



Botryandromyces, a morphology-based genus concept scrutinized by molecular data

Warre Van Caenegem¹ · André De Kesel² · Danny Haelewaters^{1,2,3,4}

Received: 3 September 2023 / Revised: 14 October 2023 / Accepted: 16 October 2023 / Published online: 13 November 2023
© The Author(s) 2023

Abstract

Laboulbeniales (*Ascomycota*) are an order of understudied, biotrophic microfungi uniquely associated with arthropods. More than 2300 species are described but only a fraction of those have been sequenced. Molecular studies have shown that cryptic diversity and phenotypic plasticity are present within the *Laboulbeniales*. Thus far, all of the 146 genera described in *Laboulbeniales* have been based on morphological characteristics; features commonly used to delineate genera are the organization of receptacle cells and the number of perithecial outer wall cells. The genus *Botryandromyces* was erected to accommodate two species, *B. heteroceri* and *B. ornatus* (type), which share similar morphological characteristics and are different from other genera in their number of perithecial outer wall cells. Here, we generated sequences of multiple loci (18S, ITS, and 28S) of *B. heteroceri* and several *Laboulbenia* species. Our phylogenetic analyses retrieved *Botryandromyces* within *Laboulbenia* with high support. The two *Botryandromyces* species are similar to related *Laboulbenia* species in their upper receptacle (i.e., cells IV and V). We propose to transfer *Botryandromyces ornatus* and *B. heteroceri* to *Laboulbenia* as *L. heteroceri* and *L. mairei* nom. nov., respectively, due to a complicated taxonomic history. These results advocate the use of molecular data and the necessity of an integrative taxonomy approach in the study of *Laboulbeniales* not only to delineate species, but also to investigate relationships among species, genera, and higher taxa as well as to understand the evolution of morphology in this group of fungi.

Keywords Integrative taxonomy · *Laboulbeniales* · Nomen novum · rDNA

Introduction

Macroscopic and microscopic morphology of sporocarps has traditionally been of great importance in identifying and describing species of fungi and classifying them into higher taxa (Bridge et al. 2005; Cao et al. 2021; Maharachchikumbura et al. 2021). The use of molecular data has challenged

several proposed morphogroups. A well-studied example within *Russulales* (*Agaricomycetes*) is the convergent evolution of sequestrate fruiting bodies in the genera *Lactarius* and *Russula*. Multiple genera were erected to accommodate these sequestrate forms, but early molecular work has indicated that these genera are polyphyletic and that these sequestrate forms independently evolved multiple times within *Lactarius* and *Russula* (Miller et al. 2001; Nuytinck et al. 2003; Eberhardt and Verbeken 2004). Similar cases can be found in other genera including *Agaricus*, *Amanita*, *Cortinarius*, and *Entoloma* (Peintner et al. 2001; Co-David et al. 2009; Justo et al. 2010; Sánchez-García et al. 2020). Similarly, morphologically defined higher taxa of *Ascomycota* have also been challenged by molecular data (Arzanlou et al. 2007; Crous et al. 2007, 2009, 2021; Wynns 2015).

A severely understudied group of *Ascomycota* is the order *Laboulbeniales* (*Laboulbeniomycetes*). These fungi obligately live on the exoskeleton of arthropod hosts. They do not form a typical hyphal system but a multicellular, 3-dimensional structure called a thallus (Haelewaters et al.

Section Editor: Cobus CM Visagie

✉ Danny Haelewaters
danny.haelewaters@gmail.com

¹ Research Group Mycology, Department of Biology, Ghent University, 9000 Ghent, Belgium

² Meise Botanic Garden, 1860 Meise, Belgium

³ Faculty of Science, University of South Bohemia, 370 05 Ceske Budejovice, Czech Republic

⁴ Institute of Entomology, Biology Centre of the Czech Academy of Sciences, 370 05 Ceske Budejovice, Czech Republic

2021a). More than 2300 species in 146 genera are currently recognized in *Laboulbeniales*. The vast majority of this diversity is described based on morphology while only a fraction has been sequenced (Haelewaters et al. 2021b). Molecular studies have presented evidence that both cryptic diversity and phenotypic plasticity are present within *Laboulbeniales* (Goldmann and Weir 2012; Goldmann et al. 2013; Haelewaters et al. 2018; Haelewaters and Pfister 2019; Van Caenegem et al. 2023a). This makes delineating the taxa of *Laboulbeniales* solely based on morphology difficult. The first molecular phylogenies of *Laboulbeniales* showed that several morphologically defined higher taxa are non-monophyletic. Antheridial characteristics appear to have a low systematic value, while features of the perithecium seem to be phylogenetically informative (Goldmann and Weir 2018; Haelewaters 2018).

The genus *Botryandromyces* was erected by Tavares and Majewski (1976) to accommodate two species that had already been described in other genera, *Botryandromyces heteroceri* (as *Misgomyces heteroceri*) (Fig. 1) and *Botryandromyces ornatus* (as *Laboulbenia heteroceratis*), which

was selected as the type species of the genus (Tavares and Majewski 1976). Both species are reported on *Heteroceridae*, mainly *Heterocerus* Fabricius 1792, but also on the following related genera: *Augyles* Schiödte, 1866; *Erus* Pacheco, 1964; *Lanternarius* Pacheco, 1964; *Littorimus* Gozis, 1885; and *Neoheterocerus* Pacheco, 1964 (Tavares and Majewski 1976; Tavares 1985; Santamaria and Pedersen 2021). Species of *Botryandromyces* are characterized by (i) sessile antheridia, clustered around the spore septum; and (ii) a perithecium with three outer wall cells in two adjacent vertical tiers and four in the other two tiers (Tavares and Majewski 1976; Tavares 1985).

In the protologue of *Laboulbenia heteroceratis*, Thaxter (1912) wrote that the production of sessile antheridia from proliferous cells had not yet been reported within the genus *Laboulbenia*. However, he stated that “the basal cells of its appendages may assume an appearance very similar to that of some of the aquatic forms on Gyrinidae.” The insertion cell of *L. heteroceratis* is concolorous with the surrounding cells. It also tends to divide into several smaller cells (Thaxter 1912). In contrast, the vast majority of species in

