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Five new species of *Gloeandromyces* (Fungi, Laboulbeniales) from tropical American bat flies (Diptera, Streblidae), revealed by morphology and phylogenetic reconstruction

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ABSTRACT

This paper describes and illustrates five new species of *Gloeandromyces* (Ascomycota, Laboulbeniales) associated with tropical American bat flies (Diptera, Streblidae). These are *Gloeandromyces cusucoensis* sp. nov. from *Trichobius uniformis* in Costa Rica and Honduras, *G. diversiformis* sp. nov. from *Strebla wiedemanni* in Costa Rica, *G. plesiosaurus* sp. nov. from *Trichobius yunkeri* in Panama, *G. pseudodickii* sp. nov. from *Trichobius longipes* in Ecuador and Panama, and *G. verbekeniae* sp. nov. from *Strebla galindoi* in Ecuador and Panama. The description of these five species doubles the number of known species in the genus. Morphological characteristics, host association, and a three-locus (18S nuc rDNA, 28S nuc rDNA, *TEF1*) phylogenetic reconstruction support placement of these taxa in the genus *Gloeandromyces*. Three of the new species are polymorphic; they have multiple morphotypes that grow in specific positions on the host integument: *G. diversiformis* f. *diversiformis*, f. *musiformis*, and f. *vanillicarpiformis; G. plesiosaurus* f. *asymmetricus* and f. *plesiosaurus*; and *G. verbekeniae* f. *verbekeniae* and f. *inflexus*. Finally, a dichotomous key to all species and morphotypes is presented.

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Arthropod-associated fungi; bat flies; integrative taxonomy; Laboulbeniomycetes; nuclear ribosomal DNA; polymorphism; *TEF1*; 9 new taxa

INTRODUCTION

Laboulbeniales (Ascomycota, Laboulbeniomycetes) is an order of obligate ectoparasitic microfungi that occur exclusively on the exoskeleton of arthropod hosts belonging to Chelicerata, Hexapoda, and Myriapoda (Haelewaters et al. 2021a). In contrast to other fungi, Laboulbeniales do not develop hyphae. Instead, through subsequent mitotic divisions, a two-celled ascospore develops into a microscopic, resilient structure of determinate growth: a thallus (Blackwell et al. 2020). The entire life cycle is completed on a single host (Haelewaters et al. 2021b). In addition, many Laboulbeniales are known to be host-specific. Even those taxa that were once thought to have a wide host range, such as *Hesperomyces virescens* Thaxt. (Haelewaters et al. 2018a, 2022b) and *Laboulbenia flagellata* Peyr. (De Weggheleire 2019; Haelewaters et al. 2019), have been shown to encompass multiple (pseudo-) cryptic species. Transmission of ascospores from one host to another primarily requires direct close contact between hosts through social activities such as grooming or mating, since indirect transmission is negligible (De Kesel 1995; Nalepa and Weir 2007).

Only 10% of the known species of Laboulbeniales occur on dipteran hosts (Weir and Hammond 1997). Within the Diptera, bat flies (Hippoboscoidea, families Nycteribiidae and Streblidae) make for interesting host-parasite interactions, as they are themselves parasites. Bat flies are exclusively and obligatorily associated with bats as blood-feeding ectoparasites (Chiroptera) (Dick and Patterson 2006). Laboulbeniales taxa associated with bat flies can therefore be considered obligate hyperparasites (parasites of parasites). Representatives of four genera of Laboulbeniales are known to have bat fly hosts: *Arthrorhynchus* Kolen., *Dimeromyces* Thaxt., *Gloeandromyces* Thaxt., and *Nycteromyces* Thaxt. (de Groot et al. 2020; Haelewaters et al. 2021b). The prevalence of Laboulbeniales on bat flies is generally very low, ranging

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from 2.2% for nycteribiid bat flies in the Eastern Hemisphere up to 7% for streblid bat flies in the Western Hemisphere (Haelewaters et al. 2021b). Throughout the 20th century, little research was done on bat fly–associated Laboulbeniales, since the multitrophic lifestyle and inconspicuous nature of these fungi make for a difficult study system. In addition, other obstacles such as the inability of the fungus to grow in axenic culture, the difficulty in DNA extraction, and gene amplification via polymerase chain reaction (PCR) hamper the research of this group of microfungi (Haelewaters et al. 2015, 2021c; Sundberg et al. 2018; Weir and Blackwell 2001). It took 85 years after Thaxter's death in 1932 before a new species of bat fly–associated Laboulbeniales was described (Haelewaters et al. 2017). Since then, renewed interest in Laboulbeniales on bat flies and intensive fieldwork have resulted in the discovery of new taxa as well as reports of new host records (Haelewaters and Pfister 2019; Haelewaters et al. 2017, 2020; Jensen et al. 2019; Liu et al. 2020; Szentiványi et al. 2018). Most of the focus of our research group has been on tropical American Laboulbeniales of bat flies, especially the genus *Gloeandromyces*.

