

Laboulbeniales (Ascomycota) of the Boston Harbor Islands II (and Other Localities): Species Parasitizing Carabidae, and the *Laboulbenia flagellata* Species Complex

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Abstract - This paper presents 13 new records of Laboulbenialean parasites on ground beetles (Coleoptera, Carabidae) from the Boston Harbor Islands National Recreation Area in Massachusetts: *Laboulbenia anoplogenii*, *L. casnoniae*, *L. clivinalis*, *L. egens*, *L. filifera*, *L. flagellata*, *L. inflata*, *L. macrotheca*, *L. pedicellata*, *L. terminalis*, *L. variabilis*, *L. vulgaris*, and *Peyritschiella geminata*. *Laboulbenia clivinalis* and *L. egens* are new country records for the US. Moreover, we present additional localities for *L. casnoniae*, *L. clivinalis*, *L. filifera*, *L. flagellata*, *L. inflata*, *L. pedicellata*, *L. variabilis*, and *L. vulgaris*. The following new country records are presented: *Laboulbenia clivinalis*, *L. filifera*, and *L. variabilis* from Canada; *L. flagellata* from the Democratic Republic of the Congo; *L. pedicellata* from Ukraine; *L. vulgaris* from Croatia and Slovenia (and the first undoubtful record from Slovakia). *Laboulbenia flagellata* was found on 11 host species in the genera *Agonum*, *Oxypselaphus*, *Patrobus*, *Platynus*, and *Pterostichus*. Using this abundant material, we performed morphometrics to test the hypothesis that *L. flagellata* is a species complex. Specimens cannot be separated based on host genus (*Agonum*, *Pterostichus*). One parameter is significant between *Pterostichus mutus* and each of the 4 *Agonum* species after applying a strong Bonferroni *P*-value correction: HIT, the ratio of height of cell I (HC1) to total thallus length (TTL). In addition, we collected fresh material to be able to add a molecular phylogenetic component to test said hypothesis. We generated ITS and nrLSU ribosomal sequences of several species of *Laboulbenia*, including isolates of *L. flagellata* from multiple hosts. Phylogenetic inference of the concatenated dataset shows that *L. flagellata* isolates from 3 host species form 2 distinct clades, providing support for our hypothesis. We also show that *L. coneglianensis* is separate from *L. flagellata*, unequivocally ending a long-standing taxonomic debate. Finally, examination of Roland Thaxter's 1891–1932 slides led to the designation of lectotypes for *L. macrothecia*, *L. terminalis*, and *P. geminata*.

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Introduction

The order Laboulbeniales (Ascomycota, Laboulbeniomycetes) is the most numerous group of ectoparasitic fungi, with ~2200 species placed in 142 genera (Reboleira et al. 2018). This order is composed of obligate, biotrophic ectoparasites of arthropods, mostly insects. Diversity and distribution of Laboulbeniales has been extensively studied only by specialists, who have often summarized their contributions in the form of monographs dedicated to Laboulbeniales of a specific country (e.g., Argentina [Spegazzini 1917], Belgium [De Kesel 1998, De Kesel and Rammeleloo 1997], Italy [Colla 1934], Poland [Majewski 1994], Spain [Santamaría 1998, 2003]). The study of Laboulbeniales in the United States has been mostly limited to the massive contributions by Roland Thaxter (1858–1932) and Richard K. Benjamin (1922–2002). Thaxter, a professor at Harvard University in Cambridge, MA, relied both on collectors (entomologists) sending him infected specimens and on the many specimens he himself collected, especially in New England (see Pfister 1982 for details of Thaxter’s collection localities). Despite geographical proximity, there is no evidence that he collected at the Boston Harbor Islands (Haelewaters et al. 2015a).

This work is a continuation of a previous study on Laboulbeniales at the Boston Harbor Islands National Recreation Area (BHI), which reported on species associated with Coccinellidae (lady beetles) and Staphylinidae (rove beetles) (Haelewaters et al. 2015a). The work on Laboulbeniales at the BHI resulted from screening insects at the entomological collection housed at the Harvard Museum of Comparative Zoology. These insects were collected for the terrestrial invertebrate All Taxa Biodiversity Inventory at the BHI (Rykken and Farrell 2013, 2018a, 2018b).

The present paper focuses on Laboulbeniales found on the speciose family Carabidae (ground beetles), which comprises 31,490 species (Bousquet 2012). Carabidae are frequently infected with Laboulbeniales, yet generally harbor a limited diversity of Laboulbeniales, particularly at the generic level. In contrast to the Staphylinidae, which are parasitized by a much larger number of genera of Laboulbeniales (49), members of Carabidae host 17 genera only (Tavares 1979): *Apatomyces* Thaxt. (Thaxter 1931), *Cesariella* W. Rossi & Santam. (Rossi and Santamaría 2008), *Cochliomyces* Speg. (Spegazzini 1912), *Corethromyces* Thaxt. (Thaxter 1931, as *Eucorethromyces* in Thaxter 1908), *Dimeromyces* Thaxt. (Thaxter 1896, 1924), *Dimorphomyces* Thaxt. (Thaxter 1920), *Dixomyces* I.I. Tav. (Tavares 1985), *Enarthromyces* Thaxt. (Thaxter 1896), *Eucantharomyces* Thaxt. (Thaxter 1908), *Euzodiomyces* Thaxt. (Scheloske 1969), *Laboulbenia* Mont. & C.P. Robin (Thaxter 1896, as *Ceraiomyces* Thaxt. in Thaxter 1908), *Misgomyces* Thaxt. (Thaxter 1908, 1931), *Ormomyces* I.I. Tav. (Tavares 1985), *Peyritschella* Thaxt. (Thaxter 1896), *Picardella* I.I. Tav. (Tavares 1985, as *Dioicomycetes* Thaxt. in Thaxter 1931), *Pseudoecteinomyces* (Rossi 1977, as *Ecteinomyces* Thaxt. in Spegazzini 1915), and *Rhachomyces* Thaxt. (Thaxter 1896, 1931).

Despite the relatively low generic diversity of Laboulbeniales, the specific diversity (numbers of species) in the genus *Laboulbenia* is high on Carabidae. In the first volume of his monograph, Thaxter (1896) listed 75 species of Laboulbeniales as parasites of Carabidae, of which 65 were species in the genus *Laboulbenia*.

In contrast, Staphylinidae hosted 50 species of Laboulbeniales, of which only 4 belonged to *Laboulbenia*. Although many species of *Laboulbenia* have been described since 1896, Thaxter's findings give us a good idea of diversification patterns of Laboulbeniales on Carabidae as compared to Staphylinidae.

The genus *Laboulbenia*

The eponymous genus of the order Laboulbeniales is also the largest in the order, with over 650 accepted species and many varieties (897 taxa; Index Fungorum 2019). Species are found worldwide on Coleoptera (beetles), Diptera (flies), Hemiptera (true bugs), Hymenoptera (Formicidae; ants), Blattodea (cockroaches), Orthoptera (crickets and allies), and Acari (mites). Most *Laboulbenia* species are parasites of Carabidae (Majewski 1994, Santamaría 1998, Tavares 1985). Despite considerable diversity, all *Laboulbenia* species share the following characteristics: 4 tiers of perithecial wall cells and an insertion cell separating the appendage system from the receptacle (Santamaría 1998).

Determinate development of each thallus causes each receptacle cell to carry important taxonomic information; thus, for convenience each cell is noted with a roman numeral. Most *Laboulbenia* species have a typically 5-celled receptacle (I–V), but some species have undivided cells III + IV or III + IV + V (especially species from Chrysomelidae and Curculionidae; Rossi et al. 2015, 2016). The vast diversity within the genus prompted first Spegazzini (1917) and then Tavares (1985) to divide *Laboulbenia* into morph groups. However, neither of the systems have been adopted by other scholars. Thaxter, the most prominent expert on the Laboulbeniales, died before completing the sixth and last part of his *Contribution towards a Monograph of the Laboulbeniaceae* that would have been dedicated to the genus *Laboulbenia*. The most comprehensive work dealing solely with *Laboulbenia* (from the Iberic peninsula) is Santamaría's (1998) monograph.

One of the most cosmopolitan and common species is *L. flagellata* Peyr. Described by Johann Joseph Peyritsch in 1873, it has been reported from more than 80 genera of Carabidae in many different countries, and on all continents except Antarctica (Santamaría 1998). The host of the holotype collection is unclear; Peyritsch (1873) mentioned 3 hosts: *Agonum ericeti* (Panzer, 1809) [as *Anchomenus marginatus*], *Bembidion (Asioperiphus) lunatum* (Duftschmid, 1812), and *Paranichus albipes* (Fabricius, 1796) [as *Anchomenus*] (Löbl and Smetana 2003). Among the many taxonomic problems in this genus, several authors have expressed the belief that *L. flagellata* may be a complex of (near-) cryptic species without clear delimitations (De Kesel and Van den Neucker 2006, Santamaría 1998). Because of considerable morphological variability, occurrence on various host genera, and the fact that the given taxa are found in dissimilar habitats, *Laboulbenia* species include many synonyms, varieties, and species of dubious position.

2, 3, ... 16 species or morphotypes?

Morphological variability in species of *Laboulbenia* and those of other genera of Laboulbeniales is expressed among host species, between sexes of the hosts, and among locations on the same host specimen. Two opposing concepts exist in

dealing with this variability. Some authors have described species that are restricted to a specific position on the host body (= position specificity) and to a given sex of the host (= sex-of-host specificity). Reported examples are the 16 species of *Chitonomyces* on *Orectogyrus specularis* Aubé, 1838 (Coleoptera, Gyrinidae) from Cameroon and 6 species of *Laboulbenia* on *Bembidion grapii* Gyllenhal, 1827 [as *picipes*] (Coleoptera, Carabidae) from Illinois (Benjamin and Shanor 1952, Thaxter 1926). This specificity goes to the extreme; for example, *Chitonomyces unciger* Thaxt. only occurs on the claw of the left metaleg of male *Laccophilus maculosus* Say, 1823 aquatic beetles (Coleoptera, Dytiscidae).

The second view treats different forms, relating to the different types of specificity (host, position, sex-of-host) as morphotypes (or growth forms) of the same biological species. Without the support of molecular data, it is almost impossible to draw species limits among morphologically similar thalli with different hosts, or among morphologically different thalli with different positions on the same host or on different sexes of the same host (sensu Scheloske 1969, 1976). As a result, in recent years researchers have described polymorphic species of Laboulbeniales for such cases (Rossi and Kotrba 2004, Rossi and Proaño Castro 2009, Santamaría and Faille 2009) or have doubted the validity of the previously described species (De Kesel and Haelewaters 2012, 2014a). Indeed, in some cases, morphotypes are morphologically so convincing that they were incorrectly given the species rank (e.g., Thaxter 1896, but see Goldmann and Weir 2012).

Although commonly accepted in mycology (Hibbett et al. 2011, 2016; Taylor et al. 2000), applying sequence-based taxonomy and phylogenetic species recognition to Laboulbeniales was long hindered by technical issues (Haelewaters et al. 2015b, Sundberg et al. 2018, Weir and Blackwell 2001). That DNA characters can provide answers to the issues of morphological variability and host specificity in Laboulbeniales was confirmed by Goldmann and Weir (2012). Using a combination of molecular, ecological, and observational data, these authors showed that the position and sex-specificity of *Chitonomyces* species on the aquatic beetle *L. maculosus* could be tied to transmission during sexual contact between hosts. Rather than 13 morphological species of *Chitonomyces*, there are 6 phylogenetic species each consisting of a pair (and 1 triplet) of position-related morphotypes. However, this copulatory transmission of ascospores cannot be generalized to all Laboulbeniales. Work needs to be done to deal with these issues in species that parasitize terrestrial hosts.

Another approach to delineate taxa involves performing morphometric analyses. Although not often used in fungal taxonomy, the statistical analysis of large sets of measurements is employed in many disciplines of biology to provide a framework for comparing morphologies (Adams et al. 2004, Zelditch et al. 2012). Morphometric techniques have proven to be particularly useful in taxonomy of closely related or hybrid species groups, for example in the *Onosma echioides* (L.) L. complex (Peruzzi et al. 2008) and *Prunus* L. section *Prunus* (Depypere et al. 2009). Such rigid analytical framework is critical when delimiting species solely on the basis of morphological data, as is often the case in palaeontology (Webster and Sheets 2010).

When researchers identify or describe thalli of Laboulbeniales, they generally measure the length and width of individual cells and structures such as the perithecium/perithecia and the appendage(s). Statistical analyses, however, have been rarely applied in Laboulbeniales taxonomy. Statistics were used to explore the morphological variability of *Laboulbenia flagellata* on different carabid hosts occupying identical or different ecological niches in Belgium (De Kesel and Van den Neucker 2006). The study showed a significant inter-correlation between all measured thallus parameters, confirming that thallus proportions of *L. flagellata* are stable and not affected by the host. However, thallus length was significantly affected by the host, the position on the host, and its habitat. Later, *Laboulbenia littoralis* De Kesel & Haelew. was described employing similar morphology-based data as well as ecological data, supporting its separation from sister species *L. slackensis* Cépède & F. Picard (De Kesel and Haelewaters 2014b).

In this paper, we present 13 records of Laboulbeniales removed from Carabidae collected at the BHI. The most commonly found species, *Laboulbenia flagellata*, was subjected to morphometric analyses. We hypothesize that thalli of *L. flagellata* from different host species would have different morphologies and that these may represent separate taxa (sensu De Kesel and Van den Neucker 2005, Haelewaters et al. 2018). We also hypothesize that thalli of single host species have different morphologies depending on the position on the host's body.

Materials

Collection and examination of insects

The Harvard Museum of Comparative Zoology houses a collection of the Carabidae from the BHI that includes 708 individuals representing 64 species. These specimens were collected for the ATBI from 13 islands using a variety of methods: litter sampling, pitfall traps, malaise traps, (UV and mercury-vapor) light traps, Berlese funnels, and collections made by hand and using an entomological net (for details, see Rykken and Farrell 2013). Names and classifications (family, subfamily, tribe, subtribe) of insect hosts follow the framework provided by Bouchard et al. (2011).