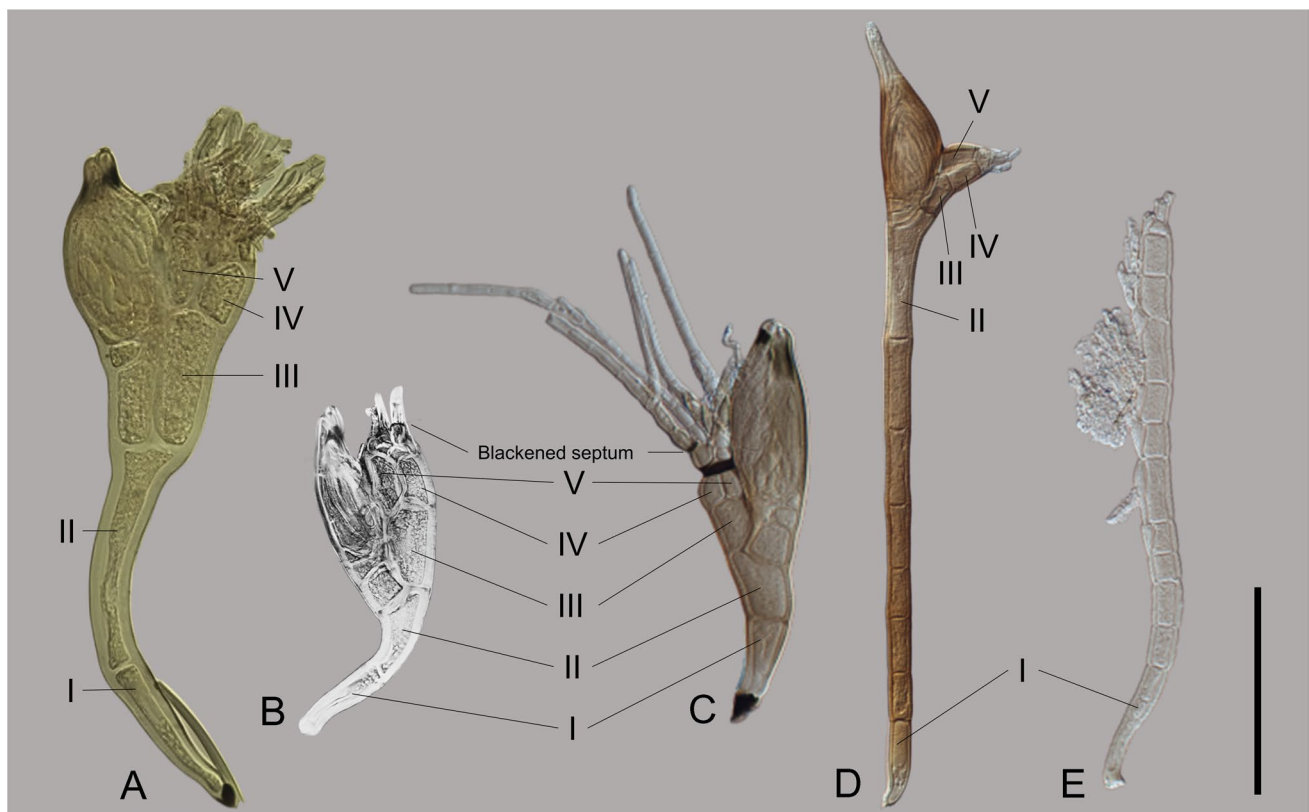


Fig. 1 *Laboulbenia* spp. **A** *Laboulbenia heteroceri*. Reprinted from Goldmann and Weir (2018), Molecular phylogeny of the *Laboulbeniomycetes* (Ascomycota), Fungal Biol. 122:87–100, with permission from Elsevier. **B** *Laboulbenia heteroceri*, reproduced and edited from Tavares and Majewski (1976), with permission from Mycotaxon. **C** *Laboulbenia slackensis*, slide D. Haelew. 4155b. **D** *Laboulbenia*

mairi, slide D. Haelew. 4847a. **E** *Laboulbenia mairi*, aberrant thallus from slide D. Haelew. 4197a. Indicated are the lower receptacle (cells I and II), the upper receptacle (cells III, IV, and V), and the blackened septum between the basal and suprabasal cell of the outer appendage. Scale bar = 100 μ m

Laboulbenia have a simple, blackened insertion cell. Thaxter (1912) did not include figures in his description, but *L. heteroceratis* is illustrated in Tavares and Majewski (1976: Fig. 2) and Goldmann and Weir (2018: Fig. 4 I).

Botryandromyces heteroceri was described as *Misgomyces heteroceri* by Maire (1920). Species of *Misgomyces* have perithecia with four outer cell walls of unequal height in each vertical tier and compound antheridia (Tavares 1985). The genus *Botryandromyces* was erected to accommodate *M. heteroceri*, as it has a perithecium with different cellular organization and simple sessile antheridia (Tavares and Majewski 1976). *Botryandromyces heteroceri* differs from *B. ornatus* by showing considerable variation in the number of cells in the lower receptacle, ranging from two to eight, and even 33 in aberrant, fili-form thalli. *Botryandromyces ornatus* always has a two-celled lower receptacle and shows a blackening on the perithecial apex, which *B. heteroceri* lacks (Tavares 1985; De Kesel 2009; Santamaria and Pedersen 2021).

Recent molecular work based on the small ribosomal subunit (18S) of the ribosomal RNA (rRNA) gene placed *B. ornatus* within *Laboulbenia*, which makes the latter a paraphyletic group (Goldmann and Weir 2018). The authors refrained from making taxonomic changes given their restricted sample size (one isolate of *Botryandromyces*, three isolates of *Laboulbenia*). Although morphological differences between the two genera are clear and well-defined, this result was not surprising given Thaxters' (1912) decision to place the taxon currently accepted as *B. ornatus* in *Laboulbenia*. Haelewaters (2018) retrieved *B. ornatus* as a sister to the genus *Laboulbenia*, which was represented by 13 isolates. Also, this analysis was only based on the 18S region.

Here, based on recently collected material, we present a phylogeny incorporating new sequence data from three loci for *B. heteroceri* and show the placement of both species of *Botryandromyces* in relation to *Laboulbenia*.

Material and methods

Collection and identification of beetles and *Laboulbeniales*, and morphological study

Specimens of *Heterocerus* Fabricius, 1792 (*Coleoptera*, *Heteroceridae*), were captured alive in 2022 and 2023 using a light trap (160w ML) in a private garden in Herzele, Belgium. Specimens were immediately screened alive for infections with *Laboulbeniales* using a dissecting microscope. Infected specimens were stored in 99% ethanol, and uninfected specimens were released back into nature. Other hosts included in this study (*Coleoptera*, *Carabidae*) were sent by entomologists or collected by W.V.C. and A.D.K. using pitfall traps and by hand, from multiple localities in Belgium, Latvia, the Netherlands, Uganda, and the USA. These specimens were

used to broaden the phylogenetic diversity and are also part of an ongoing study about the molecular diversity in the genus *Laboulbenia*. Thalli of *Laboulbeniales* were removed from their host at the point of attachment and mounted in permanent slides using the double-coverslip technique as described by Liu et al. (2020), with one modification: thalli were placed in a droplet of 1:1 Hoyer's medium:glycerin mixture instead of pure Hoyer's medium, because our Hoyer's medium dried quickly. Mounted thalli were viewed at 200–1000 \times magnification under an Olympus BH-2 microscope (Olympus, Center Valley, PA, USA). Images of thalli were made with a Nikon DS-Fi3 microscope camera mounted on an Eclipse Ni-U compound microscope (Nikon, Nelville, NY, USA), equipped with differential interference contrast optics, and processed using NIS-Elements BR 5.0.03 imaging software (Nikon). Photos were enhanced and the background was removed using cutout.pro (<https://www.cutout.pro/>) and figures were assembled in PowerPoint v.2306 (Microsoft, Redmont, WA, USA).

Studied slides are deposited at the Herbarium Universitatis Gandavensis (GENT) and Meise Botanic Garden Herbarium (BR). Hosts are stored in the Taxon Expeditions collection (TXEX) and the personal collection of Oscar Vorst.

DNA extraction, PCR amplification, and sequencing

DNA extractions were done using the REPLI-g Single Cell Kit (Qiagen, Stanford, CA, USA). All steps were performed wearing disposable latex gloves. Thalli of *Laboulbeniales* were removed from their host using a hypodermic needle, which was inserted into a glass syringe for holdfast, under a dissecting microscope. Removed thalli were placed in a droplet of glycerin on a microscope slide. The thalli were cut into multiple smaller pieces with the sharp tip of the needle. These pieces were placed in 0.2-ml PCR tubes with 4 μ l of phosphate-buffered saline (PBS). Next, we followed the instructions as indicated in the manufacturer's manual (Qiagen).

The small subunit (18S), the internal transcribed region (ITS), and the large subunit (28S) of the ribosomal RNA gene were amplified, using primer pairs NSL1/NSL2 for 18S (Haelewaters et al. 2015); ITS1f/ITS4 and ITS3/ITS4 for ITS (White et al. 1990; Gardes and Bruns 1993); and LR0R/LR5, NL1/NL4, and LIC24/LR3 for 28S (Vilgalys and Hester 1990; Hopple 1994; Kurtzman and Robnett 1997; Miadlikowska and Lutzoni 2000). PCR reactions (25 μ l total) consisted of 13.3 μ l of RedExtract *Taq* polymerase (Sigma-Aldrich), 2.5 μ l of each 10 μ M primer, 5.45 μ l of ddH₂O, and 1 μ l of DNA extract. PCR conditions followed those from Van Caenegem et al. (2023b). Gel electrophoresis was performed and PCR products were visualized using ethidium bromide staining. Purification of successful PCR products was done using 1.5 μ l of Exo-FAP (0.5 μ l exonuclease I, 1 μ l FAST alkaline phosphatase) (Thermo Fisher Scientific, Waltham, MA, USA) per 10 μ l of PCR product,

at 37 °C for 15 min, followed by deactivation at 85 °C for 15 min. The purified PCR products were sequenced at Macrogen (Amsterdam, The Netherlands) using an automated ABI 3730 XL capillary sequencer (Life Technology, Carlsbad, CA, USA). Forward and reverse sequence reads were assembled and edited in Sequencher version 5.4.6 (Gene Codes Corporation, Ann Arbor, MI, USA).

