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New and interesting Laboulbeniales (Fungi, Ascomycota) from the Netherlands, II

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With 7 figures and 2 tables

Abstract: Twenty-three species of Laboulbeniales (Fungi, Ascomycota) are reported for the first time from the Netherlands. These are *Appendiculina scaptomyzae*, *Autoicomyces aquatilis*, *Cantharomyces denigratus*, *Chitonomyces hydropori*, *Eusynaptomyces hydrobii*, *Hesperomyces coccinelloides*, *Hydrophilomyces atroseptatus*, *Laboulbenia expectata* sp. nov., *L. hyalopoda*, *L. murmanica*, *L. ophoni*, *L. thaxteri*, *Rhachomyces lavagnei*, *Rhadinomyces cristatus*, *Rhynchophoromyces anacaenae*, *Stigmatomyces crassicollis*, *S. divergatus*, *S. ephydrae*, *S. geomyzae*, *S. ptilomyiae*, *S. purpureus*, *Tavaresiella hebri*, and *Thripomyces tessinensis*. *Laboulbenia expectata* sp. nov. is

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uniquely associated with *Pterostichus vernalis* and is also reported from Belgium, Czechia, Denmark, Germany, Poland, and Spain. Morphological characteristics and a four-locus phylogenetic reconstruction recognized it as a new species in the *L. flagellata* species complex. New host species for the Netherlands are reported for *Chitonomyces bidessarius*, *Hydraeomyces halipli*, and *Zodiomyces vorticellarius*.

Keywords: 1 new species, Ascomycota, ectoparasites, arthropod-fungus interactions, Laboulbeniales, microfungi, rDNA, taxonomy

Introduction

The first published record of Laboulbeniales in the Netherlands was *Stigmatomyces baeri* H. Karst. by Boedijn (1923). It was Abraham Middelhoek who considerably increased the number of Laboulbeniales for the country (Middelhoek 1941, 1942, 1943a, 1943b, 1943c, 1943d, 1945, 1947a, 1947b, 1949). After his studies, Laboulbeniales were neglected in the Netherlands until Meijer (1975) who proposed to use Laboulbeniales to trace migration patterns of ground beetles (Coleoptera, Carabidae). Since 2012, a revived interest by Haelewaters and colleagues resulted in multiple taxonomic contributions towards the study of Laboulbeniales in the Netherlands (De Kesel & Gerstmans 2012, Haelewaters 2012, De Kesel et al. 2013, Haelewaters & De Kesel 2013, De Kesel & Haelewaters 2014b, Haelewaters et al. 2014, 2015; De Kesel & Haelewaters 2019) increasing the number of species of Laboulbeniales known in the Netherlands to 85 (Haelewaters et al. 2020). Since then, one species has been reported in the Netherlands: *Hesperomyces harmoniae* Haelew. & De Kesel (Haelewaters et al. 2022b).

Ten years after the publication of "New and interesting Laboulbeniales (Fungi, Ascomycota) from the Netherlands" in this journal, we again can add 23 unrecorded species of Laboulbeniales in the Netherlands, thanks to new material—pushing the total number of species known over 100. One of these records is an undescribed species that is here formally described as *Laboulbenia expectata* sp. nov. It is associated with *Pterostichus vernalis* (Panzer, 1796) (Coleoptera, Carabidae) and has a wide distribution in Europe.

Materials and methods

Collection of material

Arthropods were collected by the authors and collaborators using different entomological techniques (acetic acid trap, hand collection, light trap, malaise trap, pitfall trap) and stored in 70–99% ethanol. Some specimens resulted from citizen science projects organized by Taxon Expeditions and Taxon Foundation, during which mixed teams of members of the local community and biodiversity experts performed in-depth biodiversity inventories of (mostly urban) greenspaces.

Microscopy

Collected specimens were screened for the presence of Laboulbeniales using a dissecting microscope (at 50× magnification). Permanent slides were made using the double-coverslip mounting technique as described by Liu et al. (2020), with one modification: thalli were placed in a droplet of 1:1 Hoyer's medium:glycerin mixture instead of pure Hoyer's medium, because our Hoyer's medium dried quickly. Mounted thalli were viewed at 200–1000× magnification under an Olympus BH-2 microscope (Olympus, Center Valley, PA). Images were made with a Nikon DS-Fi3 microscope camera mounted on an Eclipse Ni-U compound microscope (Nikon, Nelville, NY) equipped with differential interference contrast (DIC) optics, and processed using NIS-Elements BR 5.0.03 imaging software (Nikon). Species of Laboulbeniales were identified using relevant literature (Tavares 1985, Santamaria et al. 1991, Majewski 1994, De Kesel et al. 2020, Haelewaters & De Kesel 2020, Santamaria & Pedersen 2021).

Microscopic mounts are deposited at the Herbarium Universitatis Gandavensis (GENT). Voucher host specimens are stored at the Taxon Expeditions collection (coll. TXEX), the entomological collection of the Royal Belgian Institute of Natural Sciences (coll. KBIN), and the entomological department of National Museum, Prague (coll. NMP).

DNA extractions, PCR amplification, and sequencing

For the description of *L. expectata* sp. nov., an integrative taxonomy approach was used, based on data from independent lines of evidence–morphology, ecology/host association, and molecular phylogenetic data (Maharachchikumbura et al. 2021, Haelewaters et al. 2022a). DNA extractions were done using the REPLI-g Single Cell Kit (Qiagen). Thalli of Laboulbeniales were removed from their host using a hypodermic needle inserted onto a glass syringe for holdfast under a dissecting microscope. The tip of the needle was submerged in glycerin to prevent thalli from flying away during transfer. Thalli were placed in a droplet of glycerin on a microscope slide. Appendages were often cut off to avoid contamination (as debris often sticks to the appendages). Next, thalli were cut into multiple smaller pieces with the sharp tip of the needle. These pieces were placed in 0.2-ml PCR tubes with 4 µl of phosphate-buffered saline (PBS). Next, we followed the instructions as indicated in the manufacturer's manual (Qiagen).

For PCR amplification, we focused on the small subunit (SSU), the internal transcribed region (ITS, consisting of ITS1, 5.8S, and ITS2), the large subunit (LSU) of the ribosomal RNA gene (rDNA), and the translation elongation factor 1α (*TEF1*) protein-coding gene. Primer pairs are shown in Table 1. PCR reactions (25 µl) consisted of 13.3 µl of RedExtract *Taq* polymerase (Sigma-Aldrich, St. Louis, Missouri), 2.5 µl of each 10 µM primer, 5.45 µl of ddH_2O, and 1 µl of DNA extract. The DNA extract was briefly vortexed before pipetting 1 µl in the prepared PCR tube. The following cycling conditions were used: for SSU: initial denaturation at 94 °C for 5 min; 39 cycles of denaturation at 94 °C for 30 s, annealing at 50 °C for 45 s, and extension at 72 °C for 90 s; and final extension at 72 °C

Table 1. List of primer pairs used for PCR amplification of small subunit (SSU), internal transcribed spacer (ITS), and large subunit (LSU) of the ribosomal RNA gene, and translation elongation factor 1α (*TEF1*).

Forward primer	Reverse primer	PCR product	Reference forward primer	Reference reverse primer
NSL1	NSL2	SSU	Haelewaters et al. (2015c)	Haelewaters et al. (2015c)
ITS3	ITS4	ITS2	White et al. (1990)	White et al. (1990)
NL1	NL4	LSU	Kurtzman & Robnett (1997)	Kurtzman & Robnett (1997)
LIC24R	LR3	LSU	Miadlikowska & Lutzoni (2000)	Vilgalys & Hester (1990)
EF1-1018F	EF1-1620R	TEF1	Stielow et al. (2015)	Stielow et al. (2015)
Al33_alternative_f	EF1-1620R	TEF1	Stielow et al. (2015)	Stielow et al. (2015)

for 10 min. For ITS, an initial denaturation at 94 °C for 3 min; 34 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 a final extension at 72 °C for 10 min. For LSU: initial denaturation at 94 °C for 5 min; 34 cycles of denaturation at 94 °C for 30 s, annealing at 50 °C for 45 s, and extension at 72 °C for 1 min; and final extension at 72 °C for 7 min. For *TEF1*: initial denaturation at 94 °C for 5 min; 10 cycles of denaturation at 94 °C for 50 s, annealing at 54 °C (−1 C/cycle) for 50 s, and extension at 72 °C for 1 min; followed by 40 cycles of denaturation at 94 °C for 50 s, annealing at 53 °C for 50 s, and extension at 72 °C for 1 min; and final extension at 72 °C for 7 min. Gel electrophoresis was performed and PCR products were visualized using ethidium bromide staining. Purification of successful PCR products was done using 1.5 µl of Exo-FAP (0.5 µl Exonuclease I, 1 µl FAST Alkaline Phosphatase) (Thermo Fisher Scientific, Waltham, MA, USA) per 10 µl of PCR product, at 37 °C for 15 min, followed by deactivation at 85 °C for 15 min. The purified PCR products were sequenced at Macrogen (Amsterdam, the Netherlands) using an automated ABI 3730 XL capillary sequencer (Life Technology, Carlsbad, CA, USA). Forward and reverse sequence reads were assembled and edited in Sequencher version 5.4.6 (Gene Codes Corporation, Ann Arbor, MI, USA).

Assembled sequences were deposited at the National Center for Biotechnology Information (NCBI) GenBank database, under accession numbers OR723987–OR723997 (SSU), OR752331–OR752338 (ITS), OR752339–OR752349 (LSU), and OR762491–OR762496 (*TEF1*).