Other sources of infected carabids reported in this paper were dried insect collections. Between 2013 and 2015, the first author had the opportunity to screen insects for the presence of Laboulbeniales at: American Museum of Natural History in New York, NY; Tupper Center of the Smithsonian Tropical Research Institute in Ancon, Panama; and Collection d'insectes du Québec, Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec, Québec City, QC, Canada. Some insects infected with Laboulbeniales were noted by entomologists and sent to D. Haelewaters.

Morphological studies of Laboulbeniales

We examined pinned insects under dissecting microscopes at 10–50x magnification for the presence of Laboulbeniales ectoparasites. We removed individual fungal thalli from their hosts at the foot and mounted them according to previously

described methods (Benjamin 1971, modifications in Haelewaters et al. 2015a) and observed the specimens at 400–1000x magnification for identification using relevant systematic and taxonomic sources (Santamaría 1998; Thaxter 1896, 1908, 1931). Slides are deposited at the Farlow Herbarium (FH; Harvard University, Cambridge, MA) unless otherwise indicated (CIQ = Collection d'insectes du Québec, Canada; MIUP = Museo de Invertebrados G.B. Fairchild de la Universidad de Panamá; PHREC = University of Nebraska-Lincoln Panhandle Research and Extension Center, Lincoln, NE).

***Laboulbenia flagellata*: morphometrics and statistical analysis**

We photographed 155 thalli of *Laboulbenia flagellata* using an Olympus BX40 light microscope with Olympus XC50 digital camera. Pictures of thalli may be accessed from the figshare online repository at <https://doi.org/10.6084/m9.figshare.8214128>. We employed the MicroSuite Special Edition software 3.1 (Soft Imaging Solutions GmbH) to measure taxonomically important characters. Up to 15 morphometric parameters (measurements and ratios) were taken to characterize each thallus: TTL = total thallus length, LOP = length of perithecium, LPT = LOP / TTL, WOP = width of perithecium, LWP = LOP / WOP, HC1 = height of cell I, H1T = HC1 / TTL, WC1 = width of cell I, HW1 = HC1 / WC1, HC2 = height of cell II, H2T = HC2 / TTL, WC2 = width of cell II, HW2 = HC2 / WC2, LOR = length of receptacle, LRT = LOR / TTL. Parameters for all thalli are available in Supplemental Table 1 (available online at <http://www.eaglehill.us/NENAonline/suppl-files/n26-sp9-N1560h-Haelewaters-s1>, and for BioOne subscribers, at <https://dx.doi.org/10.1656/N1560h.s1>).

We analyzed thallus data in 2 ways: (1) using mixed linear models to compare means per morphometric parameter between hosts and thallus position (Littell et al. 2006) and (2) using principal component analysis (PCA) and biplots for exploratory data analysis of morphometric parameters. For each of the 15 morphometric parameters, we fitted a mixed linear model, explaining the response variable from host species, thallus position, and their interaction. Random effects for host specimens were included because multiple observations were taken from the same specimen. The setup of the data resembles a split-plot design with specimen as whole plots (and host species as whole-plot factor) and positions within specimen as subplots (and position as sub-plot factor).

The dataset, containing 140 observations (= number of thalli for which morphometric parameters were taken), was highly unbalanced; the numbers of observations differed substantially among host species (varying from 3 to 57 thalli) and among locations (varying from 1 to 64 thalli). We restricted statistical analysis to observations of only adult thalli ($n = 99$), judged by the presence of ascospores within the perithecium. Within this subset, the number of thalli per host species varied from 2 (for *Pterostichus pensylvanicus*) to 41 (for *Agonum melanarium*). The number of thalli per location varied from 1 (for mouthparts) to 37 (for elytra). Data were available for 20 of the 49 possible combinations of host species and thallus position. For this reason, we chose to fit the mixed model to all available data (97–99

observations in total, depending on the morphometric parameter) so as to have the highest accuracy for the estimation of variance components, but to extract and report from the overall analysis only those comparisons for which enough observations were available.

We chose to make comparisons only if at least 3 observations per combination of host species and position were available. This led us to the following comparisons:

Comparison of host species within position on the host body:

Q1.1 within elytra*: compared *A. fidele*, *A. gratiosum*, *A. melanarium*,
A. viduum, *P. mutus*

Q1.2 within legs: compared *A. fidele*, *A. gratiosum*, *A. melanarium*

Q1.3 within pronotum: compared *A. fidele*, *A. gratiosum*, *A. melanarium*

Q1.4 within ventral: compared *A. fidele*, *A. melanarium*

Comparison of positions within host species:

Q2.1 within *A. fidele*: compared elytra, legs, pronotum, ventral

Q2.2 within *A. gratiosum*: compared antennae, elytra, legs, pronotum

Q2.3 within *A. melanarium*: compared elytra, legs, pronotum, ventral

*Note that only for comparison Q1.1 were enough data available to compare observations between host genera.

For all mixed models, we made plots to check for constant variance and normality of residuals. In all cases, these assumptions appeared to hold reasonably, so that we performed the analysis on the untransformed morphometric parameter.

We made comparisons using approximate *F*-tests (with degrees of freedom calculated according to the method of Kenward and Roger [1997]), followed by pairwise comparisons in case of significant *F*-tests. Mixed models and user-defined contrasts were applied using procedure MIXED of the SAS software system (version 9.3).

For a selection of morphometric parameters with significant differences between groups in the mixed linear models, we used principal component analysis (PCA) followed by exploratory biplots in an attempt to locate groups which would remain undetected in a univariate analysis. Observations were colored by host genus, host species, and thallus location. We obtained PCA and biplots using the R language and environment for statistical computing (R Core Team 2018) with the help of the 'factoextra' package (Kassambara 2015).

Molecular work and phylogenetic analyses

We realized that morphometrics and subsequent statistical analyses would not be enough to make a strong statement about the taxonomic status of *L. flagellata*. As a next step, we generated molecular phylogenetic data. A number of concerns arose. First, we had previously not experienced a lot of success isolating DNA and sequencing from *Laboulbenia* thalli. The pigment responsible for the typical darkening of many *Laboulbenia* species (melanin), apparently binds to the polymerase (Eckhart et al. 2000), thus inhibiting amplification. Recently, a modification of the REPLI-g Single Cell Kit (Qiagen, Valencia, CA) successfully resulted in sequences

from 3 ribosomal regions of Herpomycetales and Laboulbeniales representatives (Haelewaters et al. 2019). This protocol adds a whole-genome amplification (WGA) step to DNA isolation, which significantly improves success. We tested this kit for *Laboulbenia* species. Second, all of our BHI Laboulbeniales thalli originated from pinned insects. Even though dried insect collections have many values and can be an asset in biological research (e.g., Brooks et al. 2014, Haelewaters and Rossi 2017, Johnson et al. 2011), isolating DNA from Laboulbeniales preserved dry has commonly resulted in failures (Haelewaters et al. 2015b, Weir and Blackwell 2001). For this reason, we isolated DNA from thalli removed from freshly collected insect specimens preserved in 96% ethanol. The main purpose of this molecular study was to provide proof of concept, and so the origin of material is less important (details of isolates in Table 1).

Insects were collected, mostly by A. De Kesel and D. Haelewaters, and screened for Laboulbeniales under 10–50x magnification. We removed thalli at the foot using a Minuten Pin (BioQuip, Rancho Dominguez, CA, #1208SA) inserted into a wooden rod. We used between 1 and 11 thalli for DNA extraction following the manufacturer's instructions for the REPLI-g Single Cell Kit with modifications by Haelewaters et al (2019). To ensure successful lysis, we sliced every perithecium transversally once or twice using a #10 surgical blade on disposable Bard-Parker handle (Aspen Surgical, Caledonia, MI).

To gain an idea about species delimitation, we amplified the internal transcribed spacer (ITS) region of the ribosomal DNA (rDNA) as well as the partial nuclear large subunit rDNA (nrLSU). We used the following primers: ITS1f/ITS4 for the ITS, LabITS1/LR3 for partial ITS + nrLSU, and LIC24R/LR3 and LR0R/LR5 for the nrLSU locus (Gardes and Bruns 1993, Haelewaters et al. 2019, Hopple and Vilgalys 1994, Miadlikowska and Lutzoni 2000, Vilgalys and Hester 19990, White et al. 1990). PCR reactions (25 μ L total) consisted of 13.3 μ L of RedExtract Taq polymerase (Sigma-Aldrich, St. Louis, MO), 2.5 μ L of each 10- μ M primer, 5.7 μ L of ddH₂O, and 1.0 μ L of DNA extract. PCR conditions were as follows: initial denaturation at 94 °C for 3 min; 35 cycles of denaturation at 94 °C for 1 min, annealing at 50 °C for 45 s, and extension at 72 °C for 90 s; and final extension at 72 °C for 10 min. Purification and sequencing steps were outsourced to Genewiz (South Plainfield, NJ). Sequence reads were assembled and edited in Sequencher v5.0 (Gene Codes Corporation, Ann Arbor, MI). Newly generated sequences were submitted to GenBank (accession numbers in Table 1).

We constructed a concatenated ITS + nrLSU dataset to investigate the phylogenetic structure within *L. flagellata*. We aligned sequences of each locus individually using Muscle v3.7 (Edgar 2004), available on the Cipres Science Gateway v3.3 (Miller et al. 2010). The aligned sequences for each region were combined in MEGA7 (Kumar et al. 2016) to create a matrix of 2002 characters with phylogenetic data for 27 isolates. We conducted maximum likelihood inference using IQ-TREE (Nguyen et al. 2015) from the command line, under partitioned models (Chernomor et al. 2016). We statistically selected appropriate models of nucleotide substitution using jModelTest2 (Darriba et al. 2012) on Cipres, under the Akaike

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Table 1. Overview of Laboulbeniales sequences generated and/or used in this study, with indication of DNA extraction protocol (REPLI-g = REPLI-g Single Cell Kit, ISOLATE II = ISOLATE II Plant DNA Kit, Sundberg = methods of Sundberg et al. (2018), QIAamp = QIAamp DNA Micro Kit, and Extract-N-Amp = Extract-N-Amp Plant PCR Kit) and numbers of thalli used per extraction (juv = juvenile, sub = subadult, ad = adult), host species, growth position on the host, and country. All isolates of which sequences were used are listed, with GenBank accession numbers for ITS and nrLSU rDNA. “*” indicates sequences that were generated during the course of this study.

Label	Species	DNA isolation	# thalli	Host species	Position	Country	ITS	LSU
D. Haelew. 928g	<i>Hesperomyces virescens</i>	REPLI-g	1 ad	<i>Azya orbifera</i>	Sternite	Panama	MG745343	MG745343
D. Haelew. 1439a	<i>H. virescens</i>	REPLI-g	4 ad	<i>Harmonia axyridis</i>	Right elytron	USA	MN397128*	MN397128*
D. Haelew. 1346b	<i>Laboulbenia bruchii</i>	REPLI-g	2 ad	<i>Neolema adunata</i>	Left elytron	Panama	N/A	MN394843*
D. Haelew. 1456a	<i>L. collae</i>	REPLI-g	2 ad	<i>Agonum ruficorne</i>	Right elytron	Belgium	N/A	MN394844*
D. Haelew. 1456b	<i>L. collae</i>	REPLI-g	3 juv 6 ad	<i>Agonum ruficorne</i>	Right elytron	Belgium	MN397129*	MN394845*
D. Haelew. 1461a	<i>L. collae</i>	REPLI-g	2 sub	<i>Agonum ruficorne</i>	Pronotum	Belgium	MN397130*	MN397130*
D. Haelew. 1461b	<i>L. collae</i>	REPLI-g	9 ad	<i>Agonum ruficorne</i>	Left elytron	Belgium	MN397131*	MN397131*
MG029G	<i>L. coneglianensis</i>	ISOLATE II	7	<i>Harpalus affinis</i>	N/A	Poland	N/A	MN394846*
MG029H	<i>L. coneglianensis</i>	ISOLATE II	20	<i>Harpalus affinis</i>	N/A	Poland	N/A	MN394847*
D. Haelew. 1254a	<i>L. diopsideis</i>	REPLI-g	1 sub 1 ad	<i>Diopsideis longicornis</i>	Left profemur	Bénin	N/A	MN394848*
D. Haelew. 1454a	<i>L. flagellata</i>	REPLI-g	3 ad	<i>Agonum assimile</i>	Right elytron	Belgium	MN397132*	MN394849*
D. Haelew. 1454b	<i>L. flagellata</i>	REPLI-g	1 juv 5 ad	<i>Agonum assimile</i>	Right elytron	Belgium	MN397133*	MN394850*
D. Haelew. 1457a	<i>L. flagellata</i>	REPLI-g	1 ad	<i>Agonum micans</i>	Left elytron	Belgium	N/A	MN394851*
D. Haelew. 1457b	<i>L. flagellata</i>	REPLI-g S	2 sub 1 ad	<i>Agonum micans</i>	Pronotum	Belgium	MN397134*	MN394852*
D. Haelew. 1457c	<i>L. flagellata</i>	REPLI-g	1 ad	<i>Agonum micans</i>	Left antenna	Belgium	N/A	MN394853*
D. Haelew. 1458a	<i>L. flagellata</i>	REPLI-g	5 juv 2 sub	<i>Agonum assimile</i>	Elytra	Belgium	N/A	MN394854*
H85-1	<i>L. flagellata</i>	Sundberg	1	<i>Loricera pilicornis</i>	N/A	Sweden	N/A	KY350538
D. Haelew. 942b	<i>L. oiovicicola</i>	QIAamp	3 juv 5 ad	<i>Oiovelta machadoi</i>	Antennae	Brazil	N/A	MF314142
H84-1	<i>L. pedicellata</i>	Sundberg	1	<i>Dyschiramelidae</i> sp.	N/A	Sweden	N/A	KY350537
D. Haelew. 967a	<i>L. sp.</i>	Extract-N-Amp	9	<i>Rugilus similis</i>	Elytra	Panama	N/A	MN394855*
D. Haelew. 1467a	<i>L. stiliticicola</i>	REPLI-g	3 ad	<i>Disoryncha procera</i>	Right elytron	Russia	N/A	MN394856*
D. Haelew. 1342b	<i>L. systenae</i>	REPLI-g	6 ad	<i>Disoryncha procera</i>	Sternites	Panama	N/A	MN394857*
D. Haelew. 1342c	<i>L. systenae</i>	REPLI-g	3 juv 8 ad	<i>Disoryncha procera</i>	Sternites	Panama	N/A	MN394858*
D. Haelew. 1455a	<i>L. vulgaris</i>	REPLI-g	4 ad	<i>Ocys harpaloides</i>	Sternites	Belgium	MN397135*	MN397135*
D. Haelew. 1455b	<i>L. vulgaris</i>	REPLI-g	3 ad	<i>Ocys harpaloides</i>	Sternites	Belgium	MN397136*	MN397136*
D. Haelew. 1459a	<i>L. vulgaris</i>	REPLI-g	3 ad	<i>Ocys harpaloides</i>	Left elytron	Belgium	MN397137*	MN397137*
D. Haelew. 1460a	<i>L. vulgaris</i>	REPLI-g	4 ad	<i>Ocys harpaloides</i>	Pronotum	Belgium	MN397138*	MN397138*

information criterion corrected for small sample size (AICc). Ultrafast bootstrapping was implemented with 1000 replicates (Hoang et al. 2017).