Phylogenetic analyses

We used a broad selection of newly generated *Laboulbenia* sequences, supplemented with 18S and 28S sequences of *Laboulbenia* spp. and 18S sequences of other genera downloaded from NCBI GenBank. Accession numbers of sequences and additional information about the isolates can be found in Table 1. As outgroup, we used taxa from family *Dimorphomycetaceae* (*Dimeromyces*, *Nycteromyces*, and *Polyandromyces*) (Goldmann and Weir 2018).

We aligned 18S and 28S sequences by locus with the G-INS-i strategy and ITS with the E-INS-i strategy using the online version 7 of MAFFT (Katoh et al. 2005, 2019; Kuraku et al. 2013). Sequences were manually trimmed using BioEdit Sequence Alignment Editor version 7.2.6 (Hall 1999) and combined in SequenceMatrix 1.9 (Vaidya et al. 2011) to construct one concatenated dataset (18S–ITS–28S). The final dataset included five partitions: 18S, the ITS1 and ITS2 spacer regions, the 5.8S gene, and 28S. Models for nucleotide substitution were selected for each partition with ModelFinder (Kalyaanamoorthy et al. 2017) according to the corrected Akaike information criterion (AICc). A maximum likelihood (ML) reconstruction was inferred using IQ-TREE (Nguyen et al. 2015) under partitioned models (Chernomor et al. 2016). Ultrafast bootstrapping was performed with 1000 replicates (Hoang et al. 2017).

Bayesian inference was done using MrBayes (Ronquist et al. 2012), available on the CIPRES Science Gateway web portal (Miller et al. 2010). Four Markov chains were run for 80 million generations, sampling every 8000 generations. Our concatenated dataset (18S–ITS–28S) was not partitioned. The analysis was performed using the GTR substitution model, with some sites being invariable and gamma-distributed rate variation across the remaining sites (GTR+I+G) (Abadi et al. 2019). A burn-in of 8000 trees was selected.

Phylogenetic trees were visualized in FigTree version 1.4.4 (<http://tree.bio.ed.ac.uk/software/figtree/>) and edited in Inkscape (<http://www.inkscape.org>).

Results

The concatenated 18S–ITS–28S dataset included 2929 characters for 39 taxa. For the maximum likelihood analysis, selected models for each partition in the concatenated dataset

were GTR+F+I+G4 (18S, 1079 bp, -lnL = 11593.620), TPM2+F (ITS1, 352 bp, -lnL = 2028.319), K3P+I (5.8S, 129 bp, -lnL = 452.282), GTR+F+G4 (ITS2, 400 bp, -lnL = 4627.979), and GTR+F+I+G4 (28S, 969 bp, -lnL = 6939.442). The reconstructed Bayesian phylogeny of *Laboulbeniales* including the genera *Botryandromyces* and *Laboulbenia* is shown in Fig. 2 (concatenated 18S–ITS–28S dataset). The topologies of both trees resulting from the maximum likelihood and Bayesian analyses were identical. The genus *Laboulbenia* has high support (99/1), and the two species of *Botryandromyces* form a supported clade (92/0.79) within *Laboulbenia*. Together with *L. clivinalis* and *L. slackensis*, they form a well-supported clade (89/0.99).

The 18S sequence of *B. heteroceri* (D. Haelew. 4197b) shares 98.57% identity with *B. ornatus* (AW821) and 95.13–98.57% identity with other species of *Laboulbenia*, with *L. collae*, *L. notiophilii*, *L. pedicellata*, and *L. thaxteri* as the highest ranked ones. To compare these results with the divergence in the 18S region among species of *Laboulbenia*, we blasted an 18S sequence of *L. slackensis*, which shares between 100% (*L. slackensis*) and 95.50% (*L. cf. dorstii*) identity. The ITS sequence of *B. heteroceri* is highly divergent compared to those of *Laboulbenia* species, with a query cover of only 21–37% (which roughly corresponds to the conserved 5.8S region and the beginning of ITS2). It shares between 91.43 and 96.89% with other species of *Laboulbenia*, with *L. clivinalis*, *L. littoralis*, *L. pedicellata*, and *L. slackensis* as the highest ranked. The 28S sequence of *B. heteroceri* shares between 81.74 and 87.44% identity with other species of *Laboulbenia*, with *L. benjaminii*, *L. slackensis*, and *L. pedicellata* as the highest ranked. To compare these results with the divergence in the 28S region among species of *Laboulbenia*, we blasted a 28S sequence of *L. slackensis*, which shares between 100% (*L. slackensis*) and 83.92% (*L. oioveliicola*) identity.

Taxonomy

Laboulbenia Mont. & C.P. Robin, in Robin, His Nat Vég Paras Paris: 622 (1853), emend. Van Caenegem & Haelew. (hoc opus)

= *Botryandromyces* I.I. Tav. & T. Majewski, Mycotaxon 3: 195 (1976)

= *Ceraiomycetes* Thaxt., Proc Am Acad Arts Sci 36: 410 (1900)

= *Eumisgomyces* Speg., Anal Mus Nac Hist Nat B Aires 23: 176 (1912)

= *Laboulbeniella* Speg., Anal Mus Nac Hist Nat B Aires 23: 188 (1912)

= *Scalenomyces* I.I. Tav., Mycol Mem 9: 313 (1985)

= *Schizolaboulbenia* Middelh., Fungus Wagening 27: 73 (1957)

= *Thaxteria* Giard, C R Hebd Séanc Mém Soc Biol 44: 156 (1892)

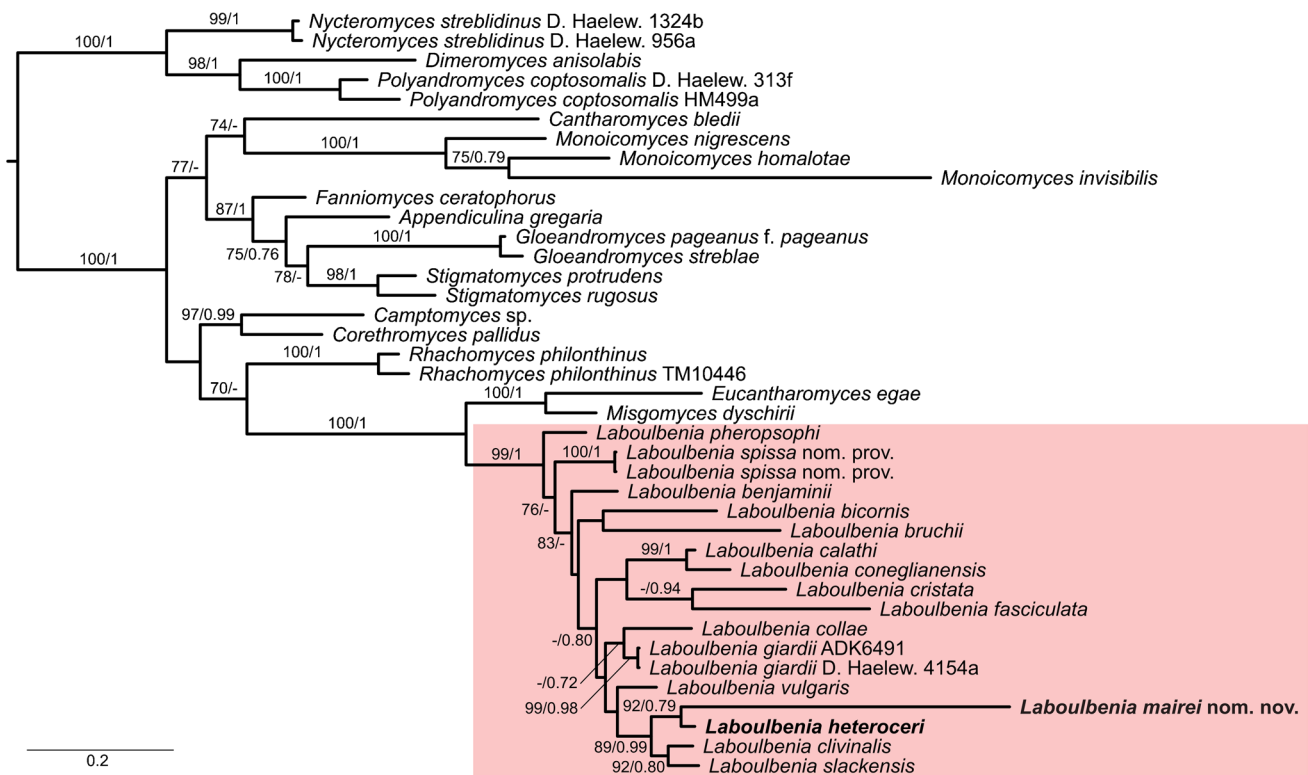


Fig. 2 Partial phylogeny of *Laboulbeniales* based on a concatenated 18S-ITS-28S dataset, with the genus *Laboulbenia* indicated in the red box. *Laboulbenia heteroceri* and *L. mairei* (in bold) are retrieved

in a well-supported clade within *Laboulbenia*. Ultrafast bootstrap values (≥ 70) and posterior probabilities (≥ 0.70) are indicated above or below the branch leading to each node

