia on wood and decaying plant material. *Pseudogymnoascus destructans*, the causal agent of white-nose syndrome in bats, is a member of this family but only known from its asexual state. Little work has been done to understand the systematics of the family Pseudeurotiaceae (Minnis and Lindner, 2013).

Biases in Sampling of Leotiomycetes

Distributional Unevenness

Geographically, Leotiomycetes are found on all continents including Antarctica, but their taxonomy has been based primarily on the diversity in the temperate Northern Hemisphere, especially in western Europe and the United States, while tropical locations are underrepresented (Fig. 3; Piepenbring *et al.*, 2018). Leotiomycetes and Helotiales in particular include some of the oldest descriptions of mycological taxa (Micheli, 1729). As the cradle for fungal taxonomy, Europe has been well-documented in terms of Leotiomycetes diversity, with early workers such as Pier Antonio Micheli (Italy, 1679–1737), Marie-Anne Libert (Belgium, 1782–1865), Elias Fries (Sweden, 1794–1878), Heinrich Rehm (Germany, 1828–1916), Émile Boudier (France, 1828–1920), and Pier Andrea Saccardo (Italy, 1845–1920). Of the 630 genera that have been placed in the class, 85% are based on species described from temperate Europe and to a lesser extent North America, whereas only 5% are based on species described from the tropics, Asia, or the temperate Southern Hemisphere, with the fewest descriptions from Africa (Haelewaters *et al.*, 2021).

Recent efforts in North America have focused mostly on macrofungi (Bruns, 2011; 2012) but there are some reports of undescribed and rare Leotiomycetes – including taxa that were previously unsequenced. Localities include New Brunswick, Canada (Quijada *et al.*, 2020), the Rocky Mountains in Colorado (C.A. Quandt, unpublished), Boston Harbor Islands National Recreation Area in Massachusetts (Haelewaters *et al.*, 2018a), and Great Smoky Mountains National Park in North Carolina and Tennessee (Hustad and Miller, 2011).

Helotiales, a Mega-Order in Disarray

Since the description of Helotiaceae (Rehm, 1896), this family has been expanded with 100s of species such that it currently is the most speciose family of the order Helotiales. Several taxa within the family, such as *Hymenoscyphus*, turned out to be polyphyletic (Stenroos *et al.*, 2010; Baral *et al.*, 2013; Baral *et al.*, 2015; Johnston *et al.*, 2019). Also higher taxa within Helotiales have been shown to be polyphyletic, although phylogenetic results from different studies heavily depend on taxon sampling and the number of loci used to estimate evolutionary relationships. Baral *et al.* (2015) found that Helotiaceae and Lachnaceae were paraphyletic based on the phylogenetic reconstruction of an ITS–LSU dataset. Johnston *et al.* (2019), on the other hand, presented a 5–15 locus tree in which both Helotiaceae and Lachnaceae were monophyletic. Taxon sampling within Helotiaceae differed between the two studies but mostly overlapped for Lachnaceae, clearly showing the importance of multiple, phylogenetically informative loci.

Baral *et al.* (2015) referred to several helotialean families and subfamilies as wastebaskets. Throughout the years, several taxa were described in these higher lineages based on morphological synapomorphies – traditionally, the morphology of ascomata (Zhang and Wang, 2015). As an example, the subfamily Encoelioidae encompassed taxa with long-lived and desiccation-tolerant apothecia. However, a five-locus phylogenetic analysis revealed that Encoelioidae was highly polyphyletic (Pärtel *et al.*, 2017).