At this time, the genus *Gloeandromyces* comprises five formally described species (Liu et al. 2020). Thaxter (1931) erected this genus to include *G. nycteribiidarum* (Thaxt.) Thaxt. and *G. streblae* (Thaxt.) Thaxt. Both taxa were originally described in the genus *Stigmatomyces* (Thaxter 1917). A third species, *Gloeandromyces pageanus* Haelew., was described by Haelewaters et al. (2017). Intensive fieldwork in Panama resulted in specimens that improved understanding of the diversity within the genus—including intraspecific morphological diversity. Haelewaters and Pfister (2019) described three morphotypes as "formae" of *G. pageanus* based on a phylogenetic reconstruction using the large subunit (28S) of the nuclear ribosomal DNA (nuc rDNA). *Gloeandromyces pageanus* f. *pageanus*, referring to the original form on *Trichobius dugesioides* bat flies (Haelewaters et al. 2017), is characterized by two horn-like projections on the perithecial venter and is found on the thorax; *G. pageanus* f. *alarum* Haelew. carries a single, almost horizontal projection on the perithecial venter and is restricted to the base of the wings of *T. joblingi* bat flies; and *G. pageanus* f. *polymorphus* Haelew. on *T. dugesioides* and *joblingi* bat flies appears to be less position-specific. The same authors also distinguished two morphotypes within the previously described *G. streblae: G. streblae* f. *sigmomorphus* Haelew. has a sigmoid habitus, a perithecial neck with a large preapical outgrowth, and is restricted to the last sternite/tergite of *T. joblingi*, whereas *G. streblae* f. *streblae* lacks any large outgrowths and is found all over the bat fly body. Although originally described from *Strebla wiedemanni* (Thaxter 1917), *G. streblae* is mostly reported on *Trichobius* species (Haelewaters and Pfister 2019). The fourth species in the genus, *G. dickii* Haelew., is recognized by its finger-like outgrowth halfway along the perithecial venter and can mostly be found on the right-hand side of the ventral abdomen of *T. joblingi* (Haelewaters and Pfister 2019). Finally, *G. hilleri* Haelew. & Pfliegler on *Mastoptera guimaraesi* bat flies is characterized by the two to three longitudinal rows of undulations on the perithecial venter (Liu et al. 2020).

In light of the global parasite plan put forward by Carlson et al. (2020), our group has invested in multitrophic fieldwork in different countries around the world, resulting in data necessary to generate sequence data, reveal undescribed diversity, resolve evolutionary relationships, discover patterns pertaining to community ecology theory, and advance the conservation of poorly studied organismal groups (Hajibabaei et al. 2007; Mueller et al. 2022; Haelewaters et al. 2022a, resubmitted). In this paper, resulting from fieldwork in Central and northern South America, we formally describe and illustrate five new species of *Gloeandromyces* through an integrated taxonomic approach, combining morphology, molecular phylogeny, and ecology.

MATERIALS AND METHODS

Fieldwork and examination of bat flies.—Bats were captured using mist nets in Costa Rica, Ecuador, Honduras, and Panama. Guidelines of the American Society of Mammalogists (Sikes and Gannon 2011) were followed for capturing and handling bats. All capturing, sampling, and exporting procedures in Costa Rica were licensed and approved by the Ministerio de Ambiente y Energía (research permit 016-2012-SINAC, export permit DGVS-270-2012); in Ecuador by the Ministerio del Ambiente (permit 023-IC-FAU-DNBAP/MA); in Honduras by the Instituto de Conservación Forestal (permit DE MP 005-2020); and in Panama by the Smithsonian Tropical Research Institute (IACUC protocols 2013-0401-2016, 2015-0206-2018, 2016-0627-2019) and the Ministerio de Ambiente (scientific permits SE/A-75-13, SE/A-21-14, SE/A-69-14, SE/A-1-15, SE/A-28-16, SE/A-18-17, SE/A-12-18; export permits SEX/A-60-15, SEX/A-101-16, SEX/A-120-16, SEX/A-52-17). Bat flies were removed from their bat hosts using featherweight forceps and preserved in 96% ethanol. Screening of bat flies for the presence of Laboulbeniales was done using a Visiscope SZB 350 stereoscope (VWR, Radnor,

Pennsylvania) under 40–50× magnification, making use of disposable needles and featherweight forceps for handling specimens.