Phylogenetic analyses

Newly generated sequences of *L. expectata* sp. nov. and other species in *Laboulbenia* Mont. & C.P. Robin were supplemented with sequences downloaded from NCBI GenBank (https://www.ncbi.nlm.nih.gov/genbank/) following the dataset in Van Caenegem et al. (2023b). All sequences included in our analyses are listed in Table 2. Only isolates of which we had at least one LSU rDNA sequence were included in phylogenetic analyses. We aligned SSU, LSU, and *TEF1* sequences by locus with the G-INS-i strategy and ITS with the E-INS-i strategy using the online version 7 of MAFFT (Katoh et al. 2005, 2019; Kuraku et al. 2013). Sequences were manually trimmed at the primer annealing sites using BioEdit Sequence Alignment Editor version 7.2.6 (Hall 1999) and concatenated in SequenceMatrix 1.9 (Vaidya et al. 2011). Our concatenated dataset (SSU–ITS–LSU–*TEF1*) included six partitions: SSU, ITS1, 5.8S, ITS2, LSU, and *TEF1*. Models for nucleotide substitution were selected for each partition with ModelFinder (Kalyaanamoorthy et al. 2017) according to the corrected Akaike Information Criterion (AICc). Maximum likelihood (ML) analyses were inferred using IQ-TREE (Nguyen et al. 2015) under partitioned models (Chernomor et al. 2016). Ultrafast bootstrapping was performed with 1000 replicates (Hoang et al. 2018). A Bayesian Inference of phylogeny was set up by running four Markov chains for 40 million generations, sampling every 4000 generations in MrBayes (Ronquist et al. 2012), available on the CIPRES Science Gateway (Miller et al. 2010). The analysis was performed using the GTR substitution model, with some sites being invariable and a gamma-distributed rate variation across the remaining sites (GTR+I+G) for each of the six partitions. A burn-in of 4000 trees was selected. Phylogenetic trees were visualized in FigTree version 1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/) and edited in Inkscape (http://www.inkscape.org). The aligned and trimmed alignment, partition file (in NEXUS format), and unedited trees (in TXT format) are available in the following GitHub directory: https://github.com/dannyhaelewaters/team-laboul/tree/main/2024_new_species_and_records_the_netherlands

Taxonomy

Appendiculina scaptomyzae (Thaxt.) Haelew. & Aime, in Haelewaters et al., Sydowia 72: 325 (2020) Fig. 1A

≡ *Stigmatomyces scaptomyzae* Thaxt., Proc. Amer. Acad. Arts Sci. 36(23): 400 (1901).

Known distribution and hosts: Described from the U.S.A. on *Scaptomyza graminum* (Fallén, 1823) (Diptera, Drosophilidae). Recorded from Canada (North America), Venezuela (South America), Austria, Czechia, Bulgaria, Denmark, Germany, Finland, France, Italy, Montenegro, Poland, Portugal, Romania, Slovakia, Spain (Europe), Burundi (Africa), and Afghanistan (Asia) on species of *Scaptomyza* Hardy, 1850, mainly *Scaptomyza pallida* (Zetterstedt, 1847) (Balazuc 1974, Máca 1987, Santamaria et al. 1991, Christian 2001, Rossi et al. 2013, Santamaria & Pedersen 2021).

Table 2. Details of all isolates used in this study. Accession numbers of sequences generated during this study are in boldface. ADK collector's labels are added to isolates from hosts collected by André De Kesel (Meise Botanic Garden).

Isolate	Species	Host species	Country	SSU	ITS	LSU	*TEF1*
LG359	*Eucantharomyces egae*	Carabidae sp.	Costa Rica	MG696305			
D. Haelew. 3758a (ADK6522)	*Laboulbenia benjaminii*	*Badister unipustulatus* Bonelli, 1813	Belgium	OR680738		OR680759	OR762491
D. Haelew. 4333a	*Laboulbenia bicornis*	Gyrinidae sp.	Uganda	OR680728		OR680748	
D. Haelew. 1346b	*Laboulbenia bruchii*	*Neolema adunata* White, 1993	Panama	MN530040		MN394843	
D. Haelew. 1007a	*Laboulbenia calathi*	*Calathus melanocephalus* (Linnaeus, 1758)	The Netherlands	MG438342		OR680755	
D. Haelw. 3037a (ADK6493)	*Laboulbenia clivinalis*	*Clivina fossor* (Linnaeus, 1758)	Latvia	OR680736		OR680757	
D. Haelew. 3038b (ADK6459)	*Laboulbenia collae*	*Paranchus albipes* (Fabricius, 1796)	Belgium	OR680732		OR680752	
D. Haelew. 3759a (ADK6524)	*Laboulbenia coneglianensis*	*Harpalus griseus* (Panzer, 1796)	Belgium	OR680734		OR680754	
D. Haelew. 3970a	*Laboulbenia cristata*	*Paederus littoralis* Gravenhorst, 1802	Belgium	OR680735		OR680756	
D. Haelew. 4363a	*Laboulbenia expectata* sp. nov.	*Pterostichus vernalis* (Panzer, 1796)	Belgium	OR723991	OR752334	OR752347	
D. Haelew. 4483a	*Laboulbenia expectata* sp. nov.	*Pterostichus vernalis*	Belgium	OR723993	OR752337	OR752345	
D. Haelew. 3044a (ADK6487)	*Laboulbenia fasciculata*	*Patrobus atrorufus* (Ström, 1768)	Belgium	OR680729		OR680749	
D. Haelew. 1454a (ADK6329)	*Laboulbenia flagellata* s.l.	*Platynus assimilis* (Paykull, 1790)	Belgium		MN397132	MN394849	OR762493
D. Haelew. 1454b (ADK6329)	*Laboulbenia flagellata* s.l.	*Platynus assimilis*	Belgium		MN397133	MN394850	OR762494

Table 2. cont.

Isolate	Species	Host species	Country	SSU	ITS	LSU	*TEF1*
D. Haelew. 1457a (ADK6337)	*Laboulbenia flagellata* s.l.	*Agonum micans* (Nicolai, 1822)	Belgium			MN394851	OR762495
D. Haelew. 1457b (ADK6337)	*Laboulbenia flagellata* s.l.	*Agonum micans*	Belgium		MN397134	MN394852	OR762496
D. Haelew. 1457c (ADK6337)	*Laboulbenia flagellata* s.l.	*Agonum micans*	Belgium			MN394853	
D. Haelew. 1458a (ADK6329)	*Laboulbenia flagellata* s.l.	*Platynus assimilis*	Belgium			MN394854	
D. Haelew. 3321c	*Laboulbenia flagellata* s.l.	*Agonum gracile* Sturm, 1824	The Netherlands	OR723996		OR752348	
D. Haelew. 3321d	*Laboulbenia flagellata* s.l.	*Agonum gracile*	The Netherlands	OR723997		OR752349	
D. Haelew. 3769a (ADK6535)	*Laboulbenia flagellata* s.l.	*Agonum muelleri* (Herbst, 1784)	Belgium	OR723990		OR752342	OR762492
D. Haelew. 3966a	*Laboulbenia flagellata* s.l.	*Platynus assimilis* (Paykull, 1790)	Belgium	OR723987	OR752331	OR752339	
D. Haelew. 4099a (ADK6459)	*Laboulbenia flagellata* s.l.	*Paranchus albipes*	Belgium	OR723988	OR752332	OR752340	
D. Haelew. 4101a (ADK6459)	*Laboulbenia flagellata* s.l.	*Paranchus albipes*	Belgium	OR723989	OR752333	OR752341	
D. Haelew. 4480a	*Laboulbenia flagellata* s.l.	*Agonum emarginatum* (Gyllenhal, 1827)	Belgium	OR723995	OR752335	OR752343	
D. Haelew. 4538a	*Laboulbenia flagellata* s.l.	*Oxypselaphus obscurus* (Herbst, 1784)	Belgium	OR723992	OR752336	OR752344	
D. Haelew. 4733a	*Laboulbenia flagellata* s.l.	*Agonum fuliginosum* (Panzer, 1809)	Belgium	OR723994	OR752338	OR752346	

Table 2. cont.

Isolate	Species	Host species	Country	SSU	ITS	LSU	*TEF1*
E11T11 (ADK6374)	*Laboulbenia flagellata* s.l.	*Oxypselaphus obscurus* (Herbst, 1784)	Latvia			MT703824	
E13T1 (ADK6428)	*Laboulbenia flagellata* s.l.	*Agonum emarginatum* (Gyllenhal, 1827)	Belgium			MT703825	
E13T11 (ADK6445)	*Laboulbenia flagellata* s.l.	*Agonum nigrum* Dejean, 1828	Belgium			MT703826	
H85-1	*Laboulbenia flagellata* s.l.	*Loricera pilicornis* (Fabricius, 1775)	Sweden			KY350538	
D. Haelew. 3052a (ADK6491)	*Laboulbenia giardii*	*Dicheirotrichus gustavii* Crotch, 1871	Belgium	OR680727		OR680747	
D. Haelew. 4154a	*Laboulbenia giardii*	*Dicheirotrichus gustavii*	Belgium	OR680726		OR680746	
AW821	*Laboulbenia heteroceri*	Heteroceridae sp.	United States of America	MG674664			
D. Haelew. 4197b	*Laboulbenia mairei*	*Heterocerus fenestratus* (Thunberg, 1784)	Belgium	OR680725		OR680745	
D. Haelew. 1009b	*Laboulbenia pheropsophi*	*Pheropsophus* sp.	Sierra Leone	MG438344		OR680760	
D. Haelew. 4131a (ADK6288)	*Laboulbenia slackensis*	*Pogonus chalceus* (Marsham, 1802)	Belgium	OR680737		OR680758	
D. Haelew. 4199c	*Laboulbenia spissa* nom. prov.	*Cyparium concolor* (Fabricius, 1801)	United States of America	OR680730		OR680751	
D. Haelew. 4199d	*Laboulbenia spissa* nom. prov.	*Cyparium concolor*	United States of America	OR680731		OR680750	
D. Haelew. 3774a	*Laboulbenia vulgaris*	*Bembidion tibiale* (Duftschmid, 1812)	The Netherlands	OR680733		OR680753	
LG487	*Misgomyces dyschirii*	Carabidae sp.	South Africa	MG696572			

New records from the Netherlands: On *Scaptomyza pallida* (Zetterstedt, 1847) (Diptera, Drosophilidae), Pampus, Gooise Meren (Noord-Holland), N 52°21'51.6" E 5°04'11.8", 15.vii.2020, leg. and det. P.H. Hoekstra, slide D. Haelew. 3801a (GENT: GENTFL00551); De Kaaistoep, Tilburg (Noord-Brabant), N 51°32'35.5" E 5°00'53.3", 06-13.viii.2020, leg. Insectenwerkgroep KNNV-afdeling Tilburg, det. J.W. van Zuijlen, slide D. Haelew. 3828a (GENT:GENTFL00559).