We performed bayesian analyses using a Markov chain Monte Carlo (MCMC) coalescent approach. We performed 4 independent runs in BEAST v1.8.4 (Drummond et al. 2012) under the following conditions: strict molecular clock, assuming a constant rate of evolution across the tree; Birth–Death Incomplete Sampling speciation model (Stadler 2009) as tree prior; the appropriate substitution models as selected by jModelTest2 (under AICc); starting from a random starting tree; and 40 million generations, with sampling frequency of 4000. We entered the resulting log files in Tracer v1.6 (Rambaut et al. 2014) to check trace plots for convergence and to adjust burn-in to achieve effective sample sizes of ≥ 200 for the majority of sampled parameters. We removed a portion of each run as burn-in and combined log files and trees files in LogCombiner v1.8.4. We used TreeAnnotator v1.8.4 to infer the Maximum Clade Credibility tree. Final trees with support values were visualized in FigTree v1.4.3 (<http://tree.bio.ed.ac.uk/software/figtree/>).

Taxonomy

***Laboulbenia anoplogenii* Thaxt.**, Proceedings of the American Academy of Arts and Sciences 35:156 (1899)

= *Laboulbenia stenolophi* Speg., Redia 10:65 (1914)

Distribution and hosts. Described on *Anoplogenius cyanescens* (Hope, 1845) [as *A. circumcinctus*] (Harpalinae, Harpalini) from China. *Laboulbenia anoplogenii* is reported from all continents but Antarctica and South America (Santamaría et al. 1991). Hosts are representatives of subfamilies Harpalinae, Pterostichinae, and Scaritinae (Haelewaters and Yaakop 2014, Santamaría et al. 1991). One report is known from *Chlaeminus* Motschulsky, 1865 (subfamily Callistinae; Sugiyama and Majewski 1985).

New records from the BHI. Norfolk County, GRAPE ISLAND, 42°16'8.9"N, 70°53'3.5"W, 5 October 2005, J. Rykken, on *Agonoleptus conjunctus* (Say, 1823) (Harpalinae, Harpalini), MCZ-ENT00600494, slides FH 00313244 (2 juvenile thalli from elytra), FH 00313245 (1 subadult thallus from right metafemur), and FH 00313246 (1 subadult thallus from left mesotibia); same data, MCZ-ENT00600496, slides FH 00313247 (3 thalli from elytra) and FH 00313248 (4 juvenile thalli from pronotum); same data, MCZ-ENT00600495, slide FH 00313249 (3 thalli from right elytron); Plymouth County, WORLD'S END, 42°15'51.9"N 70°52'37.8"E, 16 August 2006, J. Rykken, on *Stenolophus ochropezus* (Say, 1823), MCZ-ENT00600505, slide FH-D. Haelew. 1469a (2 adult thalli from right elytron); same data, MCZ-ENT00600505, slide FH-D. Haelew. 1470b (1 juvenile and 1 subadult thallus from right elytron).

Remarks. There is some disagreement about the status of *L. anoplogenii* and *L. stenolophi* (see Terada 2001). Santamaría (1989, 1998) suggested that both represent a single species based on the variability of the subdivisions of cell IV. This view was confirmed by Haelewaters and Yaakop (2014) after studying Thaxter's slides deposited at the Farlow Herbarium.

Laboulbenia anoplogenii is also associated with species in the genus *Clivina* Latreille, 1802 (Scaritinae, Clivinini). However, these are considered “accidental” hosts; they occupy the same habitat as the typical hosts for *L. anoplogenii*, and thus transmission of ascospores is possible to the unusual host insects.

***Laboulbenia casnoniae* Thaxt.**, Proceedings of the American Academy of Arts and Sciences 24:266 (1891)

Distribution and hosts. Described on *Colliuris pensylvanica* (Linnaeus, 1767) [as *Casnonia*] (Lebiinae, Odacanthini) from Connecticut, US. Although many times reported from other continents, Santamaría and Rossi’s (2006) morphological studies showed that “true” *L. casnoniae* is restricted to *C. pensylvanica* in North America.

New record from the BHI. Plymouth County, BUMPKIN ISLAND, 42°16'54.7"N, 70°54'7"W, 8 August 2006, J. Rykken, on *C. pensylvanica*, MCZ-ENT00614592, slide FH 00313149 (3 thalli from elytra).

Additional new records (non-BHI). US, NORTH CAROLINA, Mecklenburg County, Charlotte, 16 July 1968, H.P. Stockwell, on *C. pensylvanica*, D. Haelew. 867, STOCKWELL STRI-ENT 0 043 452, in coll. Smithsonian Tropical Research Institute-Tupper Center, slides FH-D. Haelew. 867a (8 adult thalli from left elytron) and MIUP-D. Haelew. 867b (2 adult thalli from tip right elytron).

Remarks. Santamaría and Rossi (2006) describe the inner appendage structure as constant and the main characteristic for the species. The basal cell of the inner appendage holds 2 simple branches that exceed the perithecial tip in length, each bearing a single antheridium at the second cell and tinged with brown at the lower portion. Earlier, thalli on European Lebiini had been identified as *L. casnoniae* by many authors but are truly *L. notiophili* Cépède & F. Picard. The structure of the inner appendage of this species is completely different, with branches/branchlets never reaching the tip of the perithecium.

Laboulbenia casnoniae is known only in the US, with records from 8 states: Illinois, Indiana, Kansas, Louisiana, Massachusetts, North Carolina, Ohio, and Tennessee (Santamaría and Rossi 2006, this paper).

***Laboulbenia clivinalis* Thaxt.** (Fig. 1A), Proceedings of the American Academy of Arts and Sciences 35:165 (1899)

Distribution and hosts. Known on *Clivina* spp. (Scaritinae, Clivinini) from many European countries. Also reported in Africa and Asia (Santamaría et al. 1991). Scheloske (1969) mentions *Patrobus atrorufus* (Strøm, 1768) (Patrobinae, Patrobini) as an accidental host in Germany.

New records from the BHI. Suffolk County, CALF ISLAND, 42°20'25.5"N, 70°53'48.9"W, 3–12 July 2007, J. Rykken, on *C. fossor* (Linnaeus, 1758), MCZ-ENT00626706, slides FH 00313108 (2 adult thalli from left elytron) and FH 00313387 (2 adult thalli from left elytron); same data, MCZ-ENT00626659, slide FH 00313578 (3 thalli from tip right elytron); same data, MCZ-ENT00626660, slides FH 00313579 (1 adult thallus from left elytron) and FH 00313580 (2 juvenile thalli from pronotum).

Additional new records (non-BHI). CANADA, Québec, Collines-de-l'Outaouais, Eardley, 25 June 2009, R. Juan, on *C. fossor*, D. Haelew. 557, in Collection d'insectes du Québec, slide CIQ-D. Haelew. 557a (5 thalli from elytra);



Figure 1. Thalli of species of *Laboulbenia*. (A) *Laboulbenia clivinalis* (slide CIQ-D. Haelew. 561b). (B) *Laboulbenia terminalis* (slide FH 00313313). (C) *Laboulbenia variabilis* (slide FH 00313311). (D) *Laboulbenia filifera* (slide FH00313389). (E) *Laboulbenia egens*, detail of appendage and perithecium (slide FH 00313112). Scale bars: A–D = 100 μ m, E = 50 μ m.

CANADA, Québec, Brome-Missisquoi, Saint-Armand, 17 August 2009, R. Juan, on *C. fossor*, D. Haelew. 558, in Collection d'insectes du Québec, slide FH-D. Haelew. 558a (9 juvenile thalli from elytra); same data, D. Haelew. 559, slides FH-D. Haelew. 559a (6 adult thalli from elytra) and FH-D. Haelew. 559b (1 adult thallus from pronotum); same data, D. Haelew. 560, slide FH-D. Haelew. 560a (1 adult and 2 juvenile thalli from elytra); same data, 18 August 2009, R. Juan, on *C. fossor*, D. Haelew. 561, in Collection d'insectes du Québec, slides FH-D. Haelew. 561a (6 thalli from elytra) and CIQ-D. Haelew. 561b (2 adult thalli from head); CANADA, Québec, Sainte-Foy, 27 June 2000, R. Juan, on *C. fossor*, D. Haelew. 562, in Collection d'insectes du Québec, slide FH-D. Haelew. 562a (1 adult thallus from right elytron); CANADA, Québec, Montréal, 16 May 1915, J.I. Beaulne, on *C. impressifrons* LeConte, 1844, D. Haelew. 563, in Collection d'insectes du Québec, slide FH-D. Haelew. 563a (4 thalli from elytra); US, KENTUCKY, Pulaski County, Somerset, 37°05'41.5608"N, 84°35'14.0964"W, 28 June 2012, B. Barnd, on *C. americana* Dejean, 1831, D. Haelew. 092, slide FH 00313165 (1 thallus from right elytron); UNITED KINGDOM, England, Yorkshire and the Humber Region, Mid-west Yorkshire Vice-County, Ripon Parks, 54°10'N 1°32'W, 16 June 2002, W. Dolling, on *C. fossor*, D. Haelew. 245, slides FH 00313399 (1 thallus from right metatrochanter) and FH 00313400 (1 juvenile thallus from left metatibia); UNITED KINGDOM, England, Yorkshire and the Humber Region, Southeast Yorkshire Vice-County, Hollym Carrs Nature Reserve, 53°41'N 0°0'E, 3 May 2013, W. Dolling, on *C. fossor*, D. Haelew. 271, slide FH 00313419 (4 thalli from left elytron).

Remarks. *Clivina* species are known to host 8 species of Laboulbeniales: *Dixomyces clivinae* (Thaxt.) I.I. Tav., *D. pallescens* (Thaxt.) I.I. Tav., *Laboulbenia anoplogenii* (accidental), *L. clivinalis*, *L. schizogenii* Thaxt., *L. timurensis* T. Majewski & K. Sugiy., *Ormomyces clivinae* (Thaxt.) I.I. Tav., and *Peyritschiella clivinae* Thaxt. A literature search reveals that from the American continents, only *D. clivinae* (Argentina, Mexico, USA), *L. pallescens* (Guatemala, Mexico), *L. schizogenii* (Ecuador, USA), and *P. clivinae* (USA) were reported from *Clivina* spp. (Proaño Castro and Rossi 2008; Thaxter 1896, 1912, 1931). Consequently, our records of *L. clivinalis* from Canada and the US (Kentucky, Massachusetts) are the first for the Americas. This is perhaps not surprising, given that *C. fossor* was introduced from Europe to North America with the first record from Montréal, QC, Canada, in 1915 (Ball and Bousquet 2001, Bousquet 1992, Lindroth 1961). However, we also found *L. clivinalis* on *Clivina americana* and *C. impressifrons*, 2 American-native species (Lorenz 2017). To make things more complicated, the record of *L. clivinalis* on *C. impressifrons* (also from Montréal) is from 1915, the same year of the first North American record of *C. fossor*. This could either mean that *L. clivinalis* was co-introduced with *C. fossor* and then shifted to native hosts (= co-invasion; Lymbery et al. 2014) or that the fungus was already present on the American continent when *C. fossor* was introduced.

***Laboulbenia egens* Speg.** (Fig. 1E), Anales de la Sociedad Científica Argentina 85:323 (1918)

= *Laboulbenia paupercula* Speg., Anales del Museo Nacional de Historia Naturel de Buenos Aires 27:59 (1915)

Distribution and hosts. Described on *Tachys* sp. indet. from Italy. Known from Tachyina beetles (Trechinae, Bembidiini) in Africa, Asia, and Europe (Santamaría et al. 1991). A single record of *L. egens* [as *L. pedicellata* Thaxt.] from the Caribbean (Guadeloupe Island) on a species of *Eotachys* Jeannel, 1941 (Trechinae, Bembidiini, Tachyina) was reported by Balazuc (1978). Majewski (1994, 2008) mentioned 2 specimens of *Bembidion octomaculatum* (Goeze, 1777) (Trechinae, Bembidiini) as hosts of *L. egens* in Poland.

New records from the BHI. Plymouth County, WORLD'S END PENINSULA, 42°16'16.4"N, 70°52'42"W, 24–30 August 2006, J. Rykken, on *Bembidion frontale* (LeConte, 1848), MCZ-ENT00626669, slide FH 00313112 (1 thallus); Norfolk County, GRAPE ISLAND, 42°16'7.4"N, 70°55'14.7"W, 25 July–1 August 2008, J. Rykken, on *B. frontale*, MCZ-ENT00626676, slide FH-D. Haelew. 1235a (2 sub-adult thalli from right elytron).