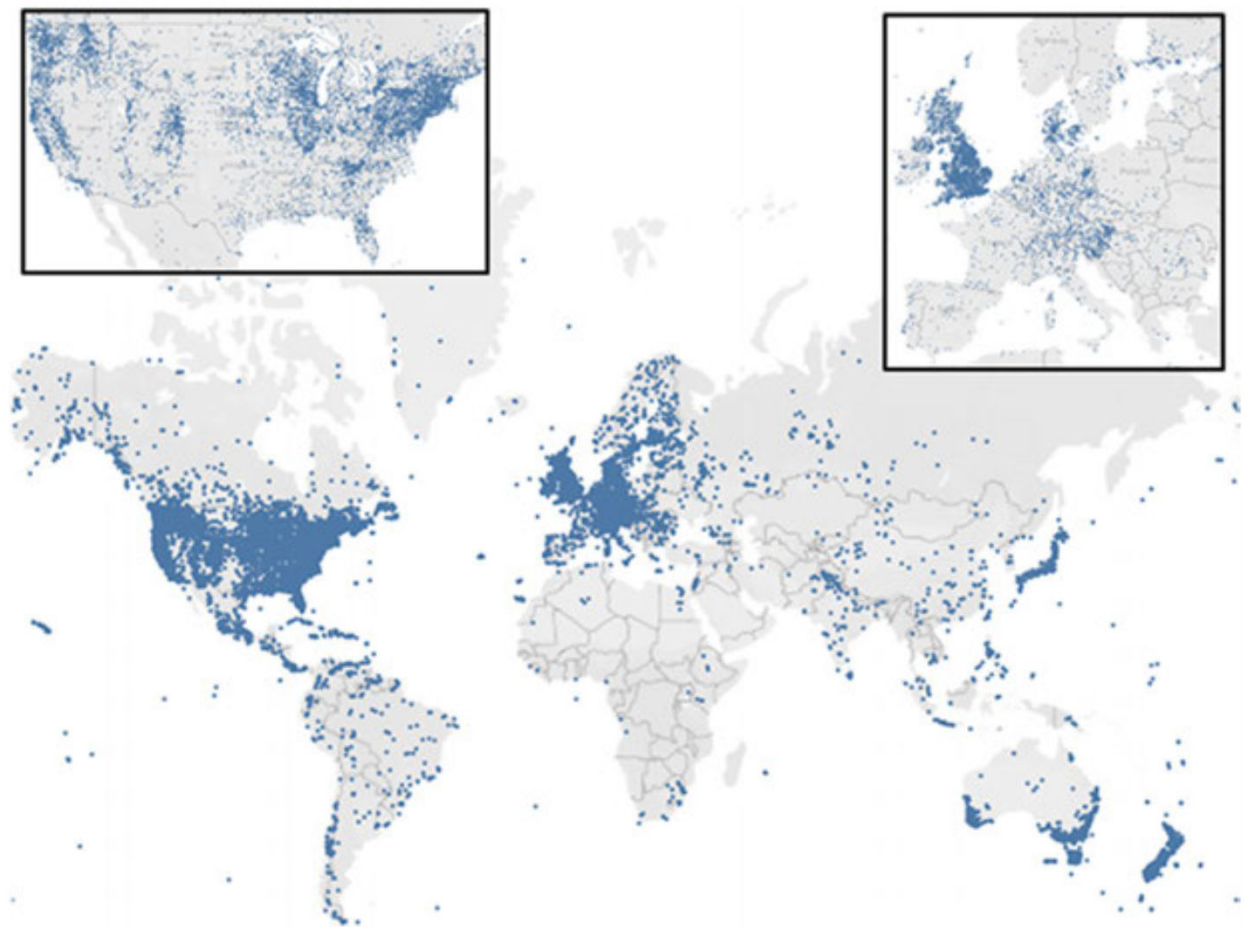


Fig. 3 Global distribution of Leotiomyces collections deposited in database herbaria, based on a dataset of 217,480 records downloaded from MyCoPortal (2020). Insets show closer views of collections from the continental US and Europe.

Species known or combined as *Encoelia* were retrieved in seven different genera in six families, two of which had to be resurrected (Cenangiaceae and Cordieritidaceae). As more multi-locus sequences and genome-scale data become available, researchers are learning that several of the characters once thought to define a higher taxon have multiple origins in the order.