Remarks: The genus *Appendiculina* Berl. was erected by Berlese (1889) to accommodate *A. entomophila* (Peck) Berl. Eventually, Thaxter (1896) synonymized *Appendiculina* with *Stigmatomyces*. Recent molecular phylogenetic analyses found that *Stigmatomyces* sensu lato (s.l.) is paraphyletic due to the position of *Gloeandromyces* Thaxt. (Haelewaters et al. 2018b, 2020, Liu et al. 2020). As a result of this, Haelewaters et al. (2020) reinstated the genera *Appendiculina* and *Fanniomyces* T. Majewski. While we acknowledge their limited sampling and the lack of sequence data for the type of *Stigmatomyces* sensu stricto (*S. baeri*), we choose to follow this taxonomic vision which has support from morphological, ecological, and molecular phylogenetic data. Currently, three species of *Appendiculina* are accepted: *A. entomophila* (Peck) Berl., *A. gregaria* (W. Rossi) Haelew. & Aime, and *A. scaptomyzae* (Thaxt.) Haelew. & Aime. A thorough revision of *Stigmatomyces* sensu lato based on an integrated approach, combining morphological, ecological, geographical, and molecular phylogenetic data, is desired, including sequencing DNA of the type species.

Autoicomyces aquatilis (F. Picard) I.I. Tav., Mycol. Mem. 9: 148 (1985) Fig. 1B

≡ *Ceratomyces aquatilis* F. Picard, Bull. Soc. Mycol. Fr. 29: 560 (1913).

Known distribution and hosts: Described from France on *Hydrochus crenatus* (Fabricius, 1792) (as *H. carinatus* Germar, 1823) (Coleoptera, Hydrochidae). Recorded from the U.S.A., Cuba (North America), Denmark, France, Germany, Hungary, Italy, Spain, Sweden, and Poland (Europe) on species of *Hydrochus* Leach, 1817 (Siemaszko & Siemaszko 1933, Santamaria et al. 1991, Majewski 1994, Santamaria 2001, Santamaria & Pedersen 2021).

New records from the Netherlands: On six specimens of *Hydrochus crenatus* (Fabricius, 1792) (Coleoptera, Hydrochidae), Zaltbommel, Brakel (Gelderland), N 51°48'12.7" E 5°03'41.5", 13.v.2015, leg. and det. A.J. Dees, slides D. Haelew. 3484a (GENT: GENTFL00246), 3485a (GENT:GENTFL00247), 3486a (GENT:GENTFL00248), 3487a (GENT:GENTFL00249), and 3487b (GENT:GENTFL00250); Leudal, Heibloem (Limburg), N 51°18'04.3" E 5°55'28.2", 04.ix.2015, leg. and det. A.J. Dees, slides D. Haelew. 3481a (GENT:GENTFL00243) and 3482a (GENT:GENTFL00244).

Cantharomyces denigratus Thaxt., Mem. Am. Acad. Arts Sci. N. S. 16: 27 (1931) Fig. 1C

Known distribution and hosts: Described from the U.K. on *Dryops griseus* (Erichson, 1847) (as *Parnus griseus* Erichson, 1847) (Coleoptera, Dryopidae). Recorded from Bel-

gium, Denmark, Poland, Spain, and Sweden (Europe) on species of *Dryops* Olivier, 1791 (Siemaszko & Siemaszko 1933, Santamaria 1992, Majewski 1994, De Kesel & Haelewaters 2014a, Huggert 2010, Santamaria & Pedersen 2021).

New records from the Netherlands: On two specimens of *Dryops similaris* Bollow, 1936 (Coleoptera, Dryopidae), Goedereede, Kwade Hoek, (Zuid-Holland), N 51°50'15.6" E 3°58'54.1", 15.ix.2017, leg. and det. A.J. Dees, slides D. Haelew. 3488a (GENT:GENTFL00251) and 3489a (GENT:GENTFL00252).

Remarks: *Cantharomyces denigratus* is often found on the last abdominal sternites of males, while *C. italicus* Speg. is more often found on the dorsal side of both sexes (De Kesel & Haelewaters 2014a). The status of these two taxa is uncertain, with some authors suggesting they might be growth forms of the same phylogenetic species (Scheloske 1969, De Kesel & Haelewaters 2014a, but see Santamaria 1992, Majewski 1994, Santamaria & Pedersen 2021). Currently, molecular data are available for neither of these species, but this question should be easily answered in the near future, as we have started to successfully sequence DNA of Laboulbeniales.

Chitonomyces bidessarius (Thaxt.) Thaxt., Mem. Am. Acad. Arts Sci. N. S. 12(3): 292 (1896) Fig. 1D

≡ *Heimatomyces bidessarius* Thaxt., Proc. Amer. Acad. Arts Sci. 28: 185 (1892).

Known distribution and hosts: Described from the U.S.A. on *Uvarus granarius* (Aubé, 1838) (as *Bidessus granarius* (Aubé, 1838)) (Coleoptera, Dytiscidae). Recorded from Denmark, Finland, France, Germany, Hungary, Poland, Sweden, the Netherlands (Europe), Cambodia, Japan, Taiwan (Asia), and Fiji (Oceania) on species of several dytiscid genera (Santamaria 1991, Majewski 1994, Santamaria & Pedersen 2021, Kong et al. 2022). In Europe, it is mostly found on *Hygrotus ineaqualis* (Fabricius, 1777). Majewski (1994) reported *Bidessus unistriatus* (Schrank, 1781) and *Hydroglyphus geminus* (Fabricius, 1792) as hosts from Poland.

New records from the Netherlands: On four specimens of *Hydroglyphus geminus* (Fabricius, 1792) (Coleoptera, Dytiscidae), Leudal, Heythuizen (Limburg), N 51°14'37.7" E 5°55'32.6", 06.ix.2015, leg. and det. A.J. Dees, slides D. Haelew. 3473a (GENT:GENTFL00233), 3474a (GENT:GENTFL00234), 3474b (GENT:GENTFL00235), 3475a (GENT:GENTFL00236), 3476a (GENT:GENTFL00237), and 3476b (GENT:GENTFL00238); Tilburg, Udenhout (Noord-Brabant), N 51°38'08.8" E 5°07'58.1", 31.iii.2018, leg. and det. A.J. Dees, slide D. Haelew. 3490a (GENT:GENTFL00253).

Remarks: *Chitonomyces bidessarius* was already known from the Netherlands on *Hygrotus impressopunctatus* (Schaller, 1783) (Haelewaters et al. 2015b, Haelewaters & De Kesel 2020). Here, we report *Hydroglyphus geminus* as a new host species in the Netherlands. This species was already reported as a host of *C. bidessarius* from Poland (Majewski 1994). Its congener *H. japonicus* (Sharp, 1873) is reported as a host in Japan (Sugiyama 1973).

Chitonomyces hydropori Thaxt., Proc. Amer. Acad. Arts Sci. 37(2): 32 (1901) Fig. 1E

Known distribution and hosts: Described from the U.S.A. on *Hydroporus niger* Say, 1823 (as *Hydroporus modestus* Aubé, 1838) (Coleoptera, Dytiscidae). Recorded from France, Germany, Hungary, Poland, Sweden (Europe), Indonesia, and Malaysia (Asia). In Europe, it is mostly found on *Hygrotus impressopunctatus* (Santamaria et al. 1991, Majewski 1994, Santamaria & Pedersen 2021). Majewksi (1994) reported *Hydroporus erythrocephalus* (Linnaeus, 1758) and *H. umbrosus* (Gyllenhal, 1808) as hosts.

New records from the Netherlands: On *Hydroporus angustatus* Sturm, 1835 (Coleoptera, Dytiscidae), Winterswijk, nature reserve Korenburgerveen (Gelderland), N 51°59'16.3" E 6°39'16.6", 07.ix.2019, leg. and det. A.J. Dees, slide D. Haelew. 3509a (GENT:GENTFL00275); on two specimens of *Hydroporus erythrocephalus*, Winterswijk, nature reserve Korenburgerveen (Gelderland), N 51°59'16.3" E 6°39'16.6", 07.ix.2019, leg. and det. A.J. Dees, slides D. Haelew. 3510a (GENT:GENTFL00276) and 3511a (GENT:GENTFL00277).

Eusynaptomyces hydrobii (T. Majewski) I.I. Tav., Mycol. Mem. 9: 220 (1985) Fig. 1F

≡ *Rhynchophoromyces hydrobii* T. Majewski, Acta Mycol. 7(2): 274 (1972).

Known distribution and hosts: Described from Poland on *Hydrobius fuscipes* (Linnaeus, 1758) (Coleoptera, Hydrophilidae). Recorded from Denmark on *Hydrobius rot-*

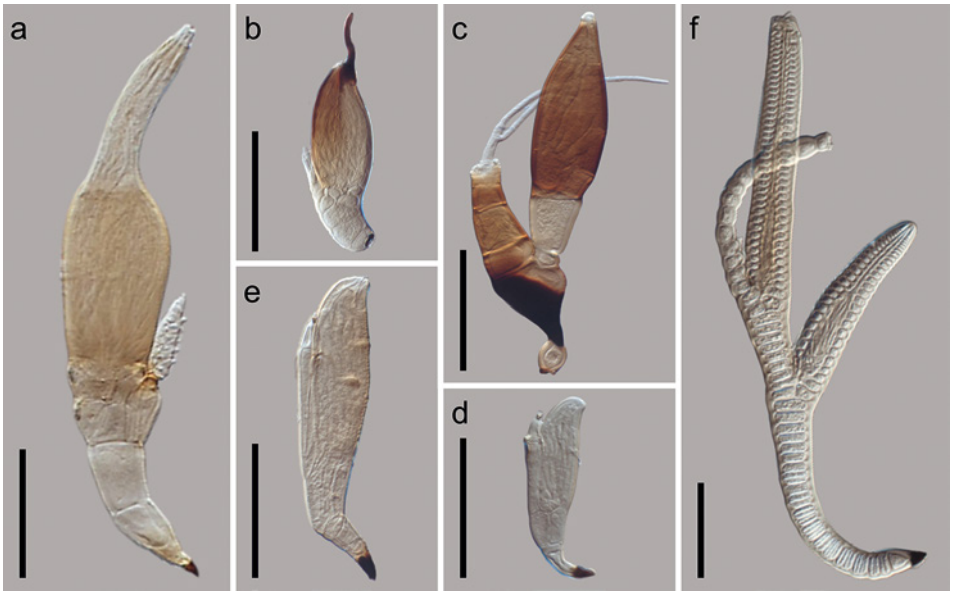

Fig. 1. a. *Appendiculina scaptomyzae*, D. Haelew. 3801a. b. *Autoicomyces aquatilis*, D. Haelew. 3482a. c. *Cantharomyces denigratus*, D. Haelew. 3488a. d. *Chitonomyces bidessarius*, D. Haelew. 3475a. e. *Chitonomyces hydropori*, D. Haelew. 3510a. f. *Eusynaptomyces hydrobii*, D. Haelew. 4420a. Scale bars: a–f = 50 µm.

tenbergii Gerhardt, 1874 (Santamaria & Pedersen 2021) and from Poland also on *Hydrophilus caraboides* (Linnaeus, 1758) (Majewski 1994).