Morphological study of Laboulbeniales.—Thalli were removed from the host flies with disposable needles of which the tip was dipped in Hoyer's medium to prevent the thalli from getting lost during transfer. Permanent mounts of thalli were made following the double slide–mounting protocol described by Liu et al. (2020). The only modification we have made is that we placed thalli in a droplet of a 1:1 mixture of glycerin and Hoyer's medium instead of in pure Hoyer's medium, because our Hoyer's medium dried too quickly to allow manipulation. Mounted specimens were examined under a compound microscope (Olympus CX21; Tokyo, Japan) at 400× magnification. Images of thalli were made using the DS-Fi3 camera (Nikon, Melville, New York) mounted on an Eclipse Ni-U microscope (Nikon) with differential interference contrast optics to enhance the contrast in the unstained slides. Images were edited in Adobe Photoshop 23.5.0 and assembled in composite figure plates using Adobe Illustrator 26.5 (San Jose, California). Unedited images used for this paper can be accessed at: https://github.com/dannyhaelewaters/teamlaboul/tree/main/gloeandromyces_paper/unedited_images. Line drawings of thalli were made with Pitt artist pens (Faber-Castell, Nürnberg, Germany) based on unedited images, scanned using an HP Scanjet G5040 scanner (Palo Alto, California), and edited with Photopea (https://www.photopea.com/).

Thalli and individual structures of the new *Gloeandromyces* species were measured using ImageJ (Abramoff et al. 2004). Measurements were reported and further processed in Excel 2210 (Microsoft, Redmond, Washington). In the descriptions, measurements of structures are reported as follows: (a–)b–c–d(–e) [n], with a, e = extreme values; b, d = mean minus/plus standard deviation; c = mean; and n = number of structures measured. Diagnoses are modeled after Sánchez-García et al. (2016), Haelewaters and Pfister (2019), and Haelewaters et al. (2022b), with an overview of unique molecular autapomorphies and motifs.

Studied slide mounts are deposited at the Herbarium Universitatis Gandavensis (GENT) and the Farlow Herbarium, Harvard University (FH). The slide collection of thallus-forming Laboulbeniomycetes at GENT is in the process of being accessioned, with each slide receiving a unique GENTFL label (F for Fungi, L for Laboulbeniomycetes).

DNA extraction, PCR amplification, and sequencing.—DNA extractions were made using the REPLI-g Single Cell Kit (Qiagen, Stanford, California) following the manufacturer's instructions. To ensure successful lysis, we cut every perithecium once transversally using a disposable surgical blade.

Partial small subunit (18S) nuc rDNA and partial 28S nuc rDNA as well as the gene encoding the translation elongation factor 1α (*TEF1*) were amplified using the following primer pairs: NSL1/NSL2 for 18S (Haelewaters et al. 2015); NL1/NL4 (Kurtzman and Robnett 1997), LR0R/LR5, and LR0R//LR7 (Hopple 1994; Vilgalys and Hester 1990) for 28S; and EF1-1018 F/EF1-1620 R and Al33_alternative_f/EF1-1620 R for *TEF1* (Stielow et al. 2015). Amplifications were performed on a T100 thermal cycler (Bio-Rad, Hercules, California) in 25-µL reactions consisting 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 following cycling conditions were used: For 18S: initial denaturation at 94 C for 5 min; 39 cycles of denaturation at 94 C for 30s, annealing at 50 C for 45s, and extension at 72 C for 90s; and final extension at 72 C for 10 min. For 28S: initial denaturation at 94 C for 5 min; 34 cycles of denaturation at 94 C for 30s, annealing at 50 C for 45s, 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 50s, annealing at 54 C (−1 C/cycle) for 50s, and extension at 72 C for 50s; followed by 40 cycles of denaturation at 94 C for 50s, annealing at 48 C for 50s, and extension at 72 C for 50s; and final extension at 72 C for 7 min.

Purification of 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, Massachusetts) per 10 µL of PCR product, at 37 C for 15 min followed by deactivation at 85 C for 15 min. Purified PCR products were sequenced using the same primers on an automated ABI 3730xl capillary sequencer (Life Technology at Macrogen, Amsterdam, The Netherlands). Forward and reverse sequence reads were assembled and edited in Sequencher 5.4.6 (Gene Codes, Ann Arbor, Michigan). Newly generated sequences were submitted to the National Center for Biotechnology Information (NCBI) GenBank database (http://www.ncbi.nlm.nih.gov/; accession numbers in TABLE 1).

Sequence alignment and phylogenetic analysis.—We downloaded all available 18S, 28S, and *TEF1* sequences of *Gloeandromyces* spp. from NCBI GenBank and combined these with our newly generated sequences for

Table 1. Details of all isolates used in this study.