Evidently, several taxa once considered as Helotiales are now recognized as members of new, distinct orders. In addition, in recent years and often based on molecular phylogenetic studies, several new families have been erected within the order and several more unnamed lineages proposed (Han *et al.*, 2014; Baral, 2016; Crous *et al.*, 2017; 2018; Pärtel *et al.*, 2017; Baral and Polhorský 2019; Johnston and Baschien, 2020). Johnston *et al.* (2019), supported by their 5–15 locus and genome-scale phylogenies, chose to recognize a larger, more inclusive definition of Helotiales in lieu of a more restricted definition, which would have necessitated the creation of several new orders. The highly diverse mega-clade Helotiales sensu Johnston *et al.* (2019) also includes the previously segregated order, Erysiphales, the powdery mildews, which encompasses more than 976 species in 20 genera (Marmolejo *et al.*, 2018; Wijayawardene *et al.*, 2020). However, not all authors agree with this proposal because of the morphological and ecological distinctiveness of powdery mildews (Ekanayaka *et al.*, 2019; A.H. Ekanayaka and K.D. Hyde in Wijayawardene *et al.*, 2020).

Understudied Ecological Niches

Historically, the majority of Leotiomyces have been described from decaying, terrestrial plant materials. However, based on environmental studies, we know that Leotiomyces, such as the psychrophilic *Pseudogymnoascus* (Rosa *et al.*, 2019) and the mycorrhizal symbiont of moss, *Rhizoscyphus*, are dominant members of polar environments (de Hoog *et al.*, 2005; Bridge and Spooner, 2012; Rosa *et al.*, 2019). Culture-based studies have isolated Leotiomyces from marine (Baral and Rămă 2015; Fryar *et al.*, 2019) and a multitude of freshwater aquatic environments (Baschien *et al.*, 2013; Tsui *et al.*, 2016). The so-called Dark Septate Endophytes, most of which are Leotiomyces, can be dominant in Alpine ecosystems. Studies based purely on DNA barcoding have suggested that Leotiomyces are dominant in many environments including peat bogs (Lamit *et al.*, 2017), the

arctic tundra, and in tropical montane soils (Tedersoo *et al.*, 2014). These studies, however, are often limited in their geographical scope and many of the detected taxa have no names.

Future Research Perspectives

Much has changed in the field of evolutionary biology since Leotiomyces has been tackled holistically, in addition to the widespread availability and low cost of whole-genome sequencing. Improved technologies and techniques including amplicon-based sequencing, single-cell genomics, metagenomics, transcriptomics, and high-throughput computing, have the ability to transform our understanding of the diversity and ecology in this class. Examples of both culture-dependent studies and environmental sequencing work suggest that Leotiomyces diversity is broader than currently understood. It is estimated that only 5–7% of Leotiomyces diversity has been formally described. This warrants a focus on taxa that are difficult to culture and undersampled geographic areas and habitats that could be diverse in Leotiomyces. Examples of such areas are tropical and subtropical regions around the world, Africa, and much of the Asian continent. Efforts are being undertaken to fill some of these distributional gaps of leotiomycetous knowledge, with fieldwork planned in southeastern Africa (Mozambique) and northern Asia (Siberia). In addition to targeted sampling of geographic areas, certain taxonomic lineages that are currently lacking molecular phylogenetic studies should be targeted in future research. Taxonomically poorly sampled regions of the class have recently produced many phylogenetically distinct genus-level and family-level clades (e.g., Somrithipol *et al.*, 2017; Quijada *et al.*, 2018), and others that remain unnamed (Johnston *et al.*, 2019). Other higher taxonomic level groups that need taxonomic revision include Lahmiales and Thelebolales, in addition to groups such as Calloriaceae, Hyaloscyphaceae, and the “Stamnaria lineage” in Helotiales and Cudoniaceae + Rhytismataceae in Rhytismatales. Finally, any molecular phylogenetic data for the 170 *incertae sedis* genera throughout the class would greatly contribute to our understanding of evolutionary relationships of Leotiomyces. It is likely that these sampling initiatives will reveal undescribed clades within the class and thereby help to resolve some of the deeper nodes that have not yet received support.

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Further Reading

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