Description: Mostly monoecious, rarely dioecious. Receptacle typically five-celled. Primary or lower receptacle consisting of two superposed cells (I and II), or composed of a uniseriate row of multiple cells. Cell II supporting on one side the perithecial stalk cell (VI) and on the other side the secondary or upper receptacle. Secondary or upper receptacle, or androstichum, typically consisting of three cells (III, IV, and V), but can be undivided (III+IV+V) or partially divided. First cell of the appendage (insertion cell) usually flattened and more or less blackened, distinguished from surrounding cells. If not flattened and blackened, then not distinguishable from the surrounding cells and surrounded by proliferating cells. Appendages variable: simple to highly branched; short to long; with or without blackened septa; hyaline, colored, or blackened; typically consisting of an inner, usually fertile, appendage and an outer, sterile, appendage. Antheridia terminal or lateral simple phialides and then usually born on the inner appendage, rarely sessile. Solitary perithecium at least free at the ventral side, with four tiers, typically with four outer wall cells of usually unequal to rarely equal size in each vertical tier or with two tiers with four unequal wall cells and two tiers with three unequal wall cells. Edited from descriptions by Tavares (1985), Majewski (1994), and Santamaria and Pedersen (2021).

Type species: *Laboulbenia rougetii* Mont. & C.P. Robin.

Laboulbenia heteroceri Thaxt. (as “*heteroceratis*”), Proc Amer Acad Arts 48: 207 (1912)

Fig. 1A, B

≡ *Botryandromyces heteroceri* (Thaxt.) I.I. Tav. & T. Majewski (as “*heteroceratis*”), Mycotaxon 3: 195 (1976)
 ≡ *Botryandromyces ornatus* I.I. Tav., Mycol Mem 9: 156 (1985)

Laboulbenia mairei Van Caenegem & Haelew., nom. nov. Fig. 1D, E

Mycobank number: MB 849899

Replaced synonym: *Misgomyces heteroceri* Maire, Bull Soc Hist Nat Afr Nord 11: 159 (1920), non *Laboulbenia heteroceri* Thaxt. (1912)

≡ *Botryandromyces heteroceri* (Maire) I.I. Tav. & T. Majewski, Mycotaxon 3: 196 (1976)

Etymology: Named after René Charles Joseph Ernest Maire, a French botanist and mycologist who made significant contributions to *Laboulbeniales* from France and North Africa.

Material examined: Belgium, East Flanders, Herzele, 50° 51' 19.4" N 3° 53' 14.3" E, 2 September 2022, on *Heterocerus fenestratus* (Thunberg, 1784) (*Coleoptera*, *Heteroceridae*), leg. W. Van Caenegem, in coll. TXEX,

Table 1 Details of all isolates used in this study

Isolate	Species	Host species	Country	GenBank accession numbers		
				18S	ITS	28S
D. Haelew. 1008a	<i>Appendiculina gregaria</i>	<i>Diopsidae</i> sp.	Sierra Leone	MG438348		
D. Haelew. 1222b	<i>Camptomyces</i> sp.	<i>Astenus</i> sp.	Tanzania	MF314140		
LG503	<i>Cantharomyces bledii</i>	<i>Staphylinidae</i> sp.	USA	MG687386		
N/A	<i>Corethromyces pallidus</i>	<i>Staphylinidae</i> sp.	USA	MG674649		
LG532	<i>Dimeromyces anisolabis</i>	<i>Spongiphoridae</i> sp.	USA	MG687388		
LG359	<i>Eucantharomyces egae</i>	<i>Carabidae</i> sp.	Costa Rica	MG696305		
D. Haelew. 1136 h	<i>Fanniomyces ceratophorus</i>	<i>Fannia canicularis</i> (Linnaeus, 1761)	USA	MG958013		
D. Haelew. 1425a	<i>Gloeandromyces pageanus</i>	<i>Trichobius dugesioides</i> Wenzel, 1966	Panama	MH040536		
D. Haelew. 1018a	<i>Gloeandromyces streblae</i>	<i>Trichobius joblingi</i> Wenzel, 1966	Nicaragua	MG438338		
D. Haelew. 3758a (ADK6522)	<i>Laboulbenia benjaminii</i>	<i>Badister unipustulatus</i> Bonelli, 1813	Belgium	OR680738	OR680744	OR680759
D. Haelew. 4333a	<i>Laboulbenia bicornis</i>	<i>Gyrinidae</i> sp.	Uganda	OR680728		OR680748
D. Haelew. 1346b	<i>Laboulbenia bruchii</i>	<i>Neolema adunata</i> White, 1993	Panama	MN530040	OR680724	MN394843
D. Haelew. 1007a	<i>Laboulbenia calathi</i>	<i>Calathus melanocephalus</i> (Linnaeus, 1758)	The Netherlands	MG438342		OR680755
D. Haelew. 3037a (ADK6493)	<i>Laboulbenia clivinalis</i>	<i>Clivina fossor</i> (Linnaeus, 1758)	Latvia	OR680736	OR680742	OR680757
D. Haelew. 3038b (ADK6459)	<i>Laboulbenia collae</i>	<i>Paranchus albipes</i> (Fabricius, 1796)	Belgium	OR680732	OR680739	OR680752
D. Haelew. 3759a (ADK6524)	<i>Laboulbenia coneglianensis</i>	<i>Harpalus griseus</i> (Panzer, 1796)	Belgium	OR680734	OR680741	OR680754
D. Haelew. 3970a	<i>Laboulbenia cristata</i>	<i>Paederus littoralis</i> Gravenhorst, 1802	Belgium	OR680735		OR680756
D. Haelew. 3044a (ADK6487)	<i>Laboulbenia fasciculata</i>	<i>Patrobus atrorufus</i> (Ström, 1768)	Belgium	OR680729	OR680723	OR680749
D. Haelew. 3052a (ADK6491)	<i>Laboulbenia giardii</i>	<i>Dicheirotichus gustavii</i> Crotch, 1871	Belgium	OR680727		OR680747
D. Haelew. 4154a	<i>Laboulbenia giardii</i>	<i>Dicheirotichus gustavii</i>	Belgium	OR680726		OR680746
AW-821	<i>Laboulbenia heteroceri</i>	<i>Heteroceridae</i> sp.	USA	MG674664		
D. Haelew. 4197b	<i>Laboulbenia mairei</i>	<i>Heterocerus fenestratus</i> (Thunberg, 1784)	Belgium	OR680725	OR680722	OR680745
D. Haelew. 1009b	<i>Laboulbenia pheropsophi</i>	<i>Pheropsophus</i> sp.	Sierra Leone	MG438344		OR680760
D. Haelew. 4131a (ADK6288)	<i>Laboulbenia slackensis</i>	<i>Pogonus chalceus</i> (Marsham, 1802)	Belgium	OR680737	OR680743	OR680758
D. Haelew. 4199c	<i>Laboulbenia spissa</i> nom. prov.	<i>Cyparium concolor</i> (Fabricius, 1801)	USA	OR680730		OR680751
D. Haelew. 4199d	<i>Laboulbenia spissa</i> nom. prov.	<i>Cyparium concolor</i>	USA	OR680731		OR680750
D. Haelew. 3774a	<i>Laboulbenia vulgaris</i>	<i>Bembidion tibiale</i> (Duftschmid, 1812)	The Netherlands	OR680733	OR680740	OR680753
LG487	<i>Misgomyces dyschirii</i>	<i>Carabidae</i> sp.	South Africa	MG696572		
D. Haelew. 1014c	<i>Monoicomyces homalotae</i>	<i>Philhygra</i> sp.	USA	MG438346		
MT004	<i>Monoicomyces invisibilis</i>	<i>Anotylus sculpturatus</i> (Gravenhorst, 1806)	Poland	KT800034		
N/A	<i>Monoicomyces nigrescens</i>	<i>Staphylinidae</i> sp.	Namibia	MG696256		
D. Haelew. 1324b	<i>Nycteromyces streblidinus</i>	<i>Trichobius joblingi</i>	Panama	MH040554		
D. Haelew. 956a	<i>Nycteromyces streblidinus</i>	<i>Trichobius parasiticus</i> Gervais, 1844	Honduras	MH040553		