Species	Isolate	Host	GenBank accession numbers		
			18S	28S	*TEF1*
Gloeandromyces cusucoensis	D. Haelew. 1884c	*Trichobius uniformis* Curran 1935	**OQ971686**	**OQ971589**	
G. cusucoensis	D. Haelew. 3194b	*Trichobius uniformis*	**OQ971687**	**OQ971590**	**OQ969945**
G. dickii	D. Haelew. 1312b	*Trichobius joblingi* Wenzel 1966	MH040546	MH040580	MT197539
G. dickii	D. Haelew. 1312c	*Trichobius joblingi*	MH040547	MH040581	
G. dickii	D. Haelew. 1323b	*Trichobius joblingi*	MG958011	MH040582	
G. dickii	D. Haelew. 1323c	*Trichobius joblingi*	MH040548	MH040583	MT197542
G. diversiformis f. *diversiformis*	D. Haelew. 1831d	*Strebla wiedemanni* Kolenati 1856	**OQ971688**	**OQ971591**	
G. diversiformis f. *diversiformis*	D. Haelew. 1833a	*Strebla wiedemanni*	**OQ971689**	**OQ971592**	
G. diversiformis f. *musiformis*	D. Haelew. 1854e	*Strebla wiedemanni*	**OQ971690**	**OQ971593**	
G. diversiformis f. *vanillicarpiformis*	D. Haelew. 1863b	*Strebla wiedemanni*	**OQ971691**	**OQ971594**	
G. diversiformis f. *vanillicarpiformis*	D. Haelew. 1863c	*Strebla wiedemanni*	**OQ971692**	**OQ971595**	
G. hilleri	D. Haelew. 1721a	*Mastoptera guimaraesi* Wenzel 1966	MT184878	MT184328	MT197534
G. hilleri	D. Haelew. 1722a	*Mastoptera guimaraesi*	MT184879	MT184892	MT197535
G. hilleri	D. Haelew. 1738b	*Mastoptera guimaraesi*	MT184880	MT184893	MT197536
G. nycteribiidarum	D. Haelew. 1319b	*Megistopoda aranea* (Coquillet 1899)	MH040533	MH040566	MT197540
G. nycteribiidarum	D. Haelew. 1334c	*Megistopoda aranea*	MH040534	MH040567	MT197533
G. nycteribiidarum	D. Haelew. 1661b	*Megistopoda aranea*	**OQ971693**	**OQ971596**	
G. nycteribiidarum	D. Haelew. 3183a	*Trichobius sparsus* Kessel 1925		**OQ971597**	
G. nycteribiidarum	D. Haelew. 3185a	*Trichobius sparsus*		**OQ971598**	
G. nycteribiidarum	D. Haelew. 3199a	*Exastinion clovisi* (Pessoa & Guimaraes 1937)	**OQ971694**	**OQ971599**	**OQ969946**
G. pageanus f. *alarum*	D. Haelew. 1306b	*Trichobius joblingi*	MH040541	MH040574	
G. pageanus f. *alarum*	D. Haelew. 1322a	*Trichobius joblingi*	MH040543	MH040577	
G. pageanus f. *alarum*	D. Haelew. 1327a	*Trichobius joblingi*	MH040544	MH040578	**OQ969947**
G. pageanus f. *pageanus*	D. Haelew. 1091b	*Trichobius dugesiodes* Wenzel 1966	MH040535	MG906798	
G. pageanus f. *pageanus*	D. Haelew. 1367b	*Trichobius dugesiodes*		MH040568	
G. pageanus f. *pageanus*	D. Haelew. 1425a	*Trichobius dugesiodes*	MH040536	MH040569	
G. pageanus f. *polymorphus*	D. Haelew. 619a	*Trichobius joblingi*	MH040537	KT800008	
G. pageanus f. *polymorphus*	D. Haelew. 1073b	*Trichobius joblingi*	MH040538	MH040570	
G. pageanus f. *polymorphus*	D. Haelew. 1089a	*Trichobius dugesiodes*	MH040539	MH040571	
G. pageanus f. *polymorphus*	D. Haelew. 1100b	*Trichobius joblingi*	MH040307	MH040572	
G. pageanus f. *polymorphus*	D. Haelew. 1272a	*Trichobius dugesiodes*	MH040540	MH040573	**OQ969948**
G. pageanus f. *polymorphus*	D. Haelew. 1315a	*Trichobius joblingi*		MH040575	**OQ969949**
G. pageanus f. *polymorphus*	D. Haelew. 1315b	*Trichobius joblingi*	MH040542	MH040576	
G. plesiosaurus f. *plesiosaurus*	D. Haelew. 3310d	*Trichobius yunkeri* Wenzel 1966	**OQ971695**	**OQ971600**	
G. pseudodickii	D. Haelew. 3417k	*Trichobius longipes* (Rudow 1871)	**OQ971696**		
G. pseudodickii	D. Haelew. 3417l	*Trichobius longipes*	**OQ971697**	**OQ971601**	**OQ117043**
G. streblae f. *sigmomorphus*	D. Haelew. 1320b	*Trichobius joblingi*	MH040545	MH040579	MT197541
G. streblae f. *streblae*	D. Haelew. 1090a	*Trichobius dugesiodes*		MH040584	
G. streblae f. *streblae*	D. Haelew. 1306c	*Trichobius joblingi*	MG958012	MH040585	MT197537
G. streblae f. *streblae*	D. Haelew. 1308b	*Trichobius dugesiodes*	MH040549	MH040586	
G. streblae f. *streblae*	D. Haelew. 1309a	*Trichobius dugesiodes*	MH040550	MH040587	MT197538
G. streblae f. *streblae*	D. Haelew. 1317a	*Trichobius joblingi*	MH040551	MH040588	
G. streblae f. *streblae*	D. Haelew. 1335c	*Trichobius joblingi*	MH040552	MH040589	MT197546
G. verbekeniae f. *verbekeniae*	D. Haelew. 1741b	*Strebla galindoi* Wenzel 1966	**OQ971698**	**OQ971602**	**OQ969950**
Hesperomyces coccinelloides	D. Haelew. 1428b	*Stethorus tenerifensis* Fürsch 1987		OL335915	
Hesperomyces. parexochomi	D. Haelew. 1690d	*Parexochomus nigripennis* Erichson 1843	MZ994884	MZ994874	
Nycteromyces streblidinus	D. Haelew. 1324b	*Trichobius joblingi*	MH040590	MH040554	MT197543
Stigmatomyces limnophorae	D. Haelew. 1802c	*Sarcophaga javanica* (Lopes 1961)	MT341792	MT341789	