Remarks. Santamaría (1998) noted that *L. egens* is “probably cosmopolitan” on members of the subtribe Tachyina. Our reports from the BHI illustrate its presence on the North American continent for the first time. *Bembidion* Latreille, 1802 has only been reported once as host genus for this parasite (Majewski 1994, 2008). All reported hosts to date belong in the Bembidiini tribe; *Bembidion frontale* and *B. octomaculatum* are representatives of the Bembidiina subtribe, all other hosts belong to the subtribe Tachyina.

A number of Asian collections have been erroneously identified as *L. tachyis* Thaxt. (Kaur et al. 1993, Sugiyama and Phanichapol 1984) even though these species are easily distinguished by the position of cell IV relative to cell V. In addition, some confusion exists between *L. egens* and some forms of *L. pedicellata* Thaxt. *Laboulbenia pedicellata* is also exclusively associated with beetles in the tribe Bembidiini. Majewski (1994) suspected there may be intermediate forms between *L. egens* and *L. pedicellata*. *Bembidion octomaculata* and *Elaphropus parvulus* (Dejean, 1831) (currently accepted name of *Tachys parvulus*) reproduce at the same time of year and occupy the same fluvial habitats (Turin 2000), which could allow for an ecological shift from one host to the other (sensu De Kesel and Haelewaters 2014b). Molecular work will confirm whether *L. egens* is indeed separate from *L. pedicellata* or represents a form within its range of natural variation.

***Laboulbenia filifera* Thaxt.** (Fig 1D), Proceedings of the American Academy of Arts and Sciences 28:165 (1893)

Distribution and hosts. Reported on several genera of subfamilies Pterostichinae and Harpalinae. Found in North America, Europe, and Asia (Majewski 2008). Described from 1 of 3 species of *Anisodactylus* Dejean, 1829 (Harpalinae, Harpalini) in the US, but no type designated.

New records from the BHI. Suffolk County, SPECTACLE ISLAND, 42°19'26"N, 70°59'10"W, 9 May 2007, J. Rykken, on female *Anisodactylus harrisii* LeConte, 1863, MCZ-ENT00600759, slides FH 00313285 (4 thalli from elytra) and FH

00313364 (many thalli from right elytron); Suffolk County, SPECTACLE ISLAND, 42°19'34.5"N, 70°59'3.8"W, 6–20 July 2007, J. Rykken, on female *Anisodactylus harrisii*, MCZ-ENT00600762, slide FH 00313389 (12 thalli from elytral margins); same data, 22–27 June 2007, J. Rykken, on female *Anisodactylus harrisii*, MCZ-ENT00600760, slides FH 00313390 (12 thalli from lateral margin of left elytron) and FH 00313391 (10 thalli from lateral margin of left elytron; Norfolk County, GRAPE ISLAND, 42°16'9.3"N, 70°55'30.6"W, 25 July–1 August 2008, A. Clark, on female *Xestonotus lugubris* (Dejean, 1829) (Harpalinae, Harpalini), MCZ-ENT00600549, slide FH 00313286 (7 thalli from elytra); Plymouth County, BUMPKIN ISLAND, 42°16'52.4"N, 70°54'8.1"W 20–27 July 2006, M. Wheat, on *Anisodactylus rusticus* (Say, 1823), MCZ-ENT00614492, slide FH 00313147 (4 thalli from elytral margins).

Additional new records (non-BHI). CANADA, Québec, Municipalité d'Oka, 16 May 1936, S. Dumont, on *Anisodactylus kirbyi* Lindroth, 1953, D. Haelew. 551, in Collection d'insectes du Québec, slide FH-D. Haelew. 551a (3 juvenile and 6 adult thalli from right elytron); CANADA, Québec, Deux-Montagnes, Parc National d'Oka, 8 May 1994, P. Bélanger, on *Anisodactylus kirbyi*, D. Haelew. 553, in Collection d'insectes du Québec, slide FH-D. Haelew. 553a (1 adult thallus from elytral tips).

Remarks. *Laboulbenia filifera* is easily recognized, by its very long outer appendage that is divided above the suprabasal cell into 2 equal branches and by its darkened perithecial tip. Our material was typically much darker in the 2 upper rows of perithecial wall cells, compared to the rest of the perithecium. *Xestonotus* LeConte, 1853 was not previously reported for this species. This genus belongs in the subtribe Anisodactylina (subfamily Harpalinae, tribe Harpalini), along with *Anisodactylus*, from which *L. filifera* was described. Majewski (1994) suggested that the European material of *L. filifera* (as well as *L. compressa* Thaxt.) may belong to *L. flagellata* Peyr.

***Laboulbenia flagellata* Peyr.**, Sitzungsberichte der Kaiserlichen Akademie der Wissenschaften Math.-naturw. Klasse Abt. I 68:247 (1873)

= *Laboulbenia elongata* Thaxt., Proceedings of the American Academy of Arts and Sciences 27:10 (1892)

Distribution and hosts. On species of *Agonum* Bonelli, 1810, *Platynus* Bonelli, 1810 (Harpalinae, Platynini), *Pterostichus* Bonelli, 1810 (Harpalinae, Pterostichini), and many other genera in subfamilies Anthiinae, Brachininae, Elaphrinae, Harpalinae, Loricarinae, Nebriinae, and Patrobinae. Majewski (1994) noted that species in more than 80 carabid genera can host *L. flagellata*. One of the most commonly reported species of *Laboulbenia*, known from all continents except Antarctica (Santamaría et al. 1991).

New records from the BHI. Norfolk County, GRAPE ISLAND, 42°16'7.4"N, 70°55'14.7"W, 2–10 July 2008, S.W. Cho, on male *Pterostichus corvinus* (Dejean, 1828), MCZ-ENT00600381, slide FH 00313264 (2 thalli from pronotum); same data, on male *Agonum fidele* Casey, 1920, MCZ-ENT00626688, slides FH

00313290 (1 thallus from elytra), FH 00313291 (2 thalli from pronotum), FH-D. Haelew. 201c (3 adult thalli from left elytron, FH-D. Haelew. 201d (1 thallus from right metatarsus), and FH-D. Haelew. 201e (4 thalli from sternites); same data, on female *Agonum fidele*, MCZ-ENT00626684, slide FH-D. Haelew. 1195a (2 adult thalli from pronotum); same data, on female *Agonum fidele*, MCZ-ENT00626685, slides FH-D. Haelew. 1196a (2 juvenile thalli from left elytron), FH-D. Haelew. 1196b (2 adult thalli from right mesofemur), FH-D. Haelew. 1196c (1 adult from left metatarsus), FH-D. Haelew. 1196d (1 thallus from proximal margin of pronotum), and FH-D. Haelew. 1196e (4 thalli from last sernite); same data, on male *Agonum fidele*, MCZ-ENT00626686, slides FH-D. Haelew. 1197a (2 thalli from left elytron) and FH-D. Haelew. 1197b (3 thalli from left protrochanter); same data, on male *Agonum fidele*, MCZ-ENT00626687, slides FH-D. Haelew. 1198b (1 subadult and 3 adult thalli from right elytron), FH-D. Haelew. 1198c (1 adult thallus from left elytron), and FH-D. Haelew. 1198d (1 adult thallus from left labial palp); same data, on female *Agonum gratiosum* (Mannerheim, 1853), MCZ-ENT00626690, slide FH-D. Haelew. 1084a (1 adult thallus from pronotum); same data, on male *Agonum gratiosum*, MCZ-ENT00626691, slide FH-D. Haelew. 1085a (3 thalli from pronotum and elytra); same data, on female *Agonum melanarium* Dejean, 1828, MCZ-ENT00600569, slide FH 00313289 (3 thalli from pronotum); same data, on female *Agonum melanarium*, MCZ-ENT00600573, slide FH-D. Haelew. 1217a (1 adult thallus from right elytron); same data, on female *Agonum melanarium*, MCZ-ENT00600572, slide FH-D. Haelew. 1218a (2 juvenile thalli from left elytral shoulder); same data, 14–22 August 2008, J. McCarron, on female *Agonum gratiosum*, MCZ-ENT00626689, slide FH-D. Haelew. 1083a (1 juvenile and 6 adult thalli from elytra around scutellum); same data, on female *Agonum melanarium*, MCZ-ENT00600574, slide FH-D. Haelew. 1216a (1 adult thallus from pronotum); same data, 30 May–12 June 2008, J. Rykken, on female *Agonum fidele*, MCZ-ENT00626683, slide FH-D. Haelew. 1194a (1 adult thallus from head); same data, on female *Agonum melanarium*, MCZ-ENT00626694, slides FH-D. Haelew. 1201a (6 thalli from pronotum), FH-D. Haelew. 1201b (8 thalli from elytra), and FH-D. Haelew. 1201c (1 thallus from left eye); Norfolk County, GRAPE ISLAND, 42°16'8"N, 70°55'13.3"W, 27 September–20 October 2005, J. Rykken, on male *Patrobis longicornis* (Say, 1823), MCZ-ENT00626654, slides FH 00313295 (3 thalli from elytra), FH 00313581 (4 thalli from elytra), and FH 00313582 (1 subadult thallus from right elytral tip); same data, on female *Patrobis longicornis*, MCZ-ENT00626709, slides FH 00313383 (2 juvenile thalli from right elytron) and FH 00313384 (2 juvenile thalli from elytra); same data, on female *Patrobis longicornis*, MCZ-ENT00626707, slide FH 00313385 (1 adult and 2 juvenile thalli from right elytron); Norfolk County, GRAPE ISLAND, 42°16'5"N 70°55'19.8"W, 20–27 October 2005, J. Rykken, on male *Pterostichus mutus* (Say, 1823), MZC-ENT00600424, slides FH 00313273 (3 thalli from elytra) and FH-D. Haelew. 480b (4 adult thalli from elytra); Norfolk County, GRAPE ISLAND, 42°16'15.3"N, 70°55'2.7"W, 30 May–12 June 2008, J. Rykken, on male *Pterostichus pensylvanicus* LeConte, 1873, MCZ-ENT00600478, slide FH-D. Haelew. 1206a (2 adult

thalli from right elytral tip); same data, on male *Pterostichus pensylvanicus*, MCZ-ENT00600479, slide FH-D. Haelew. 1207a (1 adult thallus from left prosternum); Suffolk County, CALF ISLAND, 42°20'25.5"N, 70°53'48.9"W, 31 July–7 August 2007, S. Madden, on *Pterostichus patruelis* (Dejean, 1831), MCZ-ENT00600438, slide FH 00313284 (2 thalli from elytra); same data, 3–12 July 2007, J. Rykken, on female *Agonum gratiosum*, MCZ-ENT00626693, slide FH-D. Haelew. 1087a (4 subadult thalli from pronotum); same data, on *Pterostichus patruelis*, MCZ-ENT00600446, slides FH-D. Haelew. 1215a (1 subadult thallus from right elytron) and FH-D. Haelew. 1215b (2 adult thalli from right profemur); same data, 17–24 July 2007, S. Madden, on *Pterostichus patruelis*, MCZ-ENT00600451, slides FH-D. Haelew. 1210a (1 adult thallus from right proepisternum) and FH-D. Haelew. 1210b (3 adult thalli from lateral margin of right elytron); same data, on *Pterostichus patruelis*, MCZ-ENT00600450, slide FH-D. Haelew. 1212a (1 adult and 4 subadult thalli from lateral margin of right elytron); same data, on *Pterostichus patruelis*, MCZ-ENT00600449, slide FH-D. Haelew. 1213a (6 subadult thalli from lateral margin of right elytron); same data, on *Pterostichus patruelis*, MCZ-ENT00600448, slide FH-D. Haelew. 1214a (1 adult and 2 subadult thalli from right elytron); Suffolk County, GREAT BREWSTER ISLAND, 42°19'50"N, 70°53'47.9"W, 24 July–2 August 2006, R. Becker, on female *Agonum gratiosum*, MCZ-ENT00626692, slides FH 00313292 (3 thalli from right antenna), FH-D. Haelew. 1086a (6 adult thalli from scapi of antennae), and FH-D. Haelew. 1086b (4 adult thalli from profemora and -tibiae); Plymouth County, WORLD'S END PENINSULA, 42°16'16.4"N, 70°52'42"W, 22–27 June 2006, J. Rykken, on female *Agonum melanarium*, MCZ-ENT00626700, slides FH 00313293 (2 thalli from elytra) and FH 00313294 (2 thalli from pronotum); same data, on male *Agonum melanarium*, MCZ-ENT00626696, slides FH-D. Haelew. 1203a (2 thalli from pronotum) and FH-D. Haelew. 1203b (1 adult thallus from right elytron); same data, on female *Agonum melanarium*, MCZ-ENT00626697, slides FH-D. Haelew. 1204a (2 adult thalli from pronotum), FH-D. Haelew. 1204b (1 adult thallus from left elytron), and FH-D. Haelew. 1204c (1 adult thallus from right metatrochanter); same data, on female *Agonum melanarium*, MCZ-ENT00626698, slides FH-D. Haelew. 1205a (1 adult thallus from pronotum), FH-D. Haelew. 1205b (1 adult thallus from right elytron), FH-D. Haelew. 1205c (1 adult thallus from left metatibia), and FH-D. Haelew. 1205d (3 subadult thalli from left mesocoxa); same data, 25 July–4 August 2006, R. Becker, on female *Agonum ferreum* Haldeman, 1843, MCZ-ENT00626657, slides FH 00313299 (5 thalli from elytra), FH 00313300 (4 thalli from pronotum), and FH 00313584 (8 juvenile and 2 adult thalli from left elytron); same data, 24–30 July 2006, J. Rykken, MCZ-ENT00626695, slides FH-D. Haelew. 1202a (3 thalli from pronotum), FH-D. Haelew. 1202b (8 thalli from elytra), FH-D. Haelew. 1202c (2 adult thalli from left metatrochanter), FH-D. Haelew. 1202d (3 adult thalli from right profemur), FH-D. Haelew. 1202e (12 thalli from meso- and metasternum), and FH-D. Haelew. 1202f (1 adult thalli from left mesofemur).