Table 1 (continued)

Isolate	Species	Host species	Country	GenBank accession numbers		
				18S	ITS	28S
D. Haelew. 313f	<i>Polyandromyces coptosomalis</i>	<i>Phoeacia</i> sp.	Ecuador	KT800035		
HM499a	<i>Polyandromyces coptosomalis</i>	<i>Acrosternum</i> sp.	Spain	MG438347		
TM10446	<i>Rhachomyces philonthinus</i>	<i>Philonthus</i> sp.	Poland	KT800036		
N/A	<i>Rhachomyces philonthinus</i>	<i>Staphylinidae</i> sp.	USA	MG674659		
AW-793	<i>Stigmatomyces protrudens</i>	<i>Ephyridae</i> sp.	USA	AF298232		
D. Haelew. 1138a	<i>Stigmatomyces rugosus</i>	<i>Psilopa</i> sp.	Portugal	MH040563		

Note: Accession numbers of sequences generated during this study are in boldface

slides D. Haelew. 4197a (GENT:GENTFL00780, 1 aberrant thallus from right elytron) and D. Haelew. 4197c (GENT:GENTFL00781, 5 aberrant thalli from right elytron); *ibid.*, isolate D. Haelew. 4197b (2 aberrant thalli from right elytron), GenBank accession nos. ab123456 (18S), ab123456 (ITS), and ab123456 (28S); *ibid.*, 22 August 2023, on *Heteroceris* sp., leg. W. Van Caenegem, in coll. TXEX, slide D. Haelew. 4847a (GENT, 2 adult thalli from left elytron). The Netherlands, Groningen, Lauwersoog, Marnewaard, 53° 24' N 6° 15' E, brackish lake, 6 June 1998, on *Heteroceris obsoletus* Curtis, 1828 (*Coleoptera*, *Heteroceridae*), leg. O. Vorst, in coll. Vorst, slides D. Haelew. 073a (BR MYCO 173770–43, 2 adult thalli from pronotum), D. Haelew. 073b (GENT:GENTFL01154, 3 adult thalli from dorsal head), D. Haelew. 073c (GENT:GENTFL01155, 2 adult thalli from right elytron), and D. Haelew. 073d (GENT:GENTFL01156, 1 adult thallus from right elytron); North Holland, De Cocksdorp, Polder Wassenaar, 53°10'N 4°52'E, brackish ditch, 18 May 1996, on *H. obsoletus*, leg. O. Vorst, in coll. Vorst, slides D. Haelew. 030b (GENT:GENTFL01152, 1 adult thallus from left antenna) and D. Haelew. 030c (GENT:GENTFL01152, 1 adult thallus from right elytron).

Discussion

Here, we show that the genus *Laboulbenia* is paraphyletic if *B. heteroceri* and *B. ornatus* are retained in a separate genus. Therefore, we propose to synonymize *Botryandromyces* with *Laboulbenia* and to transfer *B. ornatus* and *B. heteroceri* to *Laboulbenia* as *L. heteroceri* and *L. mairei*, respectively. When *Botryandromyces* was erected, Tavares and Majewski (1976) combined two species in the genus, as *B. heteroceratis* (Thaxt.) I.I. Tav. & T. Majewski and *B. heteroceri* (Maire) I.I. Tav. & T. Majewski. As both fungal names refer to the host genus *Heteroceris*, the correct epithet should be “*heteroceri*”; “*heteroceratis*” is an orthographic variant (Turland et al. 2018: Art. F.9). Therefore, Tavares (1985) changed the name of *B. heteroceri* (Thaxt.) I.I. Tav. & T. Majewski (as “*heteroceratis*”) to *B. ornatus*.

Because we reinstated *L. heteroceri* Thaxt., *B. heteroceri* based on *Misgomyces heteroceri* needed a replacement name in *Laboulbenia*: *Laboulbenia mairei*.

Laboulbenia mairei is positioned on a long branch in our phylogenetic tree (Fig. 2). This is mainly due to the divergence in sequences of the ITS and 28S regions between *L. mairei* and other species in the genus. For *L. heteroceri*, however, only one sequence is available: that of the conserved 18S region (Goldmann and Weir 2018). This explains the large evolutionary distance between *L. heteroceri* and *L. mairei* on the one hand and the shorter distances between *L. heteroceri* and closely related species of *Laboulbenia* on the other hand. Also, a few other species are found on relatively long branches in our phylogenetic reconstruction: *Laboulbenia bicornis*, *L. bruchii*, and *L. fasciculata*. This can, in part, be attributed to taxon sampling error. Indeed, only 14 of the 667 currently accepted species of *Laboulbenia* (Haelewaters et al. 2023) are included in our phylogenetic analysis. A revision of this genus based on molecular phylogenetic data, with increased sampling, both taxonomically (more taxa) and geographically (from a wide geographic coverage), is desirable and may result in the disintegration of *Laboulbenia* in meaningful taxonomic groups (sections, subgenera, or different genera). We conclude that the proposed transfer of *Botryandromyces* species to *Laboulbenia* is on par with our current morphological and molecular knowledge of the genus.

A few considerations arise after including these species in the genus *Laboulbenia*. The difference in perithecial outer wall cells is striking. The number of outer wall cells in each tier is a commonly used and reliable character to delineate and identify genera of *Laboulbeniales* (Tavares 1985; Majewski 1994; De Kesel et al. 2020; Santamaria and Pedersen 2021). The difference in the number of these cells was one of the main reasons why Tavares and Majewski (1976) erected *Botryandromyces*. Tavares (1985) proposed that it “was undoubtedly derived from a more typical arrangement of four cells in each row.” In addition, Tavares (1985) erected *Dixomyces* and *Scalenomyces* to accommodate a few other species, based on the number of outer wall cells in each tier and

characteristics of the appendages and the receptacle. Eventually, Rossi and Santamaria (2008) synonymized *Scalenomyces* with *Laboulbenia*, as the morphology of their newly described *L. magrinii* was similar to *S. endogaea*. Both species are known from endogean ground beetles (*Coleoptera*, *Carabidae*). Whether their specific morphology is an adaptation to their host, their host's ecology, the environment, due to random genetic drift, or other factors, is unknown. Similar thoughts can be made regarding morphological changes of *L. heteroceri* and *L. mairei* compared to phylogenetically related species (Fig. 2). Both species are found on *Heteroceridae*, while *L. clivinalis* and *L. slackensis* are found on *Carabidae*, like most species of *Laboulbenia* are. Our phylogeny provides evidence for a host shift, which might have driven the observed changes in morphology.

Remarkably, the lower receptacle of *L. mairei* often shows secondary divisions, while *L. heteroceri* consistently has a two-celled lower receptacle (Fig. 1) (Thaxter 1912; Maire 1920; Tavares and Majewski 1976). The receptacle of *L. heteroceri* resembles that of a typical species of *Laboulbenia*, which was already acknowledged by Thaxter (1912). *Laboulbenia mairei* is not the only species in the genus that has more than two cells in the lower receptacle. *Laboulbenia dohrni* and *L. partita* also have this peculiar organization of the lower receptacle but differ in other characteristics, e.g., they have a typical blackened insertion cell (Thaxter 1914; Spegazzini 1915; Tavares 1985). In *L. mairei*, the number of cells in the lower receptacle is variable and may depend on the position of thalli on the host integument or thallus age (Majewski 1994; De Kesel 2009; Santamaria and Pedersen 2021). Thalli of *L. mairei* with a typical *Laboulbenia* receptacle are illustrated by Majewski (1994).