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

phylogenetic analyses (TABLE 1). *Hesperomyces coccinelloides* (Thaxt.) Thaxt., *H. parexochomi* Mironova & Haelew., *Nycteromyces streblidinus* Thaxt., and *Stigmatomyces limnophorae* Thaxt. were selected as outgroup taxa. Sequences were aligned by locus with the E-INS-i strategy using the online version 7 of MAFFT (Katoh et al. 2019; Kuraku et al. 2013). Sequences were checked and tails were manually trimmed in BioEdit 7.2.6 (Hall 1999) and concatenated in SequenceMatrix 1.9 (Vaidya et al. 2011). Models for nucleotide substitution were selected for each locus (18S, 28S, *TEF1*) with ModelFinder (Kalyaanamoorthy et al. 2017) according to the corrected Akaike information criterion (AICc). The maximum likelihood (ML) tree was inferred from the concatenated sequences using IQ-TREE under partitioned models (Chernomor et al. 2016; Nguyen et al. 2015). Ultrafast bootstrapping was performed with 1000 replicates (Hoang et al. 2018). The phylogenetic tree was visualized in FigTree 1.4.3 (http://tree.bio.ed.ac.uk/software/figtree/) and edited in Adobe Illustrator 25.1 (San Jose, California). The final alignments and unedited tree are available for download at: https://github.com/dannyhaelewaters/teamlaboul/tree/main/gloeandromyces_paper.

RESULTS

We generated 34 *Gloeandromyces* sequences: 13 18S, 14 28S, and 7 *TEF1* (TABLE 1). Isolate D. Haelew. 3417k was excluded from further analysis because we were only able to generate an 18S sequence, which is too conserved for species delimitation. Our concatenated 18S+28S+*TEF1* data set consists of 42 18S, 47 28S, and 19 *TEF1* sequences for 47 isolates, totaling 2035 characters. Selected models for each partition in our concatenated data set are as follows: GTR+F+G4 (18S, 501 bp, −lnL = 3023.266), GTR+F+I+G4 (28S, 918 bp, −lnL = 8759.176), and TIM2+F+I (*TEF1*, 616 bp, −lnL = 4450.738). The reconstructed phylogeny is shown in FIG. 1. Ten lineages can be distinguished within *Gloeandromyces*. This number corresponds to the number of recognized species in the genus, including the five newly described ones in this paper. Support is lacking for *G. nycteribiidarum*. *Gloeandromyces pageanus* contains a highly supported subclade consisting of three isolates of *G. pageanus* f. *pageanus* (bootstrap = 99). Support for the newly described species is high (bootstrap = 94–100).