Additional new records (non-BHI). CANADA, Québec, municipalité d'Oka, 2 June 1934, S. Dumont, on *Pterostichus caudicalis*, D. Haelew. 580, in Collection

d'insectes du Québec, slide FH-D. Haelew. 580a (2 juvenile thalli from pronotum, *L. cf. flagellata*); DEMOCRATIC REPUBLIC OF THE CONGO, Orientale Province, Kisangani [as Stanleyville], 0°30'N, 25°10'E, 20 August 1909, H. Lang & J. Chapin, on *Abacetus audax* Laferte-Senectere, 1853 (Harpalinae, Abacetini), D. Haelew. 329, in coll. American Museum of Natural History, slide FH 00313551 (2 adult thalli from right elytron); US, MASSACHUSETTS, Barnstable County, Eastham, premises of Eagle Wing Inn, 7 May 2016, W.P. Pfliegler, on *Agonum* sp., D. Haelew. 1029, slide D. Haelew. 1029a (4 subadult thalli from elytra).

***Laboulbenia inflata* Thaxt.**, Proceedings of the American Academy of Arts and Sciences 27:41 (1892)

Distribution and hosts. Described from South Dakota, USA. In the original description (Thaxter 1892), *Bembidion* sp. (Trechinae, Bembidiini) was given as host, but this is likely a misidentification. Thaxter (1896) only mentioned *Bradycellus rupestris* (Say, 1823) (Harpalinae, Harpalini) as host species. In his synoptic key, Thaxter (1894) mentioned *L. inflata* to be associated with *B. rupestris*. *Laboulbenia inflata* is thus far reported on species of *Acupalpus* Latreille, 1829, *Stenolophus* Dejean, 1821, and *Bradycellus* Erichson, 1837 (Harpalinae, Harpalini) in North America (US), South America (Argentina, Galápagos Archipelago), Europe (Belgium, Bulgaria, France, Germany, Great Britain, Greece, Italy, the Netherlands, Poland, Spain), and Asia (South Korea) (De Kesel 1998, Haelewaters et al. 2014, Majewski 2008, Rossi et al. 2018).

New records from the BHI. Norfolk County, GRAPE ISLAND, 42°16'8.9"N, 70°55'11.8"W, 22 September 2005, J. Rykken, on *Acupalpus hydropicus* (Leconte, 1863), MCZ-ENT00626705, slides FH 00313302 (1 thallus from pronotum) and FH 00313365 (2 thalli from right elytron); same data, 27 September–4 October 2005, J. Rykken, on *Acupalpus nanellus* Casey, 1914, MCZ-ENT00626655, slide FH 00313303 (1 thallus from right elytron); Suffolk County, CALF ISLAND, 42°20'28.2"N, 70°53'46"W, 23–30 October 2007, J. Rykken, on *Elaphropus incurvus* (Say, 1830) (Trechinae, Bembidiini), MCZ-ENT00626644, slide FH 00313135 (1 thallus); Suffolk County, GREAT BREWSTER ISLAND, 42°20'1.7"N, 70°53'48.1"W, 14–21 June 2006, J. Rykken, on *Elaphropus vernicatus* (Casey, 1918), MCZ-ENT00626651, slide FH 00313137 (1 thallus); Plymouth County, WORLD'S END PENINSULA, 42°15'51.9"N, 70°52'37.8"E, 16 August 2006, J. Rykken, on *Stenolophus ochropezus*, MCZ-ENT00600506, slide FH-D. Haelew. 1470a (1 adult thallus from left proepisternum).

Additional new records (non-BHI). US, NEBRASKA, Scotts Bluff County, Fanning, 41°56'59"N, 103°42'18"W, “401 Zone-tillage”, 19 June 2013, R.J. Pretorius & H. Pretorius, on *Elaphropus anceps* (LeConte, 1848), D. Haelew. 237, in coll. Panhandle Research and Extension Center, slide FH-D. Haelew. 237a (3 adult thalli from elytra); same data, “106 Plowed”, 10 July 2013, R.J. Pretorius & H. Pretorius, on *Elaphropus anceps*, D. Haelew. 1480, in coll. Panhandle Research and Extension Center, slides PHREC-D. Haelew. 1480a (1 adult thallus from prosternum) and PHREC-D. Haelew. 1480b (1 adult thallus from pronotum); same data, “403 Plowed”, 19 June 2013, R.J. Pretorius & H. Pretorius, on *Elaphropus anceps*,

D. Haelew. 1481, in coll. Panhandle Research and Extension Center, slide PHREC-D. Haelew. 1481a (3 thalli from right profemur).

Remarks. *Laboulbenia inflata* is easily recognized by the black and constricted basal septa of the lower cells of its appendages. In addition, its outer appendage is simple, the inner appendage consists of 2–3 simple branches, and the perithecium-bearing thallus is paired with a small filiform thallus consisting of 6–7 superposed cells (Arndt and Desender 2002, De Kesel 1997). Dioecism in *L. inflata* has yet not been proven unequivocally. Santamaría (1996), however, cites ascospores that are built to be released in pairs, the trichogyne that grows downward (in *L. marina* F. Picard), and the presumed male thallus of which the uppermost cell functions as an antheridium as evidence of dioecy for both *L. inflata* and *L. marina*.

Thaxter (1892, 1896) reported *L. inflata* in Maine, Massachusetts, Rhode Island, and South Dakota (type). Since then, no reports of this species have been published for North America. Consequently, our records represent the first North American collections in over a century. The records from Nebraska are the first ones for this US state.

***Laboulbenia macrotheca* Thaxt.**, Proceedings of the American Academy of Arts and Sciences 30:474 (1895)

Lectotype, designated here. USA, Maine, Kittery Point, 23 June 1893, [R. Thaxter], on *Anisodactylus sanctaecrucis* (Fabricius, 1798) (Harpalinae, Harpalini), slide FH 00313740 (5 adult thalli). Typification identifier: IF556762.

Distribution and hosts. Described from *Anisodactylus sanctaecrucis* [as *Anisodactylus* “*baltimorensis*” = *baltimoriensis*] in Maine, USA, and *Anisodactylus* sp. in New Brunswick, Canada, but no type designated. Known on species of *Anadaptus* Casey, 1914, *Anisodactylus* (Harpalinae, Harpalini, Anisodactylina), *Harpalus* Latreille, 1802, *Ophonus* Dejean, 1821, and *Trichotichnus* Morawitz, 1863 (Harpalinae, Harpalini, Harpalina) in North America, Europe, and Asia (Santamaría 1993).

New records from the BHI. Suffolk County, SPECTACLE ISLAND, 6 July 2007, on *Harpalus opacipennis* (Haldemann, 1843) (Harpalinae, Harpalini), MCZ-ENT, slide FH 00313150 (5 thalli); Suffolk County, GREAT BREWSTER ISLAND, 42°19'50"N, 70°53'47.9"W, 23–30 August 2006, S. Madden, on male *Harpalus somnulentus* Dejean, 1829, MCZ-ENT00614730, slide FH 00313152 (5 thalli).

Remarks. Majewski (1994) includes this species among the synonyms of *L. flagellata*. In the absence of molecular phylogenetic data for *L. macrotheca*, we follow Santamaría’s (1998) morphological arguments to consider it a separate taxon. Since *L. flagellata* is a species complex (see Results) and considering previous results (e.g., in the genus *Hesperomyces*; Haelewaters et al. 2018), we think that currently the best practice is not to synonymize taxa without the inclusion of molecular characters.

Because Thaxter (1895, 1896, 1908) designated no type specimen, we decided to re-examine Thaxter’s slides of *L. macrotheca*, which are deposited at FH. This led to our selection of a slide to serve as lectotype.

***Laboulbenia pedicellata* Thaxt.**, Proceedings of the American Academy of Arts and Sciences 27:44 (1892)

Distribution and hosts. Described from *Bembidion* sp. in Maine, USA. On many species of the genera *Bembidion* Latreille, 1802 sensu lato (Trechinae, Bembidiini) and *Dyschirius* Bonelli, 1810 (Scaritinae, Dyschiriini). Widely distributed, with reports in many European countries (most recently from Bulgaria), North America, South America, Africa, and Asia (Santamaría et al. 1991, Majewski 2008, Rossi et al. 2018).

New records from the BHI. Suffolk County, THOMPSON ISLAND, 42°18'52.1"N, 71°0'43.1"W, 2–9 October 2006, B.D. Farrell & OEB10, on *Dyschirius globulosus* (Say, 1823), MCZ-ENT00626664, slides FH 00313134 (1 thallus), FH 00313304 (1 thallus from pronotum), and FH-D. Haelew. 1081a (2 adult thalli from left profemur); Suffolk County, THOMPSON ISLAND, 42°18'50"N, 71°0'47.1"W, 3–13 July 2007, J. Rykken, on *Dyschirius globulosus*, MCZ-ENT00626663, slide FH-D. Haelew. 1082a (from distal tip of right elytron).

Additional new records (non-BHI). US, NEW HAMPSHIRE, 16 July 1928, A. Nicolay, on *Bembidion versicolor* (LeConte, 1848), D. Haelew. 134, in coll. American Museum of Natural History, slide FH 00313343 (1 thallus from proximal third of right elytron); UKRAINE, Crimean Peninsula, Yevpatoriya [as Eupatoria], 11 May 1943, P. Rubtzov, on *Bembidion* sp., D. Haelew. 307, in coll. American Museum of Natural History, slide FH 00313446 (1 thallus from right-hand side sternite).

Remarks. This is the second report of this species in the US, after the type. Thus far, *L. pedicellata* is only known in the northeastern states of Maine (Thaxter 1892) and Massachusetts and New Hampshire (this study). The report from Ukraine represents a new country record.

Laboulbenia pedicellata belongs to a group of taxa with similar morphologies, including *L. clivinalis*, *L. gregaria* W. Rossi, *L. lichtensteinii* F. Picard, *L. littoralis* De Kesel & Haelew., *L. luxurians* Peyr., *L. parriaudii* Balazuc ex Santam., *L. patrata* Thaxt., *L. slackensis* Cépède & F. Picard, and *L. tenera* T. Majewski. These seem to occur on hosts from riparian habitats and have thalli that are recognized by (1) cells IV and V being equal in height with a vertical septum, and (2) a dark and constricted septum between the basal and suprabasal cells of the outer appendage. Huldén (1985), Majewski (1994), and Santamaría (1998) discuss the morphological variability of *L. pedicellata*; some forms may be separated as distinct species in the future.

***Laboulbenia terminalis* Thaxt.** (Fig. 1B), Proceedings of the American Academy of Arts and Sciences 30:475 (1895)

Lectotype, designated here. USA, Massachusetts, Belmont/Waltham, Waverley neighborhood, 27 September 1893, R. Thaxter, on *Pterostichus luctuosus* (Dejean, 1828) (Harpalinae, Pterostichini), slide FH 00313741 (5 thalli forming complete developmental series). Typification identifier: IF556763.

Distribution and hosts. Only known from the US (Maine and Massachusetts) on *Pterostichus luctuosus*.

New records from the BHI. Norfolk County, GRAPE ISLAND, 42°16'7.4"N, 70°55'14.7"W, 2–10 July 2008, S.W. Cho, on male *Pterostichus luctuosus*, MCZ-ENT00600395, slide FH 00313308 (3 thalli from sternite VII); Norfolk County, GRAPE ISLAND, 42°16'7.4"N, 70°55'14.7"W, 25 July–1 August 2008, A. Clark, on male *Pterostichus luctuosus*, MCZ-ENT00600397, slides FH 00313310 (1 thallus from sternite VI) and FH 00313313 (1 thallus from last sternite); same data, MCZ-ENT00600398, slide FH 00313575 (1 juvenile thallus from pronotum).

Remarks. These collections are the first of this species reported in over a century. Thaxter (1895) suggested this species was restricted to the elytral tips and the abdomen. We assume that these observations may have been premature based on an insufficient amount of material. Thaxter (1896:317) did mention that this species is “comparatively rare”. We have found 1 thallus from the host’s pronotum.

We found 3 specimens of *P. luctuosus* with this species. One of these insects (MCZ-ENT00600395) carried a triple infection by *L. terminalis*, *L. variabilis* Thaxt., and *Peyritschiiella geminata* Thaxt. The other 2 specimens carried a double infection of *L. terminalis* and *L. variabilis*. Double infections of Laboulbeniales are reported regularly: e.g., *Corethromyces henrotii* Balazuc ex Balazuc and *Diphymyces kaaistoepi* Haelew. & De Kesel [as *Corethromyces cholevae* nom. prov.] on *Choleva cisteloides* (Frölich, 1799) (De Kesel and Rammeloo 1992, De Kesel 1997); *Gloeandromyces* spp. and *Nycteromyces streblidinus* Thaxt. on *Trichobius joblingi* Wenzel, 1966 (Walker et al. 2018); *Herpomyces chaetophilus* Thaxt. and *H. periplanetae* Thaxt. on *Periplaneta americana* (Linnaeus, 1758) (Wang et al. 2016); *Hesperomyces coleomegillae* W. Rossi & A. Weir and *H. palustris* W. Rossi & A. Weir on *Coleomegilla maculata* (DeGeer, 1775) (Goldmann et al. 2013); *Laboulbenia claudaei* Santam. & Faille and *L. strigomeri* Santam. & Faille on *Strigomerus girardi* Straneo, 1991 (Santamaría and Faille 2009); and *Rickia laboulbenioides* De Kesel and *Troglomyces manfrediae* S. Colla on an unidentified Julidae millipede (C. Gerstmans and A. De Kesel, pers. observ.). Mixed infections with more than 2 species of Laboulbeniales, on the other hand, are much harder to find in the literature. The classic example, species of *Chitonomyces* Peyr. on *Laccophilus* spp. and *Orectogyrus specularis* (Dejean, 1833) (Goldmann and Weir 2012, Thaxter 1926), is often cited. Cases with species in 3 genera of Laboulbeniales occurring on a single host are excessively rare and reported only from flies (Rossi 1982) and beetles (Rossi 1992). Other examples that we are aware of include 3 species of *Dioicomycetes* Thaxt. simultaneously parasitizing *Anthicus floralis* (Linnaeus, 1758) (Thaxter 1908): *Laboulbenia barbara* Middelh. & Boelens, *L. philonthi* Thaxt., and *Peyritschiiella vulgata* (Thaxt.) I.I. Tav. on *Philonthus quisquiliarius* (Gyllenhal, 1810); and *Cantharomyces denigratus* Thaxt., *C. italicus* Speg., and *Helodiomyces elegans* F. Picard on *Dryops luridus* (Erichson, 1847) (De Kesel and Haelewaters 2014a).