New records from the Netherlands: On *Hydrobius fuscipes*, Heemskerk, Heemskerker Noordbroekpolder, (Noord-Holland), N 52°31'14.9" E 4°40'18.8", 13.iv.2022, leg. and det. B. de Boer, slide D. Haelew. 4420a (GENT:GENTFL00798).

Remarks: The Dutch material represents only the third record of *E. hydrobii* species since its description. To date, six species have been described in the genus *Eusynaptomyces* I.I. Tav., of which four in Europe (Haelewaters et al. 2024). Of those, also *E. cornutus* Santam. occurs on *H. fuscipes* (Santamaria 2006). However, this species is easily differentiated from *E. hydrobii* by its multicellular horn-like outgrowth formed by one of the rows of perithecial wall cells.

Hesperomyces coccinelloides (Thaxt.) Thaxt., Mem. Am. Acad. Arts Sci. N. S. 16: 110 (1931) Fig. 2A

≡ *Stigmatomyces coccinelloides* Thaxt., Proc. Amer. Acad. Arts Sci. 52: 704 (1917).

Known distribution and hosts: Described from Grenada on "a coccinellid allied to *Scymnus*" (Coleoptera, Coccinellidae) (Thaxter 1931). Recorded from Canada, Jamaica, Panama, the U.S.A. (North America), Brazil, Ecuador, Trinidad and Tobago (South America), Belgium, Denmark, Poland, Spain (Europe), Malaysia, and the Philippines (Asia) on species of the genera *Diomus* Mulsant, 1850, *Scymnus* Kugelann, 1794, and *Stethorus* Weise, 1885 (Thaxter 1931, Weir & Blackwell 2001, Castro & Rossi 2008, Rossi & Bergonzo 2008, De Kesel 2011, Ceryngier 2013, Haelewaters & De Kesel 2017, Haelewaters et al. 2019b, Santamaria & Pedersen, 2021, Ceryngier et al. 2024).

New records from the Netherlands: On two specimens of *Stethorus punctillum* Weise, 1891 (Coleoptera, Coccinellidae), Wageningen (Gelderland), N 51°59'19.8" E 5°40'04.4", 17.vii.2020, leg. and det. K. Verhoogt, slides D. Haelew. 3880a (GENT:GENTFL00592) and 3881a (GENT:GENTFL00593).

Remarks: *Hesperomyces coccinelloides* is reported on different species of *Diomus*, *Scymnus*, and *Stethorus* all over the globe. Ceryngier et al. (2024) suggested that *He. coccinelloides* may be a species complex based on an LSU sequence of *Hesperomyces* ex *Stethorus tenerifensis* Fürsch, 1987 sharing only 87.96% identity with that of *Hesperomyces* ex *Symnus nubilus* Mulsant, 1850. Also *Hesperomyces virescens* (Thaxt.) Thaxt. was found to be a complex of species segregated by host and geography (Haelewaters et al. 2018a, 2022b, Van Caenegem et al. 2023a). Whether similar patterns are at play in the *He. coccinelloides* complex should be investigated using an integrative taxonomy approach.

Hydraeomyces halipli (Thaxt.) Thaxt., Mem. Am. Acad. Arts Sci. (ser. 2) 12(3): 294 (1896) Fig. 2B

≡ *Heimatomyces halipli* Thaxt., Proc. Amer. Acad. Arts Sci. 27: 32 (1892).

= *Hydraeomyces venetus* Speg., Anal. Mus. nac. Hist. nat. B. Aires 27: 52 (1915).

= *Parahydraeomyces italicus* Speg., Anal. Mus. nac. Hist. nat. B. Aires 27: 70 (1915).

= *Parahydraeomyces neapolitanus* Speg., Anal. Mus. nac. Hist. nat. B. Aires 27: 70 (1915).

Known distribution and hosts: Described from the U.S.A. on *Haliplus ruficollis* (De Geer, 1774) (Coleoptera, Haliplidae). Recorded from Argentina (South America), Belgium, Bulgaria, Croatia, Czechia, Denmark, Finland, France, Germany, Hungary, Italy, the Netherlands, Poland, Russia, Slovenia, Spain, Sweden, the U.K. (Europe), Morocco, Tunisia (Africa), China, Japan, and Taiwan (Asia) on species of *Haliplus* Latreille, 1802 and *Peltodytes* Régimbart, 1879 (Santamaria et al. 1991, Majewski 1994, Weir 1994, Rossi & Máca 2006, De Kesel & Werbrouck 2008, Rossi et al. 2019, Haelewaters & De Kesel 2020, Santamaria & Pedersen 2021).

New records from the Netherlands: On two specimens of *Haliplus immaculatus* Gerhardt, 1877 (Coleoptera, Haliplidae), Overbetuwe, Zetten (Gelderland), N 51°55’05.3” E 5°40’59.7”, 09.iv.2014, leg. and det. A.J. Dees, slides D. Haelew. 3478a (GENT: GENTFL00240) and 3480a (GENT:GENTFL00242).

Remarks: *Hydraeomyces halipli* is already known from the Netherlands on *Haliplus flavicollis* Sturm, 1834 and *H. ruficollis* (De Geer, 1774) (Haelewaters & De Kesel 2020). Here, we report *H. immaculatus* as a new host species in the Netherlands.

Hydrophilomyces atroseptatus T. Majewski, Polish Bot. Stud. 7: 56 (1994) Fig. 2C

Fig. 2. a. *Hesperomyces coccinelloides*, D. Haelew. 3880a. b. *Hydraeomyces halipli*, D. Haelew. 3478a. c. *Hydrophilomyces atroseptatus*, D. Haelew. 3483a. d. *Laboulbenia hyalopoda*, D. Haelew. 3320b. e. *Laboulbenia murmanica*, D. Haelew. 3540b. Scale bars: a–e = 50 µm.

Known distribution and hosts: Described from Poland on *Limnebius aluta* (Bedel, 1881) (Coleoptera, Hydraenidae). Recorded from Denmark and Spain on species of *Limnebius* Leach, 1815 (Majewski 1994, Santamaria 2001).

New records from the Netherlands: On three specimens of *Limnebius aluta* (Bedel, 1881) (Coleoptera, Hydraenidae), Zaltbommel, Brakel (Gelderland), N 51°48'12.7" E 5°03'41.5", 13.v.2015, leg. and det. A.J. Dees, slide D. Haelew. 3483a (GENT: GENTFL00245); Winterswijk, nature reserve Korenburgerveen (Gelderland), N 51°59'16.3" E 6°39'16.6", 07.ix.2019, leg. and det. A.J. Dees, slides D. Haelew. 3512a (GENT:GENTFL00278) and 3513a (GENT:GENTFL00279).

Remarks: *Hydrophilomyces atroseptatus* was described because of a single characteristic not found in any other species in the genus: the darkened septa separating secondary branchlets. *Hydrophilomyces limnebii* Sarna & Milewska has also been reported on *Limnebius* hosts, but differs by the lack of darkened septa, a strongly bent perithecial neck, and the formation of corner cells on both sides of the appendage (Majewski 1994).

Laboulbenia expectata Van Caenegem & Haelew., **sp. nov.** Figs. 3A–3D

Index Fungorum: IF901651

Diagnosis: Different from other species in the *Laboulbenia flagellata* species complex by the combination of the following characteristics: small thallus size, higher ratio of the length of cell I to total thallus length, small and stout cell V, smaller perithecium, unevenly colored cell II and basal cell of the outer appendage, and strict association with *Pterostichus vernalis*. Unique molecular autapomorphies and motifs compared to other members of the *L. flagellata* complex in the 5.8S at positions 501 (A), 543 (T); in the ITS2 spacer at positions 549 (G), 553–556 (CGCT), 557–558 (CG) (insertion), 559 (C), 691 (G), 699 (C) (insertion), 739 (C) (insertion); and in the LSU at positions 242 (T), 425 (C), 502 (C), and 533 (T).

Type: BELGIUM: On *Pterostichus vernalis* (Panzer, 1796) (Coleoptera, Carabidae), collected in Herzele, Sint-Lievens-Esse, Duivenbos (East Flanders), N 50°50'54.60" E 3°54'51.10", 11.ii.2023, leg. and det. W. Van Caenegem, slide D. Haelew. 4483b (**holotype** GENT:GENTFL01193, 1 subadult and 3 adult thalli from left elytron), GenBank (isolate D. Haelew. 4483a, 1 adult thallus from right elytron): SSU = OR723993, ITS = OR752337, LSU = OR752345.

Etymology: From Latin, referring to the fact that we expected thalli from *P. vernalis* to represent an undescribed species based on previous molecular data for *L. flagellata* sensu lato (s.l.) on *Agonum* s.l.

Description: **Thallus:** colored unevenly, rather dull, light orange to ash gray with olivaceous shades. **Cell I**: 1.8× longer than broad, broadening upwards but with a slender appearance, slightly orange colored in the upper half near septum I–II. **Cell II**: 1.8× longer than broad, colored dull orange to grayish, less coloration on the ventral side or evenly colored. **Cell III**: 1.6× longer than broad, rectangular to trapezoidal, then slightly broadening upwards. **Cell IV**: 1.2× longer than broad, almost isodiametric. **Cell V**: 1.8× longer

than broad, wedge-shaped to triangular, bulky and small compared to cell IV. **Insertion cell**: black, flattened, slightly constricted, attached to the lower fourth of the perithecium. **Inner appendage**: basal and suprabasal cells hyaline to brown, gray, or orange, composed two to many branches, resulting from two successive dichotomies starting at the basal cell, extending beyond the perithecial tip, often proliferating and then producing many antheridia. **Antheridia**: solitary or paired, few to many in number, depending on whether the inner appendage proliferates, flask-shaped. **Outer appendage**: basal and suprabasal cells colored grey to blackish on the dorsal side, branched once at the suprabasal cells, often also on the basal cell, extending beyond the perithecial tip, but often damaged, rarely proliferating. **Cell VI**: 1.3× longer than broad, rectangular to parallelogram-shaped. **Perithecium**: 2.4× longer than broad, colored ash gray to dark brown, often with olivaceous or orange shades, rather bulky appearance, with one well-developed dorsal pre-apical spot and a often less-developed ventral pre-apical spot. **Ascospores**: two-celled, hyaline, with a slime-sheath.