TAXONOMY

Gloeandromyces Thaxt., Mem Amer Acad Arts 16:112. 1931.

Type species: Gloeandromyces streblae (Thaxt.) Thaxt., Mem Am Acad Arts Sci NS 16:113. 1931.

Basionym: Stigmatomyces streblae Thaxt., Proc Am Acad Arts Sci 52:700. 1917.

Gloeandromyces cusucoensis B. Santam., Dumolein & Haelew., sp. nov. FIGS. 2a, 4a

Index Fungorum IF900219

Typification: COSTA RICA. PROVINCIA DE PUNTARENAS: Piedras Blancas, cave 3, 8.8058056N 83.2391389W, 29 Mar 2012, on male *Trichobius uniformis* Curran 1935 (Diptera, Streblidae) collected from female *Glossophaga soricina* (Chiroptera, Phyllostomidae), *Thomas Hiller 2012CR519* (fly vial code), slide D. Haelew. 1884b (**holotype** at GENT, 3 adult thalli from left profemur); ibid., slide D. Haelew. 1884a (**isotype** at GENT, 2 adult thalli from right wing R_{2+3} vein), GenBank (isolate D. Haelew. 1884c, 4 adult thalli from left profemur): 18S = OQ971686, 28S = OQ971589.

Etymology: Referring to Cusuco National Park, an intensely studied cloud forest ecosystem in northwest Honduras (Martin et al. 2021) and the location where this species was discovered.

Diagnosis: Different from other species and formae in the genus by the pronounced constriction in the perithecial neck and the conical shape of the perithecial tip. Unique molecular autapomorphies and motifs in the 28S nuc rDNA at positions 380 (A), 497–498 (AC) (insertion), 500 (C), and 524 (C); in *TEF1* at positions 366 (A) and 411 (T).

Description: Thallus hyaline with a blackened foot; basal cell of the appendage rusty orange. Cell I 3.6× longer than broad, slightly broadening upward, carrying cells II and VI. Cell II 1.3× longer than broad, parallelogram-shaped. Cell III 1.2× broader than long, irregularly trapezoidal to quadrangular, with the outer wall convex. Basal cell of the appendage 1.9× broader than long, flattened, rectangular to pentagonal, carrying two suprabasal cells, ending in antheridial cells. Cell VI 2.1× longer than broad, asymmetrical, lens-shaped, obliquely positioned between cells II and VII. Cell VII 1.4× longer than broad, turbinate, proximal end almost in contact with cell I. Perithecium 2.9× longer than broad; venter slightly broadening upward; neck abruptly narrower than venter, constricted in the middle, tapering to a conical tip.

Measurements: Thallus (119.6–)131.6–156.8–182(–188.8) µm from foot to perithecial tip [15]. Cell I (30.0–)38.9–48.7–58.5(–65.7) × (10.9–)11–13.4–15.8(–17.5) µm [17]. Cell II (7.9–)9.6–11.9–14.2(–15.1) × (5.1–)7.5–9.3–11.1(–11.2) µm [13]. Cell III (6.4–)6.6–8.2–9.8(–11.6) × (4.5–)7.9–9.8–11.6 µm [16]. Basal cell of the appendage (3.6–)4.0–5.1–6.2(–7.9) × (7.2–)8.4–9.5–10.6(–11.7) µm [18]. Cell VI (8.4–)9.2–10.3–11.4(–12.7) × (3.1–)4.0–4.8–5.6(–6.0) µm [13]. Cell VII (5.2–)6.6–8.2–9.8(–10.1) × (4.1–)4.5–5.9–7.3(–7.8) µm [11]. Perithecium (40.0)56.1–81.0–105.9(–119.3) × (17.8–)22.9–27.5–32.1(–34.7) µm [15]. Ascospores not measured.

Additional specimens examined: COSTA RICA. PROVINCIA DE PUNTARENAS: Piedras Blancas, cave 3, 8.8058056N 83.2391389W, 28 Mar 2012, on male *T. uniformis* collected from female *G. soricina, Thomas Hiller 2012CR504* (fly vial code), slide D. Haelew. 1880a (GENT, 2 adult thalli from right protibia); ibid., 7 Feb 2012, on female *T. uniformis* collected from male *G. soricina, Thomas Hiller 2012CR140* (fly vial code), slide D. Haelew. 1890d (GENT, 1 adult thallus from right palpus); Piedras Blancas, cave 1, 8.809211N 83.238989W, 26 Mar 2012, on male *T. uniformis* collected from female *G. soricina, Thomas Hiller 2012CR474* (fly vial code), slide D. Haelew. 1885a (GENT, 2 adult thalli from right wing C vein); ibid., on male *T. uniformis* collected from female *G. soricina, Thomas Hiller 2012CR474* (fly vial code), slide D. Haelew. 1889a (GENT, 2 adult thalli from right protibia). HONDURAS. DEPARTAMENTO DE CORTÉS: Cusuco National Park, El Cortecito camp, site 33 of transect 2 (net 4), 15.5241583N