Thaxter (1895, 1896) designated no type for this species. Of several available slides present at FH of collections made in the Waverly neighborhood, MA, on the same day, slide FH 00313741 is in beautiful condition and contains a developmental series with 5 thalli. This specimen is designated above as the lectotype.

***Laboulbenia variabilis* Thaxt.** (Fig. 1C), Proceedings of the American Academy of Arts and Sciences 27:38 (1892)

Distribution and hosts. Described on several carabid species from various localities in the US. Known on species of *Chlaenius* Bonelli, 1810 (Liciniinae, Chlaeniini), *Nebria* Latreille, 1802 (Nebriinae, Nebriini), *Omophron* Latreille, 1802 (Omophroninae, Omophronini), *Patrobus* Dejean, 1821 (Trechinae, Patrobiini), *Platynus* (Platyninae, Platynini), and *Pterostichus* (Harpalinae, Pterostichini) in North and South America. Also reported from *Tetracha* spp. (Cicindelinae, Megacephalini) in Ecuador (Arndt et al. 2003, Thaxter 1908).

New records from the BHI. Norfolk County, GRAPE ISLAND, 42°16'7.4"N, 70°55'14.7"W, 25 July–1 August 2008, A. Clark, on male *Pterostichus caudicalis* (Say, 1823), MCZ-ENT00600357, slides FH 00313265 (1 thallus from left leg), FH 00313266 (5 thalli from elytra), and FH-D. Haelew. 1145a (1 adult thallus from right elytron); same data, on male *Pterostichus caudicalis*, MCZ-ENT00600358, slides FH 00313267 (2 thalli from prosternum), FH 00313268 (4 thalli from elytra), and FH 00313269 (1 thallus from right mesoleg); same data, on male *Pterostichus caudicalis*, MZC-ENT00600359, slides FH 00313270 (3 thalli from elytra), FH 00313271 (3 thalli from gena), and FH 00313272 (2 thalli from left proleg); same data, 2–10 July 2008, S.W. Cho, on male *Pterostichus caudicalis*, MCZ-ENT00600353, slide FH-D. Haelew. 481b (from left mesofemur); same data, on female *Pterostichus corvinus*, MCZ-ENT00600369, slide FH 00313263 (5 thalli from elytra); same data, on male *Pterostichus corvinus*, MCZ-ENT00600361, slide FH-D. Haelew. 1208a (2 juvenile thalli from proximal margin of right elytron); same data, on male *Pterostichus corvinus*, MCZ-ENT00600362, slides FH-D. Haelew. 1209a (1 juvenile thallus from right clypeo-ocular prolongation) and FH-D. Haelew. 1209b (1 juvenile thallus from left elytron); same data, 25 July–1 August 2008, A. Clark, on female *Pterostichus luctuosus*, MCZ-ENT00600389, slide FH 00313275 (5 thalli from elytra); same data, on female *Pterostichus luctuosus*, MCZ-ENT00600390, slides FH 00313276 (4 thalli from elytra) and FH 00313277 (1 thallus from prosternum); same data, on female *Pterostichus luctuosus*, MCZ-ENT00600391, slides FH 00313280 (2 thalli from elytra), FH 00313281 (2 thalli from right epipleuron), and FH 00313282 (1 thallus from right proleg); same data, on male *Pterostichus luctuosus*, MCZ-ENT00600397, slides FH 00313311 (3 thalli from left elytron) and FH 00313312 (1 thallus from left mesofemur); same data, on male *Pterostichus luctuosus*, MCZ-ENT00600398, slides FH 00313574 (1 juvenile and 3 adult thalli from left mesofemur) and FH 00313576 (2 adult thalli from right metasternum); same data, 2–10 July 2008, S.W. Cho, on male *Pterostichus luctuosus*, MCZ-ENT00600394, slide FH 00313305 (1 thallus from right mesofemur); same data, on male *Pterostichus luctuosus*, MCZ-ENT00600395, slides FH 00313307 (3 thalli from right proepisternum) and FH 00313309 (2 thalli from mesepisternum); same data, 8–16 October 2008, J. Rykken, on male *Patrobus longicornis*, MCZ-ENT00626708, slides FH 00313378 (1 thallus from distal part of right elytron), FH 00313379 (6 thalli from lateral margin of right elytron), and FH 00313382 (1 adult thallus from proximal margin of right elytron); Norfolk County, GRAPE ISLAND, 42°16'8"N, 70°55'13.3"W, 27 September–20 October 2005, J. Rykken, on female

Pterostichus luctuosus, MCZ-ENT00600384, slide FH 00313274 (2 thalli from elytra); same data, on female *Pterostichus luctuosus*, MCZ-ENT00600385, slide FH 00313577 (3 adult thalli from elytral shoulders); same data, on male *Patrobis longicornis*, MCZ-ENT00626654, slides FH 00313581 (1 juvenile thallus from elytra) and FH 00313582 (1 juvenile thallus from tip of right elytron).

Additional new records (non-BHI). CANADA, Québec, municipalité d'Oka, 2 June 1934, S. Dumont, on *Pterostichus caudicalis*, D. Haelew. 580, in Collection d'insectes du Québec, slide FH-D. Haelew. 580b (3 adult thalli from junction of left metafemur and -tibia); CANADA, Québec, Ville de Québec, 28 August 1952, J.-P. Laplante, on *Pterostichus caudicalis*, D. Haelew. 581, in Collection d'insectes du Québec, slide FH-D. Haelew. 581a (5 juvenile thalli from left elytron); CANADA, Québec, municipalité de Nicolet, 2 September 1963, no collector, on *Pterostichus caudicalis*, D. Haelew. 582, in Collection d'insectes du Québec, slides FH-D. Haelew. 582a (4 adult thalli from elytral tips) and CIQ-D. Haelew. 582b (3 adult thalli from right metafemur); CANADA, Québec, Nicolet-Bécancour, 1 June 1968, C. Chantal, on *Chlaenius cordicollis* Kirby, 1837 (Harpalinae, Chlaeniini), D. Haelew. 866, STOCKWELL STRI-ENT 0 043 373, in coll. Smithsonian Tropical Research Institute-Tupper Center, slides FH-D. Haelew. 866a (3 thalli from elytra) and MIUP-D. Haelew 866b (3 thalli from elytra); US, SOUTH CAROLINA, Calhoun County, Congaree River, 20 April 1968, H.P. Stockwell, on *Chlaenius aestivus* Say, 1823, D. Haelew. 856, STOCKWELL STRI-ENT 0 043 370, in coll. Smithsonian Tropical Research Institute-Tupper Center, slides FH-D. Haelew. 865a (3 adult thalli from elytra) and MIUP-D. Haelew. 865b (1 adult thallus from right metatibia).

Remarks. In North America, *L. variabilis* has been reported from California, Connecticut, Louisiana, "Maine to Florida", Nebraska, New York, South Dakota, Texas, Utah, Virginia, and Washington in the US as well as from Cuba and Mexico (Thaxter 1892, 1896, 1908). In South America, collections are known from Argentina, Brazil, Chile, and Ecuador (Arndt et al. 2003; Thaxter 1896, 1908). Here we report the first records from Canada, removed from 2 host species—*Pterostichus caudicalis* and *Chlaenius cordicollis*.

On 7 host specimens of 2 species, we observed mixed infections of *L. variabilis* and at least 1 other species of Laboulbeniales. On 2 specimens of *Patrobis longicornis* (MCZ-ENT00626654, MCZ-ENT00626708), we found both *L. flagellata* and *L. variabilis*. On *Pterostichus caudicalis*, we found mixed infections with *L. terminalis* and *L. variabilis* (3 specimens: MCZ-ENT00600394, MCZ-ENT00600397, MCZ-ENT00600398); with *L. variabilis* and *Peyritsiella geminata* (1 specimen: MCZ-ENT00600390); or with all 3 species (1 specimen: MCZ-ENT00600395).

Laboulbenia variabilis as currently circumscribed is easily recognized. Cells IV and V carry apically a number of small cells that serve as basal cells for the numerous appendages. The appendages can be branched, with the lower cells somewhat inflated, divided by dark and constricted septa, and the other cells towards the distal end slenderer and tapering. However, *L. variabilis* is a potential problematic taxon regarding species delimitation. It has been reported from many host species

and genera in different subfamilies. As is the case for *L. flagellata*, it may be that different hosts (species or genera) carry distinct cryptic or near-cryptic phylogenetic species. This taxon shows considerable variation in size (although this can even be observed on the same host specimen). Thaxter (1896:351) highlighted “specimens on *Pterostichus caudicalis* measuring over a millimeter and a half, while many individuals on *Omophron* are less than 200 μm in length”. Finally, morphological features are also variable among thalli, such as the perithecial shape and position. *Laboulbenia variabilis* is a good candidate for dedicated species-level taxonomic work, which should include collection of fresh material, a morphometric approach with consideration of previously neglected morphological characters, and generation of ITS and nrLSU rDNA sequences.

In his description of *L. variabilis*, Thaxter (1892) did not designate a type, neither did he describe what he thought of as the “typical” host. Thaxter’s collection at FH consists of about 70 slides of *L. variabilis*. Many of them are in bad condition and have missing metadata; in several occasions, no host name is provided. For this reason, we decided not to designate a lectotype. Instead, we think designating an epitype (fide Turland et al. 2018) would be best practice, after detailed study and with associated DNA sequence data.

***Laboulbenia vulgaris* Peyr.**, Sitzungsberichte der Kaiserlichen Akademie der Wissenschaften Mathematisch-Naturwissenschaftliche Classe Abt. I 68:248 (1873)

Distribution and hosts. On numerous species of Trechinae (mostly *Bembidion* sensu lato, *Trechus* Clairville, 1806 sensu lato, and *Trechoblemus* Ganglbauer, 1891) and other hosts of different subfamilies of Carabidae (Santamaría et al. 1991). Widely distributed, with records in North and South America, Europe, Africa, and Asia.

New records from the BHI. Norfolk County, GRAPE ISLAND, 42°16'7.4"N, 70°55'14.7"W, 25 July–1 August 2008, A. Clark, on *Bembidion graciliforme* Hayward, 1897, MCZ-ENT00626677, slides FH 00313296 (4 thalli from elytra) and FH 00313297 (4 thalli from left mesoleg); same data, MCZ-ENT00626678, slide FH 00313298 (2 thalli from elytra).

Additional new records (non-BHI). CROATIA, Split-Dalmatia County, Dugopolje, Mosor Mountains, Maklutača špilja [cave], 29 August 2010, D. Čeplík, on *Duvalius (Eduvalius) erichsonii netolitzkyi* Müller, 1908 (Trechinae, Trechini), D. Haelew. 136, in pers. coll. Dávid Čeplík, slides FH 00313195 (2 adult thalli from left elytron) and FH 00313196 (2 adult thalli from right elytron); same data, D. Haelew. 137, in pers. coll. Dávid Čeplík, slides FH 00313197 (2 adult thalli from left elytron), FH 00313198 (2 adult thalli from proximal third of left elytron), and FH 00313199 (2 adult thalli from right elytron); SLOVAKIA, Košice Region, Slovenský kras, Silická planina plateau, Závozná priepasť [cave], 48°33'36.26"N, 20°28'47.08"E, 8 May 2000, D. Čeplík, on *Duvalius hungaricus sziliczensis* (Csiki, 1912) (Carabidae, Trechinae), D. Haelew. 140, in pers. coll. Dávid Čeplík, slides FH 00313205 (4 juvenile and 7 adult thalli from distal third of right elytron) and FH 00313206 (3 juvenile, 1 subadult and 2 adult thalli from right elytron); SLOVENIA, Savinja Statistical Region, Studence, Steska cave, 370 m a.s.l., April 1995, M.

Egger, on *Anophthalmus hitleri* Scheibel, 1933 (Trechinae, Trechini), D. Haelew. 141, slides FH 00313207 (1 thallus from pronotum) and FH 00313208 (3 adult thalli from right elytron); UNITED KINGDOM, England, Yorkshire and the Humber Region, South-east Yorkshire Vice-County, Elstronwick, Brook Farm, 53°46'N, 00°07'W, 10 October 1994, W. Dolling, on *Ocys harpaloides* (Audinet-Serville, 1821) (Trechinae, Bembidiini), D. Haelew. 273, slides FH 00313421 (6 adult thalli from elytra) and FH 00313422 (5 adult slides from pronotum); CANADA, Québec, Harrington, 1 July 1971, A. Larochelle, on *Trechus apicalis* Motschulsky, 1845 (Carabidae, Trechinae, Trechini), D. Haelew. 571, in Collection d'insectes du Québec, slide FH-D. Haelew. 571a (1 subadult and 3 adult thalli from pronotum); same data, D. Haelew. 572, in Collection d'insectes du Québec, slide FH-D. Haelew. 572a (1 subadult thallus from pronotum); same data, D. Haelew. 573, in Collection d'insectes du Québec, slides FH-D. Haelew. 573a (2 adult thalli from pronotum) and CIQ-D. Haelew. 573b (3 juvenile and 6 adult thalli from elytra); CANADA, Québec, Saint-Augustin-de-Desmaures, 6 June 1954, J.-P. Laplante, on *Trechus apicalis*, D. Haelew. 574, in Collection d'insectes du Québec, slide FH-D. Haelew. 574a (4 juvenile thalli from left elytron); CANADA, Québec, Charlevoix-Est, Baie-Sainte-Catherine, 17 August 1992, P. Bélanger, on *Trechus apicalis*, D. Haelew. 575, in Collection d'insectes du Québec, slide FH-D. Haelew. 575a (5 thalli from pronotum and right elytron); CANADA, Québec, Les Appalaches, Municipalité de Saint-Jacques-de-Leeds, 17 July 1992, no collector, on *Trechus apicalis*, D. Haelew. 576, in Collection d'insectes du Québec, slide FH-D. Haelew. 576a (3 thalli from elytra); CANADA, Québec, L'Île d'Anticosti, Port-Menier, 13 July 1971, A. Larochelle, on *Bembidion bruxellense* Wesmael, 1835 (Carabidae, Trechinae, Bembidiini), D. Haelew. 578, in Collection d'insectes du Québec, slides FH-D. Haelew. 578a (6 thalli from pronotum) and CIQ-D. Haelew. 578b (1 juvenile and 2 adult thalli from elytra); same data, D. Haelew. 579, in Collection d'insectes du Québec, slide FH-D. Haelew. 579a (2 subadult thalli from right elytron).