The upper receptacle of *L. heteroceri* and *L. mairei* is reminiscent of the ones from species in the *Laboulbenia luxurians* group as defined by Tavares (1985). Similar to the species in this group, the height of their cells IV and V is equal; the vertical septum between these cells reaches cell III. *Laboulbenia clivinalis* and *L. slackensis* also belong to this group and form a well-supported clade with *L. heteroceri* and *L. mairei* in our phylogeny (Fig. 2). In addition, most species of this group (e.g., *L. clivinalis* and *L. slackensis*) have a blackened septum between the basal and supra-basal cells of their outer appendage. *Laboulbenia heteroceri* also has a blackened septum in this position (Fig. 1). Species in this group are commonly found on hosts that live in humid environments like sandy or muddy river banks, seashores, and wet grasslands. *Carabidae* (hosts for *L. clivinalis*, *L. pedicellata*, and *L. slackensis*) and *Heteroceridae* (hosts for *L. heteroceri* and *L. mairei*) are often found together in these environments (Holeski and Graves 1978; A. De Kesel, pers. obs.). This shared habitat preference makes host shifts of

Laboulbeniales between those two families likely (Rossi 2011; De Kesel and Haelewaters 2014).

The morphology of *Dixomyces clivinae* and *D. pallescens* is similar to that of *L. heteroceri* and *L. mairei*. They were transferred from *Laboulbenia* in which they were originally described by Thaxter (1896, 1908) to *Dixomyces* by Tavares (1985). Both *Dixomyces* species were described from carabid beetles, suggesting that the adjusted morphology was already present on carabid hosts. No sequence data of these species are available, but we hypothesize that *D. clivinae* and *D. pallescens* may also need to be transferred back to *Laboulbenia*.

Thaxter (1912) reported morphological differences between the holotype of *L. heteroceri* from Argentina and thalli found on beetles collected in KS, USA. Several studies also reported differences in length between the holotype of *L. mairei* from Algeria and specimens from Europe. Reasons behind these differences are unknown but they have been attributed to either inaccurate measurements or environmental differences (Scheloske 1969; Tavares and Majewski 1976; Majewski 1994; Weir 1994). Given that cryptic diversity in *Laboulbeniales* is proven using molecular data (Haelewaters et al. 2018, 2019) and that both *L. heteroceri* and *L. mairei* are reported from different genera of *Heteroceridae*, it is only a matter of time and effort to confirm or reject whether there are multiple cryptic species hidden under these two names. Host specimens should be freshly collected to sequence species of *Dixomyces* and *Scalenomyces*, *L. heteroceri*, and *L. mairei* and resolve these outstanding taxonomic issues.

Conclusions

Based on molecular phylogenetic data, we synonymized *Botryandromyces* with *Laboulbenia* and emended the description of *Laboulbenia* to include that (1) the perithecial outer wall can have either four cells in each of the four vertical tiers, or two tiers with four cells and two tiers with three cells, and (2) the lower receptacle can be either two-celled or multi-celled. The species formerly placed in *Botryandromyces* (now known as *Laboulbenia heteroceri* and *L. mairei*) are morphologically similar to species of the *Laboulbenia luxurians* species group. Their hosts also occupy the same habitats, which increases the chance of a host shift. Although there is a major difference in the morphology of the perithecium, the equal size of cells IV and V and the presence of a blackened septum in the outer appendage correspond to their phylogenetic position inside this species group. Future research should focus on adding sequence data for morphologically described genera related to *Laboulbenia*, host shifts within the *L. luxurians* species group, and cryptic diversity in *L. heteroceri* and *L. mairei*.

Acknowledgements We thank Menno Schilthuizen (Leiden University) for the identification of host specimens, Konstanze Bensch (Mycobank) for help with taxonomy, and Lauren Goldmann (State University of Cortland) and Noni Korf (MycoTaxon Ltd.) for guidance on the reproduction of figures.

Author contribution Conceptualization: W.V.C. and D.H. Methodology, investigation, visualization, and writing—original draft: W.V.C. Resources and supervision: D.H. Writing—review and editing: W.V.C., A.D.K., and D.H. All authors read and approved the final version of the manuscript.

Funding This study received support from a U.S. National Science Foundation grant (DEB-2127290) and a Research Foundation—Flanders Senior Postdoctoral Fellowship (1206024N) to DH.

Data availability Unedited images, final alignments, and unedited tree are available through GitHub: https://github.com/dannyhaelewaters/teamlaboul/tree/main/botryandromyces_paper. Newly generated sequences were submitted to the National Center for Biotechnology Information (NCBI) GenBank database (<https://www.ncbi.nlm.nih.gov/genbank/>), under the following accession numbers: OR680722–OR680760.

Declarations

Ethics approval Not applicable.

Conflict of interest The authors declare no competing interests.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