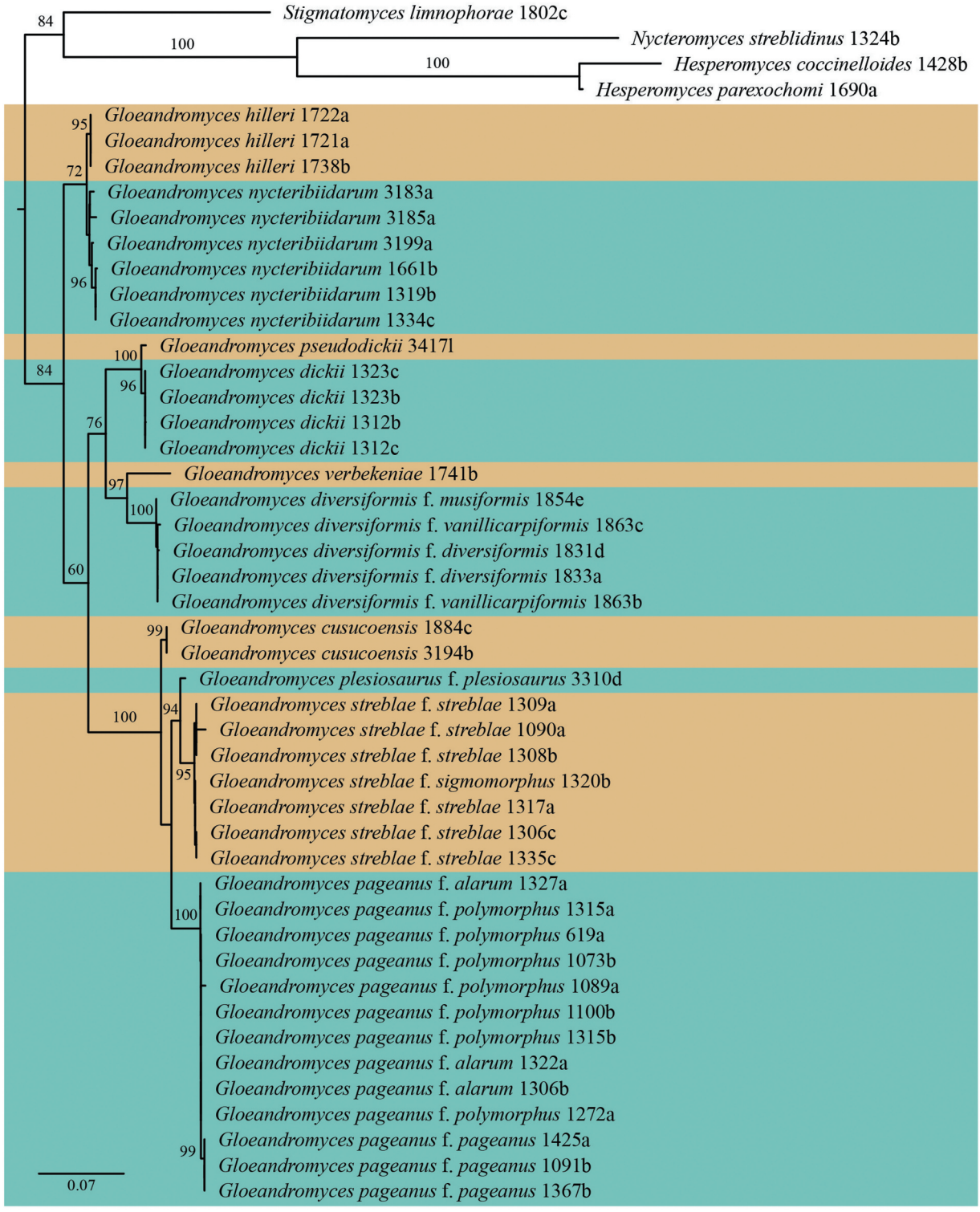

Figure 1. Phylogenetic placement of the new species in the genus *Gloeandromyces*, reconstructed from a combined data set of 18S, 28S, and *TEF1* sequences. The topology is the result of maximum likelihood inference performed with IQ-TREE. For each node, ML bootstrap support (≥70) is presented above or below the branch leading to that node. Color scheme from https://colorbrewer2.org by C. A. Brewer, Department of Geography, Pennsylvania State University.