Measurements: Thallus: (221.1–)255.6–308.4–361.3(–534.7) µm from foot to perithecial tip [25]. **Cell I**: (39.6–)45.6–52.6–59.7(–98.1) × (23.7–)26.1–29.9–33.8(–41.2) µm [25]. **Cell II**: (44.6–)57.7–78.7–99.8(–186.3) × (35.9–)38.9–44.1–49.2(–60.7) µm [25]. **Cell III**: (27.5–)33.8–44.3–54.7(–80.6) × (21.4–)24.5–28.0–31.5(–44.7) µm [25]. **Cell IV**: (22.4–)27.4–31.8–36.3(–41.8) × (21.1–)24.5–27.6–30.7(–37.5) µm [25]. **Cell V**: (13.6–)14.3–16.5–18.7(–22.6) × (7.2–)8.0–9.5–11.1(–14.2) µm [25]. **Insertion cell**: (20.4–)21.1–23.1–25.1(–38.3) µm [22]. **Basal cell of the inner appendage**: (11.4–)12.9–

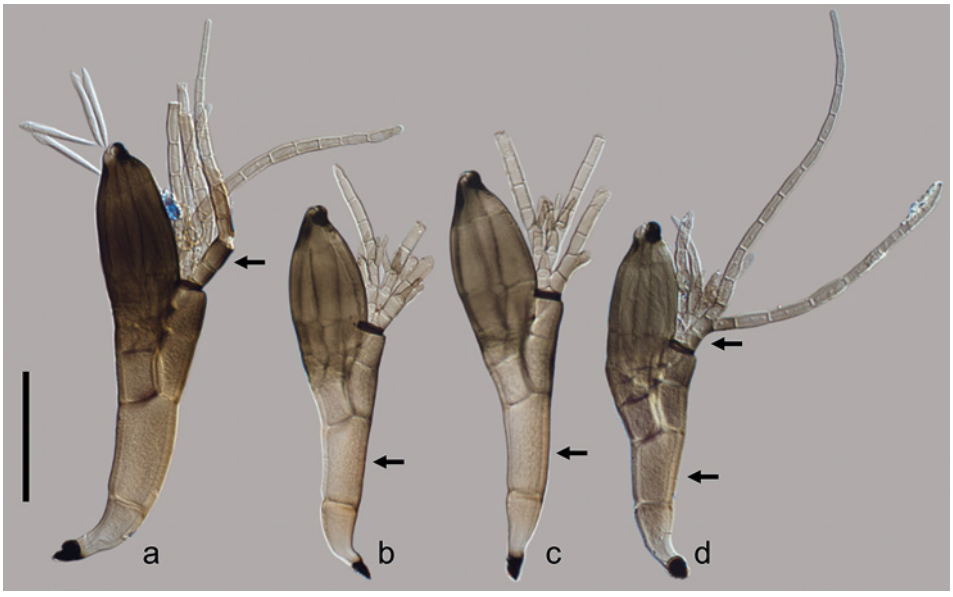

Fig. 3. *Laboulbenia expectata* sp. nov. from *Pterostichus vernalis*, a. D. Haelew. 4483b (holotype), b, c. D. Haelew. 4556a. d. D. Haelew. 4523a. Arrows indicate the unevenly colored cell II and the blackened dorsal side of the outer appendage. Scale bar = 100 µm.

14.7–16.5(–17.3) µm [25]. **Basal cell of the outer appendage**: (17.0–)18.0–19.6–21.1 (–21.7) [12]. **Cell VI**: (21.6–)29.6–39.2–48.7(–77.1) × (21.1–)25.5–30.3–35.0(–47.6) µm [12]. **Perithecium**: (100.9–)115.3–133.2–151.0(–166.5) × (41.1–)48.2–56.6–65.0(–77.9) µm [25]. **Ascospores**: (65.2–)65.6–68.7–71.7(–73.2) × (6.0–)6.2–6.8–7.3(–7.5) µm [8].

Additional specimens examined: BELGIUM: Sint-Truiden (Limburg), N 50°51’04.0” E 5°12’49.5”, 27.vi–15.vii.2022, on *P. vernalis*, leg. K. Van Vooren, det. W. Van Caenegem, in coll. KBIN, slide D. Haelew. 4363b (GENT:GENTFL00909); GenBank (isolate D. Haelew. 4363a, 1 adult thallus from left elytron): SSU = OR723991, ITS = OR752334, LSU = OR752347; Jemeppe-sur-Sambre (Namur), N 50°26’55.83” E 4°39’02.95”, 8.viii.2010, on *P. vernalis*, leg. C. Gerstmans (CG165), det. A. De Kesel, slides D. Haelew. 3047b (GENT:GENTFL00817) and 3047c (GENT:GENTFL00818). CZECHIA: Opava District, Velké Hoštice (Moravian-Silesian Region), 19.iii.1989, on *P. vernalis*, leg. Z. Malinka, det. P. Veselý, in coll. NMP, slide D. Haelew. 3680a (GENT:GENTFL00471). THE NETHERLANDS: Etten-Leur (Noord-Brabant), N 51°33’48.4” E 4°39’57.1”, 27.vi–15.vii.2022, on *P. vernalis*, leg. K. Van Vooren, det. W. Van Caenegem, in coll. KBIN, slides D. Haelew. 4556a (GENT:GENTFL01074) and 4560a (GENT:GENTFL01078); Amsterdam (Noord-Holland), N 52°22’42.9” E 4°46’23.3”, 9.v–27.v.2022, on *P. vernalis*, leg. K. Van Vooren, det. W. Van Caenegem, in coll. KBIN, slide D. Haelew. 4474b (GENT:GENTFL00993); ibid., 27.vi–15.vii.2022, on *P. vernalis*, leg. K. Van Vooren, det. W. Van Caenegem, in coll. KBIN, slides D. Haelew. 4540a (GENT:GENTFL01051), 4540b (GENT:GENTFL01052), 4569a (GENT:GENTFL01087), and 4569b (GENT:GENTFL01088); Stichtse Vecht, Maarssen (Utrecht), N 52°07’47.4” E 5°03’53.1”, 27.vi–15.vii.2022, on *P. vernalis*, leg. K. Van Vooren, det. W. Van Caenegem, in coll. KBIN, slide D. Haelew. 4379a (GENT:GENTFL00914); Rijswijk (Zuid-Holland), N 51°57’03.0” E 5°21’17.9”, 27.vi–15.vii.2022, on *P. vernalis*, leg. K. Van Vooren, det. W. Van Caenegem, in coll. KBIN, slide D. Haelew. 4523a (GENT:GENTFL01033).

Known distribution and hosts: Reported on *Pterostichus vernalis* in Belgium, Czechia, Denmark, Germany, the Netherlands, Poland, and Spain (Scheloske 1969, Santamaria 1985, Majewski 1994, Haelewaters & De Kesel 2020, Santamaria & Pedersen 2021). Previous records of this new species were referred to as *L. flagellata*.

Remarks: *Laboulbenia expectata* is described based on 25 adult and several juvenile and subadult thalli. We generated sequences from two isolates, both from Belgium, included in our phylogenetic analyses (Fig. 4). All records of *L. flagellata* s.l. on *P. vernalis* belong to this species. Records of *L. flagellata* s.l. from other *Pterostichus* species should be studied using molecular phylogenetic data. It is possible that more undescribed species are “hidden” among these. *Laboulbenia expectata* seems to be absent in Finland; Huldén (1983) examined 361 specimens of *P. vernalis* and did not find any infected individuals.

Laboulbenia flagellata is recognized as a species complex and exhibits enormous morphological variation depending on growth position, host species, and habitat (De Kesel & Van den Neucker 2005, Haelewaters et al. 2019a). This variation is visible in *L. expectata*. There are some morphological characteristics that are on average different from *L. flagel-*

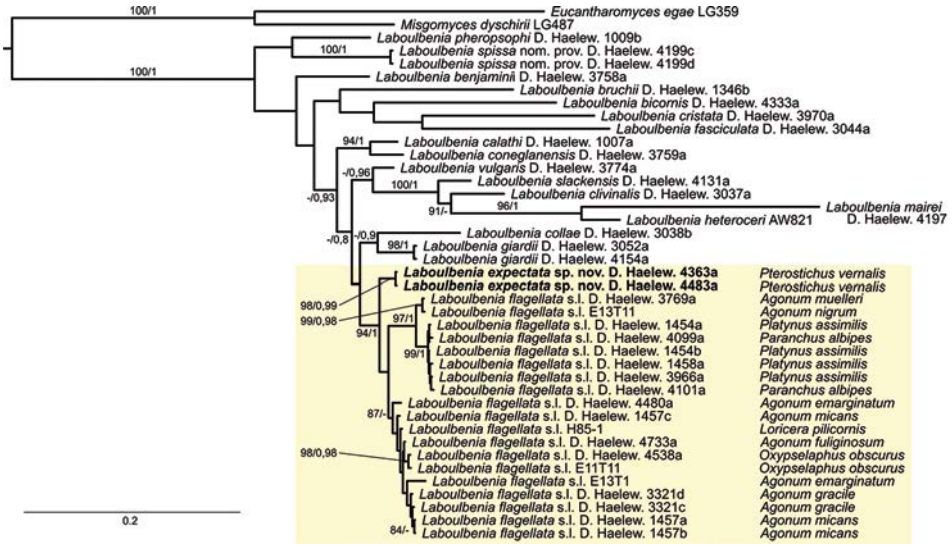

Fig. 4. Phylogeny of the genus *Laboulbenia*, reconstructed from a concatenated SSU–ITS–SSU–*TEF1* dataset. The topology is the result of maximum likelihood inference performed with IQ-TREE. The *L. flagellata* species complex indicated in yellow. *Laboulbenia* expectata sp. nov. is shown in bold. Ultrafast bootstrap values (≥ 70) and Bayesian posterior probabilities (≥ 0.7) are indicated above or below the branch leading to each node.

lata s.l., e.g., its smaller size, a higher ratio of the length of cell I to total thallus length, the small and stout cell V, the smaller perithecium, and the unevenly colored cell II and basal cell of the outer appendage. The combination of these morphological characters and the strict host association with *Pterostichus vernalis* allows us to identify this species more easily. Sequencing the ITS spacer region provides a definitive identification. This is the first taxon within the *L. flagellata* species complex that is described properly using an integrative taxonomy approach.