- Abadi S, Azouri D, Pupko T, Mayrose I (2019) Model selection may not be a mandatory step for phylogeny reconstruction. *Nat Comm* 10:1–11. <https://doi.org/10.1038/s41467-019-08822-w>
- Arzanlou M, Groenewald JZ, Gams W, Braun U, Shin HD, Crous PW (2007) Phylogenetic and morphotaxonomic revision of *Ramichloridium* and allied genera. *Stud Mycol* 58:57–93. <https://doi.org/10.3114/sim.2007.58.03>
- Bridge PD, Spooner BM, Roberts PJ (2005) The impact of molecular data in fungal systematics. *Adv Bot Res* 42:33–67. [https://doi.org/10.1016/S0065-2296\(05\)42002-9](https://doi.org/10.1016/S0065-2296(05)42002-9)
- Cao B, Haelewaters D, Schoutteten N, Begerow D, Boekhout T, Giachini AJ, Gorjón SP, Gunde-Cimerman N, Hyde KD, Kemler M, Li GJ, Liu DM, Liu XZ, Nuytincck J, Papp V, Savchenko A, Savchenko K, Tedersoo L, Theelen B, Thines M, Tomšovský M, Toome-Heller M, Urón JP, Verbeken A, Vizzini A, Yurkov AM, Zamora JC, Zhao RL (2021) Delimiting species in *Basidiomycota*: a review. *Fungal Divers* 109:181–237. <https://doi.org/10.1007/s13225-021-00479-5>
- Chernomor O, von Haeseler A, Minh BQ (2016) Terrace aware data structure for phylogenomic inference from supermatrices. *Syst Biol* 65:997–1008. <https://doi.org/10.1093/sysbio/syw037>
- Co-David D, Langeveld D, Noordeloos ME (2009) Molecular phylogeny and spore evolution of *Entolomataceae*. *Persoonia* 23:147–176. <https://doi.org/10.3767/003158509X480944>
- Crous PW, Braun U, Schubert K, Groenewald JZ (2007) Delimiting *Cladosporium* from morphologically similar genera. *Stud Mycol* 58:33–56. <https://doi.org/10.3114/sim.2007.58.02>
- Crous PW, Lombard L, Sandoval-Denis M, Seifert KA, Schroers HJ, Chaverri P, Gené J, Guarro J, Hirooka Y, Bensch K, Kema GHJ, Lamprecht SC, Cai L, Rossman AY, Stadler M, Summerbell RC, Taylor JW, Ploch S, Visagie CM, Yilmaz N, Frisvad JC, Abdel-Azeem AM, Abdollahzadeh J, Abdolrasouli A, Akulov A, Alberts JF, Araújo JPM, Ariyawansa HA, Bakshi M, Bendiksby M, Ben Hadj Amor A, Bezerra JDP, Boekhout T, Câmara MPS, Carbia M, Cardinali G, Castañeda-Ruiz RF, Celis A, Chaturvedi V, Collemare J, Croll D, Damm U, Decock CA, de Vries RP, Ezekiel CN, Fan XL, Fernández NB, Gaya E, González CD, Gramaje D, Groenewald JZ, Grube M, Guevara-Suarez M, Gupta VK, Guarnaccia V, Haddaji A, Hagen F, Haelewaters D, Hansen K, Hashimoto A, Hernández-Restrepo M, Houbraken J, Hubka V, Hyde KD, Iturriaga T, Jeewon R, Johnston PR, Jurjević Ž, Karalti İ, Korsten L, Kuramae EE, Kušan I, Labuda R, Lawrence DP, Lee HB, Lechat CLL, Li HY, Litovka YA, Maharachchikumbura SSN, Marin-Felix Y, Kemkuignou BM, Matočec N, McTaggart AR, Mičoch P, Mugnai L, Nakashima C, Nilsson RH, Nougour SR, Pavlov IN, Peralta MP, Phillips AJL, Pitt JI, Polizzi G, Quaedvlieg W, Rajeshkumar KC, Restrepo S, Rhaïem A, Robert J, Robert V, Rodrigues AM, Salgado-Salazar C, Samson RA, Santos ACS, Shivas RG, Souza-Motta CM, Sun GY, Swart WJ, Szoke S, Tan YP, Taylor JE, Taylor PWJ, Tiago PV, Váczy KZ, van de Wiele N, van der Merwe NA, Verkley GJM, Vieira WAS, Vizzini A, Weir BS, Wijayawardene NN, Xia JW, Yañez-Morales MJ, Yurkov A, Zamora JC, Zare R, Zhang CL, Thines M (2021) *Fusarium*: more than a node or a foot-shaped basal cell. *Stud Mycol* 98:100116. <https://doi.org/10.1016/j.simyco.2021.100116>
- Crous PW, Summerell BA, Carnegie AJ, Wingfield MJ, Hunter GC, Burgess TI, Andjic V, Barber PA, Groenewald JZ (2009) Unravelling *Mycosphaerella*: do you believe in genera? *Persoonia* 23:99–118. <https://doi.org/10.3767/003158509X479487>
- De Kesel A (2009) *Botryandromyces* and *Ecteinomyces* (*Laboulbeniales*) in Belgium. *Sterbeekia* 29:23–26
- De Kesel A, Gerstmans C, Haelewaters D (2020) Catalogue of the *Laboulbeniomyces* of Belgium. *Sterbeekia* 36:3–143
- De Kesel A, Haelewaters D (2014) *Laboulbenia slackensis* and *L. littoralis* sp. nov. (*Ascomycota, Laboulbeniales*), two sibling species as a result of ecological speciation. *Mycologia* 106:407–414. <https://doi.org/10.3852/13-348>
- Eberhardt U, Verbeken A (2004) Sequestrate *Lactarius* species from tropical Africa: *L. angiocarpus* sp. nov. and *L. dolichoaulis* comb. nov. *Mycol Res* 108:1042–1052. <https://doi.org/10.1017/S0953756204000784>
- Gardes M, Bruns TD (1993) ITS primers with enhanced specificity for basidiomycetes – application to the identification of mycorrhizae and rusts. *Mol Ecol* 2:113–118. <https://doi.org/10.1111/j.1365-294X.1993.tb00005.x>
- Goldmann L, Weir A (2012) Position specificity in *Chitonomyces* (*Ascomycota, Laboulbeniomyces*) on *Laccophilus* (*Coleoptera, Dytiscidae*): a molecular approach resolves a century-old debate. *Mycologia* 104:1143–1158. <https://doi.org/10.3852/11-358>
- Goldmann L, Weir A (2018) Molecular phylogeny of the *Laboulbeniomyces* (*Ascomycota*). *Fungal Biol* 122:87–100. <https://doi.org/10.1016/j.funbio.2017.11.004>
- Goldmann L, Weir A, Rossi W (2013) Molecular analysis reveals two new dimorphic species of *Hesperomyces* (*Ascomycota*,

- Laboulbeniomyces*) parasitic on the ladybird *Coleomegilla maculata* (Coleoptera, Coccinellidae). Fungal Biol 117:807–813. <https://doi.org/10.1016/j.funbio.2013.10.004>
- Haelewaters D (2018) Studies of the *Laboulbeniomyces*: diversity, evolution, and patterns of speciation. PhD dissertation, Harvard University. <https://dash.harvard.edu/handle/1/40049989>
- Haelewaters D, Blackwell M, Pfister DH (2021a) *Laboulbeniomyces*: intimate fungal associates of arthropods. Annu Rev Entomol 66:257–276. <https://doi.org/10.1146/annurev-ento-013020-013553>
- Haelewaters D, De Kesel A, Gorczak M, Bao K, Gort G, Zhao SY, Pfister DH (2019) *Laboulbeniales* (Ascomycota) of the Boston Harbor Islands II (and other localities): species parasitizing *Carabidae*, and the *Laboulbenia flagellata* species complex. Northeast Nat 25:110–149. <https://doi.org/10.1656/045.025.s906>
- Haelewaters D, De Kesel A, Pfister DH (2018) Integrative taxonomy reveals hidden species within a common fungal parasite of ladybirds. Sci Rep 8:15966. <https://doi.org/10.1038/s41598-018-34319-5>
- Haelewaters D, Gorczak M, Kaishian P, De Kesel A, Blackwell M (2021b) *Laboulbeniomyces*, enigmatic fungi with a turbulent taxonomic history. In: Zaragoza Ó, Casadevall A (eds) Encyclopedia of Mycology, vol 1. Elsevier, Oxford, pp 263–283. <https://doi.org/10.1016/B978-0-12-819990-9.00052-4>
- Haelewaters D, Gorczak M, Pfliegler WP, Tartally A, Tischer M, Wrzosek M (2015) Bringing *Laboulbeniales* into the 21st century: enhanced techniques for extraction and PCR amplification of DNA from minute ectoparasitic fungi. IMA Fungus 6:363–372. <https://doi.org/10.5598/ima fungus.2015.06.02.08>
- Haelewaters D, Matthews TJ, Wayman JP, Cazabonne J, Heyman F, Quandt CA, Martin TE (2023) *Laboulbeniomyces* as a case study for biodiversity shortfalls in poorly studied groups. J Biogeogr. <https://doi.org/10.1111/jbi.14725>
- Haelewaters D, Pfister DH (2019) Morphological species of *Gloeandromyces* (Ascomycota, *Laboulbeniales*) evaluated using single-locus species delimitation methods. Fungal Syst Evol 3:19–34. <https://doi.org/10.3114/fuse.2019.03.03>
- Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser 41:95–98
- Hoang DT, Chernomor O, von Haeseler A, Minh BQ, Vinh LS (2017) UFBoot2: improving the ultrafast bootstrap approximation. Mol Biol Evol 35:518–522. <https://doi.org/10.1093/molbev/msx281>
- Holeski PM, Graves RC (1978) An analysis of the shore beetle communities of some channelized streams in Northwest Ohio (Coleoptera). Gt Lakes Entomol 11: 23–36. <https://doi.org/10.22543/0090-0222.1315>
- Hopple JS (1994) Phylogenetic investigations in the genus *Coprinus* based on morphological and molecular characters. PhD dissertation, Duke University
- Justo A, Morgenstern I, Hallen-Adams HE, Hibbett DS (2010) Convergent evolution of sequestrate forms in *Amanita* under Mediterranean climate conditions. Mycologia 102:675–688. <https://doi.org/10.3852/09-191>
- Kalyaanamoorthy S, Minh B, Wong T, von Haeseler A, Jermin L (2017) ModelFinder: fast model selection for accurate phylogenetic estimates. Nat Methods 14:587–589. <https://doi.org/10.1038/nmeth.4285>
- Katoh K, Kuma K, Toh H, Miyata T (2005) MAFFT version 5: improvement in accuracy of multiple sequence alignment. Nucleic Acids Res 33:511–518. <https://doi.org/10.1093/nar/gki198>
- Katoh K, Rozewicki J, Yamada KD (2019) MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. Brief Bioinformatics 20:1160–1166. <https://doi.org/10.1093/bib/bbx108>
- Kuraku S, Zmasek CM, Nishimura O, Katoh K (2013) aLeaves facilitates on-demand exploration of metazoan gene family trees on MAFFT sequence alignment server with enhanced interactivity. Nucleic Acids Res 41:22–28. <https://doi.org/10.1093/nar/gkt389>
- Kurtzman CP, Robnett CJ (1997) Identification of clinically important ascomycetous yeasts based on nucleotide divergence in the 5' end of the large-subunit (26S) ribosomal DNA gene. J Clin Microbiol 35:1216–1223. <https://doi.org/10.1128/jcm.35.5.1216-1223.1997>
- Liu J, Haelewaters D, Pfliegler WP, Page RA, Dick CW, Aime MC (2020) A new species of *Gloeandromyces* from Ecuador and Panama revealed by morphology and phylogenetic reconstruction, with a discussion of secondary barcodes in *Laboulbeniomyces* taxonomy. Mycologia 112:1192–1202. <https://doi.org/10.1080/00275514.2020.1781496>
- Maharachchikumbura SSN, Chen Y, Ariyawansa HA, Hyde KD, Haelewaters D, Perera RH, Samarakoon MC, Wanasinghe DN, Bustamante DE, Liu JK, Lawrence DP, Cheewangkoon R, Stadler M (2021) Integrative approaches for species delimitation in *Ascomycota*. Fungal Divers 109:155–179. <https://doi.org/10.1007/s13225-021-00486-6>
- Maire R (1920) Troisième contribution à l'étude des *Laboulbeniales* de l'Afrique du Nord. Bull Soc Hist Nat Afr Nord 11(123–138):143–170
- Majewski T (1994) The *Laboulbeniales* of Poland. Polish Bot Stud 7:1–466
- Miadlikowska J, Lutzoni F (2000) Phylogenetic revision of the genus *Peltigera* (lichen-forming *Ascomycota*) based on morphological, chemical, and large subunit nuclear ribosomal DNA data. Int J Plant Sci 161:925–958. <https://doi.org/10.1086/317568>
- Miller M, Pfeiffer WT, Schwartz T (2010) Creating the CIPRES Science Gateway for inferences of large phylogenetic trees. Proc Gateway Comp Environ Workshop 14:1–8. <https://doi.org/10.1109/GCE.2010.5676129>
- Miller SL, McClean TM, Walker JF, Buyck B (2001) A molecular phylogeny of the *Russulales* including agaricoid, gasteroid and pleurotoid taxa. Mycologia 93:344–354. <https://doi.org/10.1080/00275514.2001.12063166>
- Nguyen LT, Schmidt HA, von Haeseler A, Minh BQ (2015) IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Mol Biol Evol 32:268–274. <https://doi.org/10.1093/molbev/msu300>
- Nuytink J, Verbeke A, Delarue S, Walley R (2003) Systematics of European sequestrate lactarioid *Russulaceae* with spiny spore ornamentation. Belg J Bot 136:145–153
- Peintner U, Boughner N, Castellano M, Moncalvo JM, Moser M, Trappe J, Vilgalys R (2001) Multiple origins of sequestrate fungi related to *Cortinarius* (*Cortinariaceae*). Am J Bot 88:2168–2179. <https://doi.org/10.2307/3558378>
- Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Syst Biol 61:539–542. <https://doi.org/10.1093/sysbio/sys029>
- Rossi W (2011) New species of *Laboulbenia* from Ecuador, with evidence for host switch in the *Laboulbeniales*. Mycologia 103:184–194. <https://doi.org/10.3852/10-117>
- Rossi W, Santamaria S (2008) New *Laboulbeniales* parasitic on endogean ground beetles. Mycologia 100:636–641. <https://doi.org/10.3852/07-081R>
- Sánchez-García M, Ryberg M, Khan FK, Varga T, Nagy LG, Hibbett DS (2020) Fruiting body form, not nutritional mode, is the major driver of diversification in mushroom-forming fungi. Proc Natl Acad Sci U S A 117:32528–32534. <https://doi.org/10.1073/pnas.1922539117>
- Santamaria S, Pedersen J (2021) *Laboulbeniomyces* (*Fungi, Ascomycota*) of Denmark. Eur J Taxon 781:1–425. <https://doi.org/10.5852/ejt.2021.781.1583>