Remarks. The presence of *L. vulgaris* in Croatia and Slovenia is reported here for the first time. Additionally, our report from Slovakia is the first undoubted record from this country. Stadelmann and Poelt (1962) reported *L. vulgaris* on *Bembidion millerianum* Heyden, 1883 from "Tschechoslowakei: Beskiden". Only a small part of the Beskid Mountains stretches to what is now Slovakia, but we cannot know for certain where that cited host specimen was collected.

***Peyritschella geminata* Thaxt.**, Proceedings of the American Academy of Arts and Sciences 29:101 (1894)

Lectotype, designated here. USA, Maine, Kittery Point, 1 September 1893, [R. Thaxter], on *Pterostichus luctuosus* (Dejean, 1828) (Harpalinae, Pterostichini), slide FH 00313743 (2 adult thalli). Typification identifier: IF556764.

Distribution and hosts. Described from *Pterostichus luctuosus* and *P. patruelis* (Harpalinae, Pterostichini) in Maine, USA. Thaxter (1896) added an additional record from *Pterostichus multipunctatus* (Dejean, 1828) [as *P. erythropus*] in Massachusetts, USA. This host species, however, has a European distribution (Lorenz 2019) and thus likely represents a misidentification. Reported in Poland only

once from a single individual of *Pterostichus nigrita* (Paykull, 1790), based on 3 thalli of which only 1 was adult (Majewski 1999, 2003).

New records from the BHI. Norfolk County, GRAPE ISLAND, 42°16'7.4"N, 70°55'14.7"W, 25 July–1 August 2008, A. Clark, on female *Pterostichus lucuosus*, MCZ- ENT00600390, slides FH 00313277 (1 thallus from prosternum), FH 00313278 (2 thalli from left proepisternum right behind procoxa), and FH 00313279 (1 thallus from metepisternum); Norfolk County, GRAPE ISLAND, 42°16'7.4"N, 70°55'14.7"W, 2–10 July 2008, S.W. Cho, on male *Pterostichus lucuosus*, MCZ-ENT00600395, slides FH 00313306 (1 thallus from mesoepisternum) and FH 00313307 (1 thallus from right proepisternum).

Remarks. Thaxter (1894, 1896) designated no type for this species. We re-examined Thaxter's original slides of *P. geminata* and selected the best-looking slide (FH 00313743) as lectotype.

Results

Morphometrics and statistical analysis of *L. flagellata*

Results from the mixed modeling part of the analysis are shown in Supplemental Table 2.1 showing general results from the mixed models, and Supplemental Table 2.2 showing results for the specific comparisons Q1.1 to Q2.3 (both Supplemental Tables available online at <http://www.eaglehill.us/NENAonline/suppl-files/n26-sp9-N1560h-Haelewaters-s2>, and for BioOne subscribers, at <https://dx.doi.org/10.1656/N1560h.s2>). Uncorrected *P*-values are given. Note that only for 8 out of the total 105 comparisons (7 questions × 15 morphometric parameters), *P* < 0.05 was obtained, close to the number of significant results to be expected if all significant results would be false positives. The following significant differences were obtained:

- Elytra measurements between host species regarding H1T (Q1.1): pairwise comparisons yielded significant differences between *P. mutus* and each of the 4 *Agonum* species (*P* < 0.0013 for all 4 pairwise comparisons); *P. mutus* measurements were higher.
- Pronotum measurements between *Agonum* host species regarding:
 - ◆ H1T (Q1.3): pairwise comparisons yielded a significant difference between *A. gratiosum* and *A. melanarium* (*P* = 0.0016).
 - ◆ HW2 (Q1.3): pairwise comparisons yielded a significant difference between *A. gratiosum* and *A. melanarium* (*P* = 0.0055) and between *A. fidele* and *A. gratiosum* (*P* = 0.050).
 - ◆ LOR (Q1.3): pairwise comparisons yielded a significant difference between *A. gratiosum* and *A. melanarium* (*P* = 0.010).
- *Agonum fidele* measurements between positions regarding H1T (Q2.1): pairwise comparisons yielded significant differences between elytra and legs (*P* = 0.0013) and between elytra and ventral (*P* = 0.0018).
- *Agonum gratiosum* measurements between positions regarding LPT (Q2.2): pairwise comparisons yielded significant differences between antennae and each of other 3 locations (all *P* < 0.02); antennae measurements were higher.

- *Agonum gratiosum* measurements between positions regarding H2T (Q2.2): pairwise comparisons yielded significant differences between antennae and elytra ($P = 0.016$) and between elytra and pronotum ($P = 0.046$); elytra measurements were higher.
- *Agonum gratiosum* measurements between positions regarding LRT (Q2.2): pairwise comparisons yielded significant differences between antennae and each of 3 other locations (all $P < 0.03$); antennae measurements were higher.

Application of a strong Bonferroni correction of the P -value ($P\text{-value} \times 105$ comparisons) yielded one significant result: only H1T shows a significant difference between *P. mutus* and each of the 4 *Agonum* species.

A principal component analysis of the morphometric parameters LPT, H1T, H2T, LOR, and LRT, which gave significant results in the mixed model part (above), on the correlation scale resulted in 2 principal components (PCs) that accounted for 82.2% of the total variation in the dataset: PC1 for 66.7% of variation explained and PC2 for 15.5% of variation explained. Figures 2–4 show biplots of the first 2 PCs, with observations colored by host genus (Fig. 2), host species (Fig. 3), and thallus position on the host body (Fig. 4). Variable representations of LOR and HW2 are on top of each other, meaning that they are highly correlated. The variable representation of H1T is orthogonal (uncorrelated) to LPT and LRT.

No obvious separation of observations with respect to genus can be seen. In Figure 3, observations from *A. gratiosum* have relatively strong negative scores for PC1 (indicating high values for LPT and low values for LRT, HW2, HW2, and LOR). The few observations for *P. mutus* all have negative values for PC1, whereas the few observations on *P. patruelis* have positive values for PC1. In Figure 4, the observations on antennae, which were high for LPT, can be easily recognized.

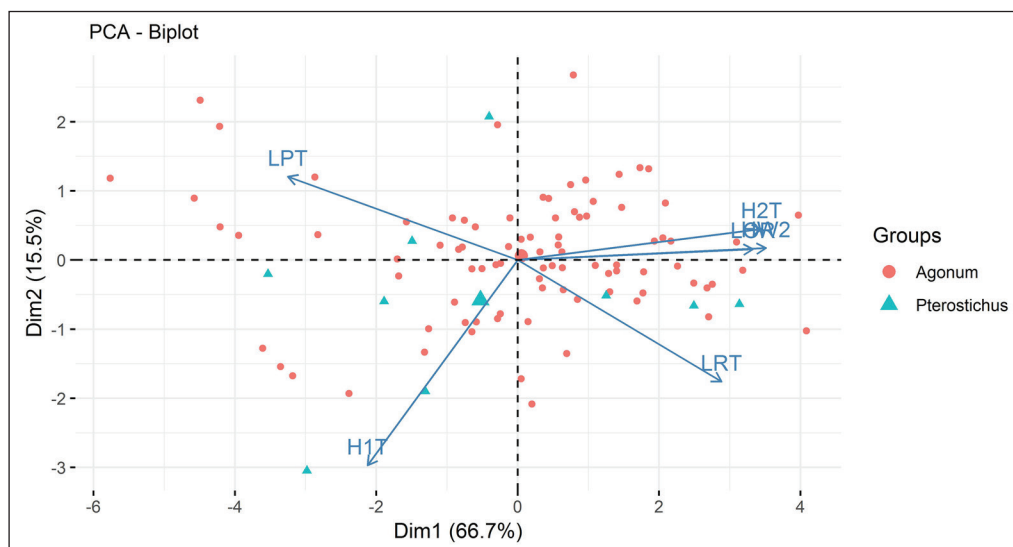


Figure 2. Biplot from principal component analysis (PCA) of results from analysis of host genus.

Molecular phylogenetic analyses

We generated 33 rDNA sequences for 23 isolates of *Laboulbenia* species. Two *Laboulbenia* sequences were downloaded from NCBI GenBank. In our final dataset, *L. flagellata* sequences were included of isolates originating from 3 hosts: *Agonum micans* Nicolai, 1822, *Limodromus assimilis* (Pontoppidan, 1763), and *Loricera pilicornis* (Fabricius, 1775). In addition, sequences of *L. coneglianensis* Speg. removed from *Harpalus affinis* (Schrank, 1781) were included, which makes for an

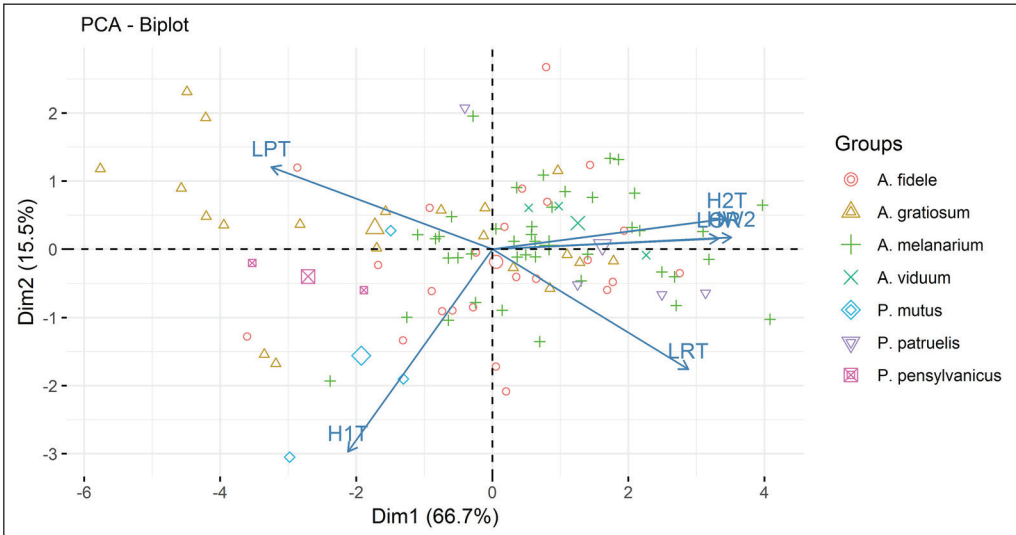


Figure 3. Biplot from principal component analysis (PCA) of results from analysis of host species.

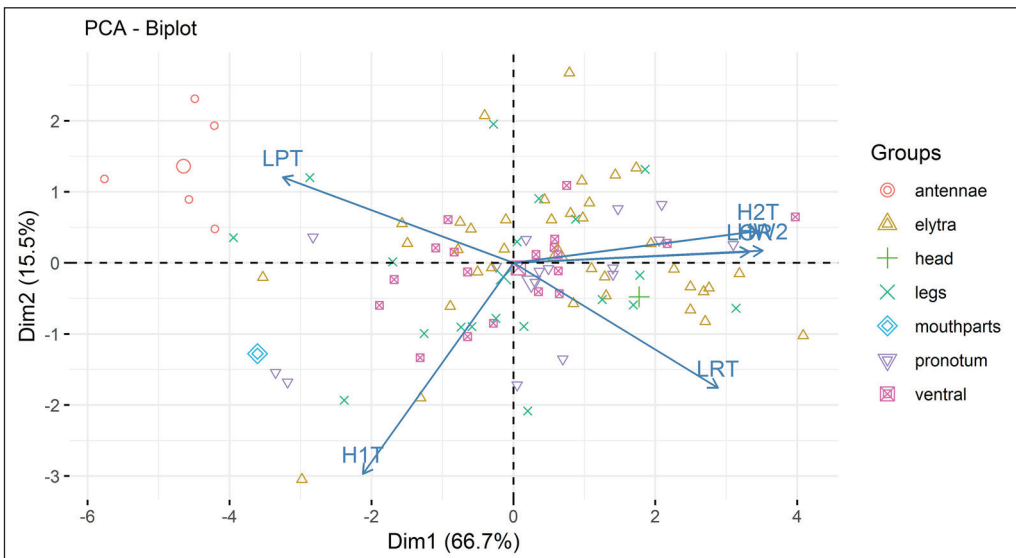


Figure 4. Biplot from principal component analysis (PCA) of results from analysis of thallus position on the host.

interesting case study because *L. coneglianensis* has been considered a synonym of *L. flagellata* by Balazuc (1974) and Majewski (1994). However, after a comparative morphological study by Terada (1998), all European authors, including Majewski (2003), now reject this synonymy. Our ITS dataset consisted of 12 isolates and 1194 characters, of which 739 were constant and 346 were parsimony-informative. Our nrLSU dataset consisted of 27 isolates and 808 characters, of which 499 were constant and 236 were parsimony-informative. Models of nucleotide substitution selected by jModelTest 2 (under AICc) were GTR + G for ITS (-lnL = 3986.6147) and TrN + G for nrLSU (-lnL = 3432.4289).

In both the ML and Bayesian analyses, 12 species of *Laboulbenia* can be recognized (Figs. 5, 6). *Laboulbenia flagellata* forms 2 distinct clades with very high support: one clade consisting of isolates removed from *Limodromus assimilis*, and the second with isolates removed from *Agonum micans* and *Loricera pilicornis*. *Laboulbenia collae* T. Majewski from a related host, *Paranchus albipes* (Fabricius, 1796) [as *Agonum ruficorne* (Goeze, 1777)], is obviously separated from *L. flagellata*. Also *L. coneglianensis* from *Harpalus affinis* is a separate species, placed sister to *L. stillicicola* Speg. with high support (BS = 88, pp = 1). These results from molecular phylogenetic analyses are the first to support the idea that behind the wide host range of *L. flagellata* we may find multiple species.