Pterostichus vernalis has also been reported as a host species for *L. argutoris* Cépède & F. Picard and *L. rigida* Thaxt. *Laboulbenia argutoris* was reported on *P. vernalis* from Italy (Spegazzini 1914), which according to Picard (1917) represents *L. rigida*. However, the illustration in Spegazzini (1914) shows an outer appendage that is broken above the suprabasal cell. It seems that it may have been branched but it would be too speculative as to use this poor illustration for a definitive identification. *Laboulbenia rigida* was described from the North American *Pterostichus patruelis* (Dejean, 1831) (Thaxter 1895) and afterwards reported but not illustrated from France, Poland, and Switzerland on *P. vernalis* (Picard 1917, Baumgartner 1923, Siemaszko & Siemaszko 1932, Bánhegyi 1940, Lepesme 1941). Also Spegazzini (1914) reported *L. rigida* from Italy, but on *Broscosoma baldense* Rosenhauer, 1846 (Carabidae, Broscini). Huldén (1985) concluded that this Italian material on *Broscosoma* belongs to *Laboulbenia broscosomatis* (as *L. broscosomae*); it differs from *L. rigida* by its more rounded and clumsy habitus and its association with a different host genus.

We agree with Majewski (1994) who expressed doubts about European records of *L. rigida* on *P. vernalis*. While Majewski (1994) treated these records as *L. flagellata*, we now know that this taxon represents a species complex (Haelewaters et al. 2019a). In our opinion, *L. rigida* does not occur on *Pterostichus* spp. in Europe – similar to the situation with *L. casnoniae* Thaxt. (Rossi & Santamaria 2006). In addition to their non-overlapping distributions, there is a morphological difference between *L. expectata* and *L. rigida*: the outer appendage of *L. expectata* is branched above the suprabasal cell (typical for all species in the *L. flagellata* group), while that of *L. rigida* is simple.

Laboulbenia hyalopoda De Kesel, Sterbeeckia 18: 17 (1998) Fig. 2D

Known distribution and hosts: Described from Belgium on *Paradromius linearis* (Olivier, 1795) (Coleoptera, Carabidae). Recorded from Denmark, Latvia, Spain (Canary Islands), Sweden, and the U.K. (Europe) on *P. linearis* (De Kesel 1998, De Kesel & Krastina-De Kesel 2006, Lazenby 2017, Santamaria & Pedersen 2021).

New records from the Netherlands: On *Paradromius linearis*, Castricum (Noord-Holland), 6.ix.2011, foredune, leg. and det. P.S. van Wielink, slide D. Haelew. 104b (FH); Zandvoort, north side of nature bridge over Voormalige Trambaan and Zandvoortselaan (Noord-Holland), N 52°22’00.99” E 4°33’48.22”, 6.v.2019, pitfall trap series 5, leg. and det. M. Boeken, slide D. Haelew. 3320b (GENT:GENTFL00154); *ibid.*, D. Haelew. 3649 (no slides); Zandvoort, north side of nature bridge over Voormalige Trambaan and Zandvoortselaan (Noord-Holland), N 52°22’03.34” E 4°33’48.78”, 6.iv.2020, pitfall trap series 6, leg. and det. M. Boeken, D. Haelew. 3367 (no slides); Zandvoort, south side of nature bridge over Voormalige Trambaan and Zandvoortselaan (Noord-Holland), N 52°22’00.44” E 4°33’47.99”, 10.viii.2020, pitfall trap series 4, leg. and det. M. Boeken, D. Haelew. 3368 (no slides).

Remarks: The perithecial apex of our material deviates from the typical horn-like outgrowth as described by De Kesel (1998) and shown by Santamaria & Pedersen (2021). The Dutch material (GENT:GENTFL00154) shows a more blunt and rounded perithecial apex, but other characters such as the almost hyaline foot, the free insertion cell, and the very specific position of growth on the last abdominal sternites match the original description.

Laboulbenia murmanica Huldén, Karstenia 23(2): 57 (1983) Fig. 2E

Known distribution and hosts: Described from Russia on *Bembidion transparens* (Gebler, 1830) (Coleoptera, Carabidae). Recorded from Belgium, Denmark, Lithuania, Poland, and the U.K. (Europe) on *B. assimile* Gyllenhal, 1810, *B. biguttatum* (Fabricius, 1779), *B. doris* (Panzer, 1796), *B. transparens*, and *B. varium* (Olivier, 1795) (Santamaria et al. 1991, Majewski 1994, Markovskaja 2004, Haelewaters & De Kesel 2020, Santamaria & Pedersen 2021).

New records from the Netherlands: On *Bembidion assimile* Gyllenhal, 1810 (Coleoptera, Carabidae), Voorne aan Zee, Oostvoorne (Zuid-Holland), N 51°55’12.0” E

4°03’36.0”, 30.iii.2020, leg. and det. M. Schilthuizen, in coll. TXEX (TXEX.COL.225), slides D. Haelew. 3540a (GENT:GENTFL00309) and 3540b (GENT:GENTFL00310).

Remarks: Host specimen D. Haelew. 3540 carried a double infection of *L. murmanica* and *L. vulgaris* Thaxt. *Laboulbenia murmanica* differs from *L. vulgaris* by the distinct constriction between cells II and III–VI and the outer appendage that does not exceed beyond the perithecial apex (Haelewaters & De Kesel 2020, Santamaria & Pedersen 2021).

Laboulbenia ophoni Thaxt., Proc. Amer. Acad. Arts Sci. 35(9): 190 (1899) Fig. 5A

Known distribution and hosts: Described from Italy on *Harpalus sulphuripes* Germar, 1823 (Coleoptera, Carabidae). Recorded from Algeria (Africa), Belgium, Denmark, Finland, France, Germany, Hungary, Lithuania, Poland, Romania, Slovakia, Spain, Sweden, Switzerland, Russia, and the U.K. (Europe) mainly on species of *Harpalus* Latreille, 1802 and *Ophonus* Dejean, 1821, but also on *Amara* Bonelli, 1810, *Carterus* Dejean, 1830, *Dolichus* Bonelli, 1810, and *Scybalicus* Schaum, 1862 (Santamaria et al. 1991, Majewski 1994, Markovskaja 2004, Haelewaters & De Kesel 2020, Santamaria & Pedersen 2021).

New records from the Netherlands: On *Harpalus rubripes* (Duftschmid, 1812) (Coleoptera, Carabidae), Zandpoort, south side of nature bridge over Voormalige Trambaan and Zandvoortselaan (Noord-Holland), N 52°22’00.44” E 4°33’47.99”, 5.vi.2017, pitfall trap series 4, leg. and det. M. Boeken, slide D. Haelew. 1381a (GENT:GENTFL00040).

Laboulbenia thaxteri Cépède & F. Picard, Bull. Biol. Fr. Belg. 42: 260 (1909) Fig. 5B

Known distribution and hosts: Described from France on *Asaphidion flavipes* (Linnaeus, 1760) (Coleoptera, Carabidae). Recorded from Austria, Belgium, Bulgaria, Denmark, England, Greece, Italy, Latvia, Lithuania, Poland, Romania, Russia, Spain, Sweden, Switzerland, the U.K. (Europe), and Algeria (Africa) on species of *Asaphidion* Gozis, 1886 (Santamaria et al. 1991, Majewski 1994, Rossi et al. 2019, Haelewaters & De Kesel 2020, Santamaria & Pedersen 2021).

New records from the Netherlands: On six specimens of *Asaphidion curtum* (Heyden, 1870) (Coleoptera, Carabidae), Amsterdam, Herengracht 480 (Noord-Holland), N 52°21’53.3” E 4°53’30.8”, 17–24.viii.2020, leg. Taxon Expeditions participants, det. M. Schilthuizen, in coll. TXEX (TXEX.COL.334–TXEX.COL.339), slides D. Haelew. 3550a (GENT:GENTFL00320), 3551a (GENT:GENTFL00321), 3551b (GENT:GENTFL00322), 3551c (GENT:GENTFL00323), 3552a (GENT:GENTFL00324), 3553a (GENT:GENTFL00325), 3554a (GENT:GENTFL00326), 3554b (GENT:GENTFL00327), 3555a (GENT:GENTFL00328), 3555b (GENT:GENTFL00329), 3555c (GENT:GENTFL00330), and 3555d (GENT:GENTFL00331).

Remarks: *Laboulbenia thaxteri* has two different growth forms (elongated and stout), depending on the position of growth. The Dutch material illustrated in Fig. 5B (GENT:GENTFL00320) shows the stout form, which grows on the pronotum of the host.

The outer appendage is broken off, but the inner appendage that consists of a basal cell with a single antheridium, a diagnostic character, can be clearly observed.

Rhachomyces lavagnei (F. Picard) W. Rossi, Mycologia 74(6): 1025 (1982) Fig. 5C

≡ *Dimeromyces lavagnei* F. Picard, Bull. Sci. Fr. Belg. 50: 441 (1917).

Known distribution and hosts: Described from France on *Microlestes minutulus* (Goeze, 1777) (Coleoptera, Carabidae). Recorded from Algeria, South Africa (Africa), Türkiye (Asia), Italy, and Spain (Europe) on species of *Microlestes* Schmidt-Goebel, 1846 (Santamaria et al. 1991, Rossi 1992, Rossi 2016).

New records from the Netherlands: On two specimens of *Syntomus foveatus* (Geoffroy, 1785) (Coleoptera, Carabidae), Zandvoort, National Park Zuid-Kennemerland, pitfall series 7 (Noord-Holland), N 52°22'05.89" E 4°33'48.88", 5.vi.2017, leg. and det. M. Boeken, slides D. Haelew. 1387a (GENT:GENTFL00046) and 1391b (FH).