- Scheloske HW (1969) Beiträge zur Biologie, Ökologie und Systematik der *Laboulbeniales* (*Ascomycetes*) unter besondere Berücksichtigung des Parasit-Wirt-Verhältnisses. *Parasitol Schriften* 19:1–176
- Spegazzini C (1915) *Laboulbeniales* ritrovate nelle collezioni di alcuni musei italiani. *Anal Mus Nac Hist Nat B Aires* 26:451–511
- Tavares II (1985) *Laboulbeniales* (*Fungi, Ascomycetes*). *Mycol Mem* 9:1–627
- Tavares II, Majewski T (1976) *Siemaszkoa* and *Botryandromyces*, two segregates of *Misgomyces* (*Laboulbeniales*). *Mycotaxon* 3:193–208
- Thaxter R (1896) Contributions towards a monograph of the *Laboulbeniaceae*: Part I. *Mem Am Acad Arts Sci N S* 12:187–429
- Thaxter R (1908) Contribution toward a monograph of the *Laboulbeniaceae*: Part II. *Mem Am Acad Arts Sci N S* 13:217–469. <https://doi.org/10.2307/25058090>
- Thaxter R (1912) New or critical *Laboulbeniales* from the Argentine. *Proc Am Acad Arts Sci* 48:155–223. <https://doi.org/10.2307/20022824>
- Thaxter R (1914) *Laboulbeniales* parasitic on *Chrysomelidae*. *Proc Am Acad Arts Sci* 50:15–50. <https://doi.org/10.2307/20025507>
- Turland NJ, Wiersema JH, Barrie FR, Greuter W, Hawksworth DL, Herendeen PS, Knapp S, Kusber WH, Li DZ, Marhold K, May TW, McNeill J, Monro AM, Prado J, Price MJ, Smith GF (2018) International Code of Nomenclature for algae, fungi, and plants (Shenzhen Code) adopted by the Nineteenth International Botanical Congress Shenzhen, China, July 2017. *Regnum Vegetabile* 159. Koeltz Botanical Books, Glashütten. <https://doi.org/10.12705/Code.2018>
- Vaidya G, Lohman DJ, Meier R (2011) SequenceMatrix: concatenation software for the fast assembly of multi-gene datasets with character set and codon information. *Cladistics* 27:171–180. <https://doi.org/10.1111/j.1096-0031.2010.00329.x>
- Van Caenegem W, Blondelle A, Dumolein I, Santamaria B, Dick C, Hiller T, Liu J, Quandt CA, Villarreal Saucedo R, Verbeken M, Haelewaters D (2023a) Five new species of *Gloeandromyces* (*Fungi, Laboulbeniales*) from tropical American bat flies (*Diptera, Streblidae*), revealed by morphology and phylogenetic reconstruction. *Mycologia* 115:714–737. <https://doi.org/10.1080/00275514.2023.2230114>
- Van Caenegem W, Ceryngier P, Romanowski J, Pfister DH, Haelewaters D (2023b) *Hesperomyces* (*Fungi, Ascomycota*) associated with *Hyperaspis* ladybirds (*Coleoptera, Coccinellidae*): rethinking host specificity. *Front Fungal Biol* 3:1040102. <https://doi.org/10.3389/ffunb.2022.1040102>
- Vilgalys R, Hester M (1990) Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *J Bacteriol* 172:4238–4246. <https://doi.org/10.1128/jb.172.8.4238-4246.1990>
- Weir A (1994) Further records of *Laboulbeniales* from collections of British *Coleoptera*. *Mycol Res* 98:433–444. [https://doi.org/10.1016/S0953-7562\(09\)81201-X](https://doi.org/10.1016/S0953-7562(09)81201-X)
- White TJ, Bruns T, Lee S, Taylor J (1990) Analysis of phylogenetic relationships by amplification and direct sequencing of ribosomal RNA genes. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) *PCR protocols: a guide to methods and applications*. Academic Press, San Diego, pp 315–322. <https://doi.org/10.1016/B978-0-12-372180-8.50042-1>
- Wynns AA (2015) Convergent evolution of highly reduced fruiting bodies in *Pezizomycotina* suggests key adaptations to the bee habitat. *BMC Evol Biol* 15:145. <https://doi.org/10.1186/s12862-015-0401-6>

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.