Discussion

To our knowledge, this study is only the second to apply standard geometric morphometrics methods such as principal component analysis (PCA) to Laboulbeniales (after Haelewaters et al. 2018). Our dataset was based on a relatively small number of thalli, many of them juvenile, and data were lacking for certain combinations of host species and thallus position. As a result, only a subset of possible comparisons of diagnostic characteristics was tested. Measuring more thalli—several hundred—from multiple host species, will allow for more robust analyses and the application of diagnostic traits to differentiate morphologically closely related species (sensu De Kesel and Haelewaters 2014b, De Kesel and Van den Neucker 2006). We are planning to undertake such extensive analysis in the future based on existing collections (R. Thaxter collection at FH; T. Majewski collection at KRAM) and on our own collections of fresh material.

To our surprise, the single significant difference (after applying a strong Bonferroni correction) concerned H1T. Using this character in formal species descriptions would be a novelty in *Laboulbenia* taxonomy. Overall, ratios of measurements are rarely used. Most laboulbeniologists separate species by the combination of lengths (heights) and widths of individual cells and structures (e.g., receptacle, perithecium). Although these results are preliminary, it may eventually be an argument for considering other, neglected characters in Laboulbeniales taxonomy. In an age when we are discovering that many microscopic taxa with worldwide distributions represent complexes of multiple, often cryptic or near-cryptic species, it is increasingly important to explore new morphological features and tools to evaluate their validity. Baur and Leuenberger (2011:824) stipulated that “[a] taxonomist trying to

distinguish between two most similar species will be happy about any discriminating character”, even if it is the ratio of 2 seemingly unrelated measurements.

Our phylogenetic work suggests that *L. flagellata* is not a single phylogenetic species. Isolates removed from 3 host species form 2 clades. Isolates from *A. micans* and *L. pilicornis* form a single clade. This result could be due to the limited length for some of the obtained sequences. Interestingly, *L. flagellata* isolates taken from *Agonum micans* and *Limodromus assimilis*, both collected from under bark of the same logs, are separated. This observation is unexpected because under controlled conditions and in confined areas, carabidicolous *Laboulbenia* species are known to

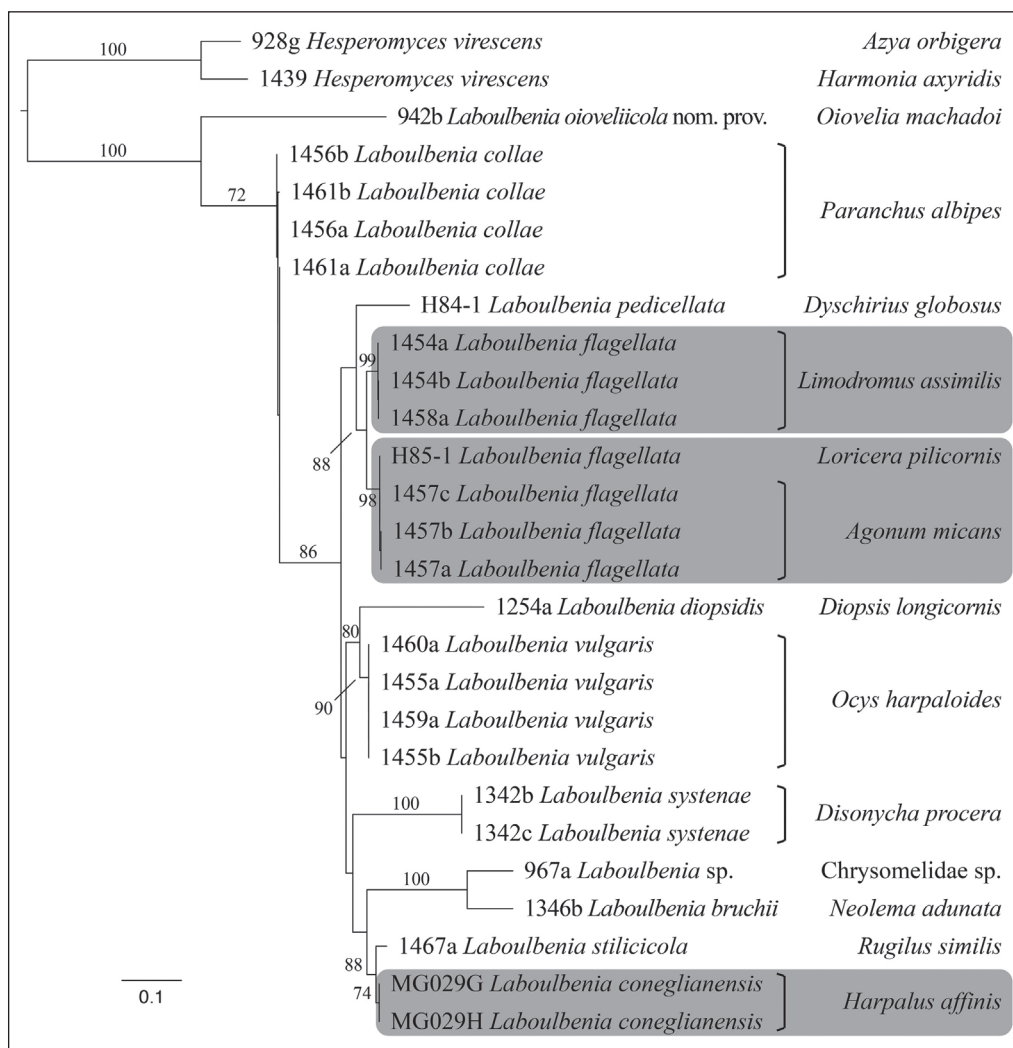


Figure 5. Phylogeny of *Laboulbenia* isolates, with *Hesperomyces virescens* as outgroup, reconstructed from the ITS + nrLSU concatenated dataset. The topology is the result of maximum likelihood inference performed with IQ-TREE. For each node, the ML bootstraps (if ≥ 70) are presented above the branch leading to that node. Host species are presented at the right of the phylogeny. Gray shading added for isolates of particular interest here.

transmit ascospores easily among (carabid) hosts (De Kesel 1996). Although both host species seem to co-occur in the same microhabitats, our preliminary molecular results point towards sympatric speciation, which suggests that there is little to no transmission and isolation between the *Laboulbenia* populations from *A. micans* and *L. assimilis*. Laboratory experiments in containers with stacks of bark under controlled conditions (A. De Kesel, unpubl. data) show that both species avoid contact. Separation under natural conditions is hard to demonstrate, but *L. assimilis* is significantly larger than *A. micans* (Benisch 2019) and selects larger and drier parts of the log. Because of its size, it also occupies areas with more space between bark

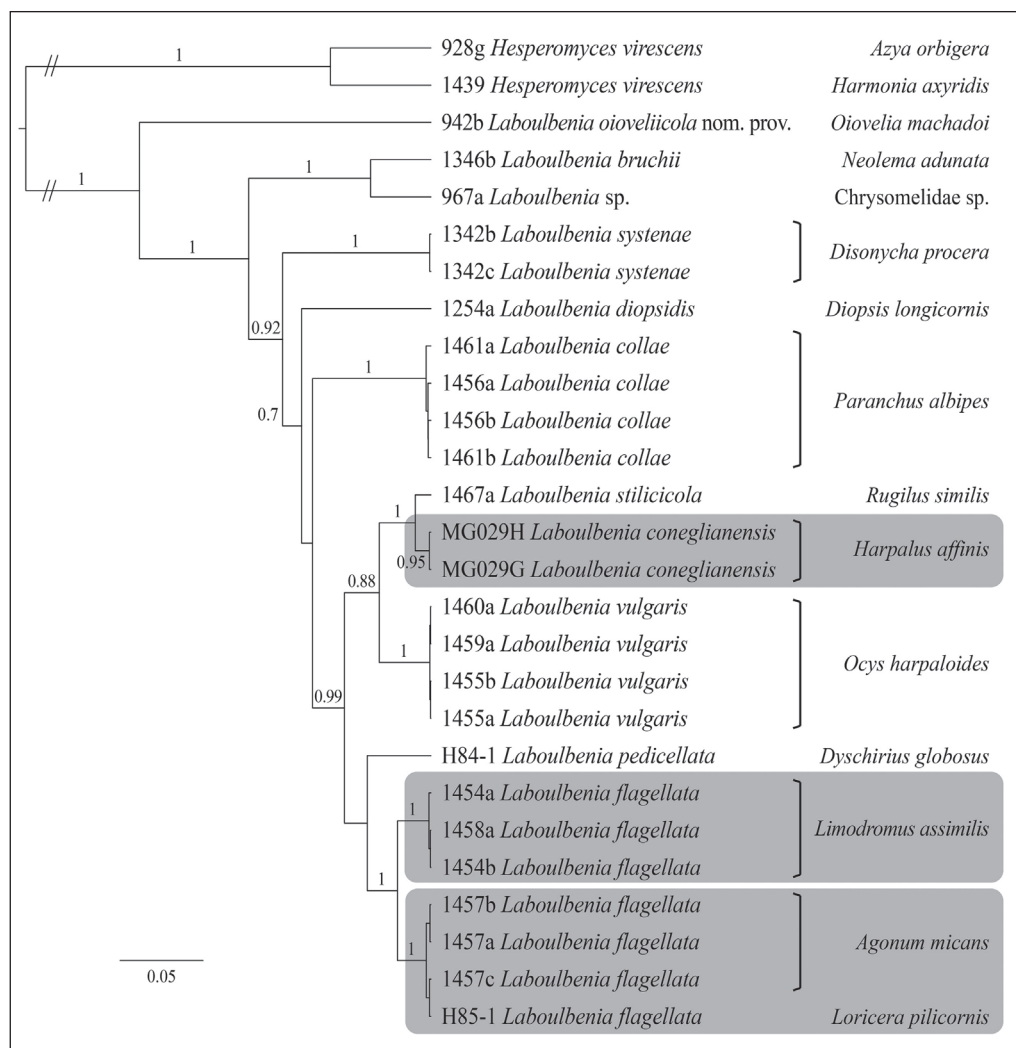


Figure 6. Cladogram of *Laboulbenia* isolates, with *Hesperomyces virescens* as outgroup, reconstructed from the ITS + nrLSU concatenated dataset. The topology is the result of Bayesian inference performed with BEAST. For each node, the posterior probabilities (if ≥ 0.7) are presented above the branch leading to that node. Host species are presented at the right of the phylogeny. Gray shading added for isolates of particular interest here.

and wood. Being smaller, *A. micans* uses much narrower spaces from the same logs and seems to prefer the damper zones closer to the ground. More work is needed, but we hypothesize that there may be reproductive isolation between the *L. flagellata* populations from *L. assimilis* and *A. micans* due to differential habitat choice and parasite transmission of the host species (sensu De Kesel 1996).

Thalli of *L. flagellata* from antennae can be easily separated from thalli removed from other positions (Fig. 4). In *A. gratiosum*, the only host species for which we collected thalli from the antennae, these thalli significantly differ from thalli from the other positions (elytra, legs, pronotum) in 3 ratios: LPT, H2T, and LRT. When we only consider TTL, thalli from the antennae and the pronotum are significantly smaller compared to thalli from the elytra and legs (GLMM: $\chi^2 = 10.41$, $df = 3$, $P = 0.01538$). Position-induced polymorphisms can be the result of either characters of the cuticle such as local variations of thickness or differential nutritional supplies, or due to local “stress” factors resulting from host activities, which may physically affect thalli during development (De Kesel and Van den Neucker 2005). Antennae have thinner cuticles at their joints (Loudon et al. 2014) and they are important sensory organs and thus exposed to external forces. It is not hard to believe that these organs represent a very different environment for colonization by *Laboulbenia* thalli.

The separation of *L. coneglianensis* from *L. flagellata* has been a matter of ongoing taxonomic debate (Balazuc 1974, De Kesel 1997, Majewski 1994, Santamaría 1998). Here we provide the first evidence from molecular phylogeny showing that *L. coneglianensis* deserves the status of species, confirming morphological observations by Terada (1998). Santamaría (1998) suggested that *L. coneglianensis* should be limited to thalli occurring on species in the genera *Harpalus* and *Ophonus* (Harpalinae, Harpalini). We note that it is not impossible that thalli from *Ophonus* spp. might represent another taxon. Given recent findings of host-related diversity in other Laboulbeniales taxa, it is imperative to expand the existing sequence data with collections of *L. coneglianensis* from hosts other than *H. affinis*. In our analysis, this species is most closely related to *L. stilicicola* Speg., a taxon with a totally different habitus and host range (*Rugilus similis*; Staphylinidae, Paederinae). In general, following the results from this study and previous ones, and given common issues such as morphological variability, cryptic diversity, and polymorphism in Laboulbeniales, we think it is best to no longer synonymize taxa without the inclusion of molecular (phylogenetic) data.

Laboulbenia flagellata and other plurivorous and cosmopolitan species of *Laboulbenia* are found on multiple hosts, many of them living in different habitats. Spatial and ecological disparity probably separates *Laboulbenia* populations, especially because dispersal potential in Laboulbeniales and their hosts is low (De Kesel 1995, 1996). These conditions support the hypothesis that eurytopic species such as *L. flagellata* are in fact complexes of morphologically similar or cryptic species (De Kesel and Van den Neucker 2006, Santamaría 1998). To test such hypotheses properly, we propose a combination of morphological and molecular studies. We used this approach in our assessment of *Hesperomyces virescens*, which was found to be a complex of species segregated by host (Haelewaters et al. 2018). Now that as we are

routinely generating high-quality rDNA sequences of *Laboulbenia* (S. De Weggheleire and A. De Kesel, unpubl. data), we will finally be able to tackle the largest genus of the Laboulbeniales—nearly a century after Thaxter died.

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