Remarks: This is the first record of *Rhachomyces lavagnei* on *Syntomus foveatus*. Both host genera *Microlestes* and *Syntomus* are classified in the same tribe, Lebiini. Species of these two genera occur in the same habitat, which increases the chance of ascospore transmission. Thalli of *R. lavagnei* from species of *Microlestes* and *Syntomus* are morphologically identical, but cryptic species might be hidden under this name, separated by host (Haelewaters et al. 2018a, 2022b). Both studied host specimens carried a double infection with *Laboulbenia metableti* Scheloske (Haelewaters & De Kesel 2020).

Rhadinomyces cristatus Thaxt., Proc. Amer. Acad. Arts Sci. 28: 180 (1893) Fig. 5D

≡ *Corethromyces cristatus* (Thaxt.) Thaxt., Proc. Amer. Acad. Arts Sci. 48(7): 180 (1912).

Known distribution and hosts: Described from the U.S.A. on *Tetartopeus nitidulus* (J.L. LeConte, 1880) (as *Lathrobium nitidulum* J.L. LeConte, 1880) (Coleoptera, Staphylinidae). Recorded from Belgium, France, Latvia, Poland, Spain, Ukraine, the U.K. (Europe), and Japan (Asia) on species of *Lathrobium* Gravenhorst, 1802 and *Tetartopeus* Czwalina, 1888 (Santamaria et al. 1991, Majewski 1994, De Kesel & Krastina-De Kesel 2006, Haelewaters & De Kesel 2020, Santamaria & Pedersen 2021).

New records from the Netherlands: On two specimens of *Lathrobium* cf. *geminum* Kraatz, 1857 (Coleoptera, Staphylinidae), Voorne aan Zee, Oostvoorne, N 51°55'12.0" E 4°04'12.0", 28.xii.2020, leg. and det. M. Schilthuizen, slides D. Haelew. 3634a (GENT:GENTFL00441), 3635a (GENT:GENTFL00442), and 3635b (GENT:GENTFL00443).

Remarks: The status of *R. cristatus* is uncertain. Some authors treat it as a separate species (Majewski 1994, Haelewaters & De Kesel 2020), whereas others consider it a synonym of *R. pallidus* Thaxt. (Santamaria & Pedersen 2021). Santamaria & Pedersen (2021) studied the type slide of *R. pallidus* but were unable to locate the type material of *R. cristatus* and concluded that it is impossible to separate both species based on overlapping morphological variability. If *R. cristatus* is treated as a separate species instead of a synonym of *R. pallidus*, then the Dutch material represents a new record for the Netherlands.

Fig. 5. a. *Laboulbenia ophoni*, D. Haelew. 1381a. b. *Laboulbenia thaxteri*, D. Haelew. 3550a. c. *Rhachomyces lavagnei*, D. Haelew. 1387a. d. *Rhadinomyces cristatus*, D. Haelew. 3634a. Scale bars: a–d = 50 µm.

If *R. cristatus* is treated as a synonym of *R. pallidus*, then this material represents a new host record from the Netherlands. Thus far, *R. pallidus* was only reported from the Netherlands on *Lathrobium elongatum* (Linnaeus, 1767) (Haelewaters & De Kesel 2020). *Lathrobium geminum* has already been reported as a host of *R. pallidus* in Belgium and Denmark. Molecular work, in conjunction with the study of Thaxter's slides of *R. cristatus* (at FH), is necessary to clarify the status of both taxa.

Rhynchophoromyces anacaenae Scheloske, Parasitol. Schriftenr. 19: 143 (1969)
Fig. 6A

Known distribution and hosts: Described from Germany on *Anacaena limbata* (Fabricius, 1792) (Coleoptera, Hydrophilidae). Recorded from Belgium, Bulgaria, Denmark, France, Germany, Hungary, Italy, Poland, Spain, and Sweden (Europe) on species of *Anacaena* C.G. Thomson, 1859 (Santamaria et al. 1991, Majewski 1994, Rossi et al. 2019, Haelewaters & De Kesel 2020, Santamaria & Pedersen 2021).

New records from the Netherlands: On *Anacaena limbata* (Coleoptera, Hydrophilidae), Tilburg, Udenhout (Noord-Brabant), N 51°37'46.5" E 5°08'23.7", 07.vi.2019, leg. and det. A.J. Dees, slides D. Haelew. 3508a (GENT:GENTFL00273) and 3508b (GENT:GENTFL00274); Tilburg, Udenhout (Noord-Brabant), N 51°37'46.5" E 5°08'23.7", 28.xii.2019, leg. and det. A.J. Dees, slide D. Haelew. 3515a (GENT:GENTFL00280); on *Anacaena lutescens* (Stephens, 1829) (Coleoptera, Hydrophilidae), Tilburg, Udenhout

(Noord-Brabant), N 51°37’46.5” E 5°08’23.7”, 23.iii.2019, leg. and det. A.J. Dees, slides D. Haelew. 3496a (GENT:GENTFL00262) and 3496b (GENT:GENTFL00263).

Stigmatomyces crassicollis Thaxt., Proc. Amer. Acad. Arts Sci. 52(10): 661 (1917) Fig. 6B

= *Stigmatomyces papuanus* var. *leiostoma* Maire, Bull. Soc. Hist. Nat. Afr. N. 11: 137 (1920).

= *Stigmatomyces longicollis* Thaxt., Proc. Amer. Acad. Arts Sci. 52(10): 660 (1917).

= *Stigmatomyces hackmanii* Huldén, Karstenia 23(2): 66 (1983).

Known distribution and hosts: Described from Jamaica on *Limosina* sp. (Diptera, Sphaeroceridae). Recorded from Costa Rica (North America), Bolivia (South America), Belgium, Denmark, Germany, Italy, Poland, Portugal, Slovakia, Spain, the U.K. (Europe), Algeria (Africa), and New Zealand (Oceania) on species of *Leptocera* Olivier, 1813 s.l. (Diptera, Sphaeroceridae) (Santamaria et al. 1991, Majewski 1994, Haelewaters & De Kesel 2020, Santamaria & Pedersen 2021).

New records from the Netherlands: On two specimens of *Leptocera caenosa* (Rondani, 1880) (Diptera, Sphaeroceridae), Tilburg, nature reserve De Kaaistoep (Noord-Brabant), N 51°32’23.2” E 5°00’34.4”, 14–21.x.2015, pitfall trap in dead crayfish, leg. H. Spijkers & P.S. van Wielink, det. J.W. van Zuijlen, slides D. Haelew. 1407a (FH) and 1408a (FH). On *Leptocera finalis* (Collin, 1956) (Diptera, Sphaeroceridae), Zaandam (Noord-Holland), N 52°25’56.7” E 4°48’49.5”, 25–31.v.2017, leg. A. de Boer, det. E. de Bree, slide D. Haelew. 3826a (GENT:GENTFL00557).

Remarks: *Stigmatomyces crassicollis* has been reported on many different species of flies, mainly on species of *Leptocera* s.l. (a complete list of hosts is given in Santamaria & Pedersen 2021). The morphology of this species can be highly variable. Santamaria & Pedersen (2021) suggested *S. crassicollis* could be a complex of several species.

Stigmatomyces ephydrae L. Mercier & R.A. Poiss., Bull. Soc. Zool. Fr. 52: 226 (1927) Fig. 6C

= *Stigmatomyces bottnicus* Huldén, Karstenia 23(2): 64 (1983).

= *Stigmatomyces setacerae* Huldén, Karstenia 23(2): 68 (1983).

Known distribution and hosts: Described from France on *Ephydra riparia* Fallén, 1813 (Diptera, Ephydridae). Recorded from Canada, the U.S.A. (North America), Brazil (South America), Denmark, Finland, Italy, Portugal, Russia, the U.K. (Europe), China, Kyrgyzstan (Asia), and New Zealand (Oceania) on species of *Ephydra* Fallén, 1810, *Glenanthe* Haliday, 1839, and *Setacera* Cresson, 1930 (Santamaria et al. 1991, Santamaria & Pedersen 2021).

New records from the Netherlands: On *Ephydra riparia* (Diptera, Ephydridae), Reimerswaal, nature reserve Yerseke Moer (Zeeland), N 51°30’10.8” E 4°00’30.6”,

18.v.2021, leg. and det. N.-J. Dek, slides D. Haelew. 3428a (GENT:GENTFL00202) and 3428b (GENT:GENTFL00203).

Remarks: The known distribution of *S. ephydrae* is disjunct (see discussion of Wallacean shortfall in Haelewaters et al. 2024). Whether specimens from Brazil, the Netherlands, and New Zealand belong to the same species should be treated with caution. Van Caenegem et al. (2023a) found a geographical pattern in cryptic species within the *He. virescens* complex: two species of *Chilocorus*, one from North America and the other from northern Africa, were found to be infected by two phylogenetically distinct species of *Hesperomyces*. *Stigmatomyces ephydrae* could present a similar pattern and can only be resolved using an integrative taxonomy approach (Aime et al. 2021).

Stigmatomyces geomyzae W. Rossi & Cesari, G. Bot. Ital. 113(5–6): 384 (1980) [1979]
Fig. 6D

Known distribution and hosts: Described from Italy on *Geomyza tripunctata* (Fallén, 1823) (Diptera, Opomyzidae). Reported from Denmark, Portugal, Spain, the U.K. (Europe) on species of *Geomyza* Fallén, 1810 and *Opomyza petrei* Mesnil, 1934 (Santamaria et al. 1991, Rossi et al. 2013, Santamaria & Pedersen 2021).

New records from the Netherlands: On *Geomyza breviseta* Czerny, 1928 (Diptera, Opomyzidae), Bloemendaal, Overveen, National Park Zuid-Kennemerland (Noord-Holland), N 52°23’50.11” E 4°34’51.96”, 3.vii.2017, pitfall trap series 21, leg. M. Boeken, det. J.W. van Zuijlen, slide D. Haelew. 1384a (GENT:GENTFL00043).

Remarks: Santamaria & Pedersen (2021) reported that *S. geomyzae* is uniquely found on *Geomyza* species. Previously, Rossi et al. (2013) reported *O. petrei* as a host of *S. geomyzae*. Both recorded host genera *Geomyza* and *Opomyza* belong to the same family (Opomyzidae). More research, including molecular phylogenetic methods, is needed to elucidate the host specificity of *S. geomyzae*. There is some doubt regarding the identification of the Dutch host specimen. The genital shape and the chaetotaxy suggest *G. breviseta*, whereas the wing pattern is more similar to that of *G. apicalis* (Meigen, 1830).

Stigmatomyces ptilomyiae Thaxt., Mem. Am. Acad. Arts Sci. N. S. 16: 154 (1931)
Fig. 6E

= *Stigmatomyces micrandrus* var. *atissae* Thaxt., Proc. Amer. Acad. Arts Sci. 52(10): 681 (1917).

Known distribution and hosts: Described from Jamaica on *Ptilomyia parva* (Williston, 1896) (as *Ptilomyia enigma* Coquillett, 1900) (Diptera, Ephydridae). Reported from Grenada (North America), Brazil (South America), France, Italy, Portugal (the Azores) (Europe), and Sierra Leone (Africa) on species of *Atissa* Haliday, 1837 (Diptera, Ephydridae) and *Ptilomyia* Coquillett, 1900 (Santamaria et al. 1991, Bergonzo et al. 2004, Rossi et al. 2013, Santamaria & Pedersen 2021).

New records from the Netherlands: On *Atissa pygmaea* (Haliday, 1833) (Diptera, Ephydridae), Yerseke Moer, Yerseke (Zeeland), N 51°29'39.5" E 4°00'50.0", 04. viii.2021, leg. and det. N.-J. Dek, slide D. Haelew. 3929a (GENT:GENTFL00596).

Remarks: The situation of *S. ptilomyiae* is similar to the one of *S. ephydrae* discussed above. Cryptic species could be hidden under this name. Fresh material removed from hosts of both genera and from different countries and continents should be studied using an integrative taxonomy approach.

Stigmatomyces purpureus Thaxt., Proc. Amer. Acad. Arts Sci. 36: 404 (1901) Fig. 6E
= *Stigmatomyces scatellae* S.W.T. Batra, Am. J. Bot. 50: 986 (1963).

= *Stigmatomyces purpureus* f. *scatellae* (S.W.T. Batra) Balazuc, in Santamaría et al., Treb. Inst. Bot. Barcelona 14: 51 (1991).

Known distribution and hosts: Described from the U.S.A. on *Scatella stagnalis* (Fallén, 1813) (Diptera, Ephydridae). Reported from Canada (North America), Denmark, Finland, France, Italy, Poland, Spain, the U.K. (Europe), Algeria (Africa), India, Türkiye (Asia), and New Zealand (Oceania) on species of *Scatella* Robineau-Desvoidy, 1830 (Santamaria et al. 1991, Majewski 1994, Santamaria & Pedersen 2021).

Fig. 6. a. *Rhynchophoromyces anacaenae*, D. Haelew. 3515a. b. *Stigmatomyces crassicollis*, D. Haelew. 3826a. c. *Stigmatomyces ephydrae*, D. Haelew. 3428a. d. *Stigmatomyces geomyzae*, D. Haelew. 1384a. e. *Stigmatomyces ptilomyiae*, D. Haelew. 3929a. f. *Stigmatomyces purpureus*, D. Haelew. 3974a. Scale bars: a–f = 50 µm.

New records from the Netherlands: On *Scatella lutosa* (Haliday, 1833) (Diptera, Ephydridae), Kapelse Moer, Kapelle (Zeeland), N 51°29'07.7" E 4°00'00.7", 19.vii.2021, leg. and det. N.-J. Dek, slide D. Haelew. 3974a (GENT:GENTFL00747).

Remarks: Similar to *S. ephydrae* and *S. ptilomyiae*, *S. purpureus* has been reported all over the world, on many species of *Scatella*. Whether all these records belong to a single phylogenetic species should be studied using an integrative taxonomy approach.

Tavaresiella hebri T. Majewski, Acta Mycol. 16(1): 148 (1980) Fig. 7A

Known distribution and hosts: Described from Poland on *Hebrus ruficeps* Thomson, 1871 (Hemiptera, Hebridae). Recorded from Denmark and Spain (Europe) on *H. ruficeps* (Majewski 1994, Santamaria & Pedersen 2021).

New records from the Netherlands: On *Hebrus ruficeps* (Hemiptera, Hebridae), Veenedaal, De Blauwe Hel (Utrecht), N 52°00'44.97" E 05°34'16.57", 16.xii.2020, leg. and det. A.J. Dees, slide D. Haelew. 3872a (GENT:GENTFL00583).

Remarks: In their overview of the distribution for *T. hebri*, Santamaria & Pedersen (2021) mentioned Costa Rica, referencing Goldmann & Weir (2018). However, Goldmann & Weir (2018) included *Triceromyces hebri* (on Hebridae, Costa Rica) in their phylogenetic analysis, not *Tavaresiella hebri*. Both species can be easily distinguished from each other based on morphology (Benjamin 1986, 1993).

Thripomyces tessinensis T. Majewski, Polish Bot. Stud. 1: 125 (1991) Fig. 7B

Known distribution and hosts: Described from Poland on *Hydraena pygmaea* G.R. Waterhouse, 1833 (Coleoptera, Hydraenidae). Only known from the type location.

New records from the Netherlands: On *Hydraena britteni* Joy, 1907 (Coleoptera, Hydraenidae), Tilburg, Udenhout (Noord-Brabant), N 51°37'46.7" E 5°08'25.4", 31.iii.2018, leg. and det. A.J. Dees, slide D. Haelew. 3493a (GENT:GENTFL00256).

Remarks: This is the first record of this species since its description by Majewski (1991). Here we present an image of this species for the first time. We observed only four thalli that are not in good condition. To date, only two species of *Thripomyces* have been described, with *T. italicus* Speg. being the type species. The identification of the Dutch material is based on the total length of the two mature thalli (295 µm), the length of the perithecium (64–76 µm), and the perithecial apex (indistinct in *T. tessinensis*). The shape of cells of the receptacle may not be a reliable characteristic – Santamaria (1993) describes it as variable for *T. italicus*. Also the total thallus length of *T. italicus* is highly variable: 350–1000 µm by Thaxter (1931), 132–640 µm by Santamaria (1993). Based on current information, it is unclear to us whether *T. italicus* and *T. tessinensis* represent a single species or two (or even more) separate species.

Zodiomyces vorticellarius Thaxt. (as *Z. vorticellaria*), Proc. Amer. Acad. Arts Sci. 25: 263 (1891) Fig. 7C

Known distribution and hosts: Described from the U.S.A. on *Cymbiodyta lacustris* (LeConte, 1890) (as *Hydrocombus lacustris* LeConte, 1890) (Coleoptera, Hydrophilidae). Reported from Cuba, Mexico (North America), Argentina, Trinidad and Tobago, Venezuela (South America), Belarus, Belgium, Bulgaria, Czechia, Denmark, Germany, Greece, Hungary, France, Italy, the Netherlands, Poland, Spain, Sweden, Türkiye, and the U.K. (Europe), Cameroon (Africa), Cambodia, and South Korea (Asia) on species of *Cymbiodyta* Bedel, 1881, *Enochrus* C.G. Thomson, 1859, *Helochares* Mulsant, 1844, *Hydrobiomorpha* Blackburn, 1888, and *Sternolophus* Solier, 1834 (Santamaria et al. 1991, Majewski 1994, Santamaria & Pedersen 2021, Kong et al. 2022).

New records from the Netherlands: On two specimens of *Helochares obscurus* (O. F. Müller, 1776) (Coleoptera, Hydrophilidae), Wijk bij Duurstede, Overlangbroek (Utrecht), N 51°59’18.7” E 5°22’34.3”, 11.v.2019, leg. and det. A.J. Dees, slide D. Haelew. 3498a (GENT:GENTFL00264); Wijk bij Duurstede, Overlangbroek (Utrecht), N 51°59’55.4” E 5°22’41.9”, 11.v.2019, leg. and det. A.J. Dees, slide D. Haelew. 3499a (GENT:GENTFL00265).

Remarks: *Zodiomyces vorticellarius* was already reported in the Netherlands (Haelewaters et al. 2012a). The material studied here represents a new host species in the Netherlands. Santamaria & Pedersen (2021) mentioned the possibility that *Z. vorticellarius* is a

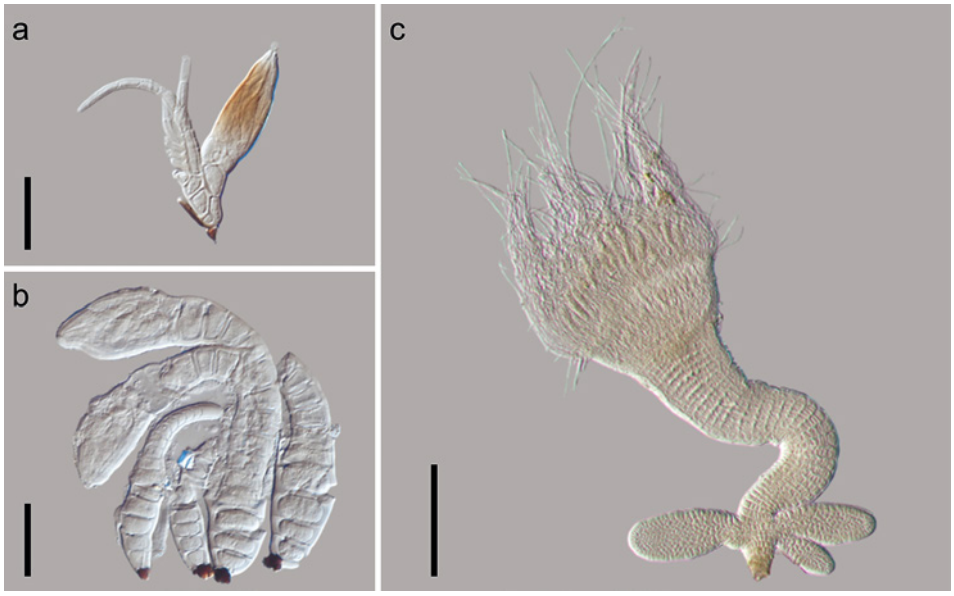

Fig. 7. a. *Tavaresiella hebri*, D. Haelew. 3872a. b. *Thripomyces tessinensis*, four thalli from D. Haelew. 3493a. c. *Zodiomyces vorticellarius*, D. Haelew. 3499a. Scale bars: a–b = 50 µm, c = 100 µm.

species complex, as it is reported on representatives in different genera of Hydrophilidae all over the world. An integrative taxonomy approach should elucidate this hypothesis.

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