88.2871472W, 1407 m above sea level (a.s.l.), 21 Jul 2019, on male *T. uniformis* collected from male *G. soricina*, *Zeltia López-Gallego, Elena Uribe DH2957* (fly vial code), slides D. Haelew. 3194a (GENT, 1 subadult thallus from left wing R_1 vein), D. Haelew. 3194c (GENT, 2 subadult thalli from right mesotibia), and D. Haelew. 3194d (GENT, 5 subadult thalli from right protibia), GenBank (isolate D. Haelew.

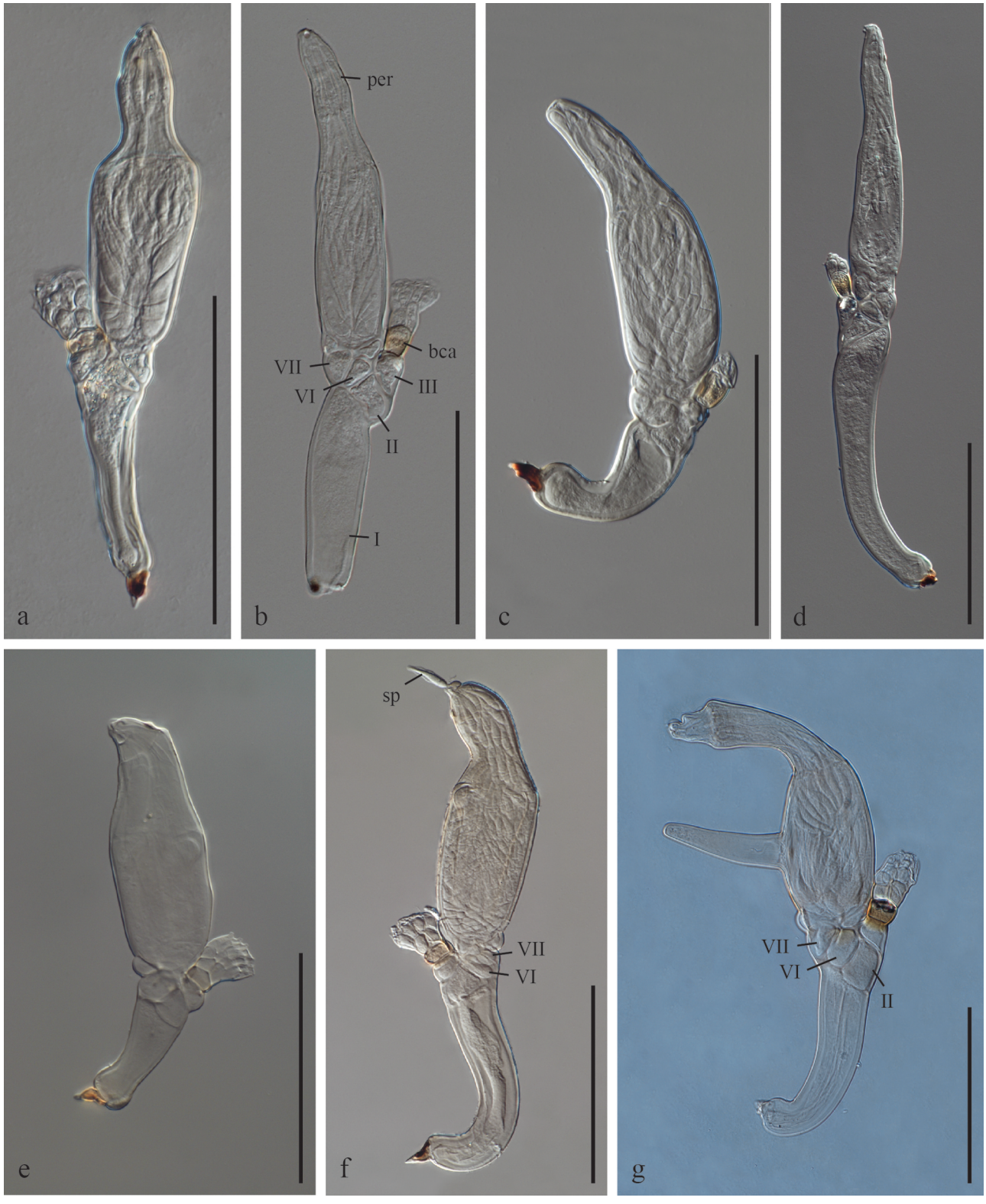

Figure 2. New species of *Gloeandromyces*. a. *Gloeandromyces cusucoensis*, slide D. Haelew. 1884a (isotype). b. *Gloeandromyces diversiformis* f. *diversiformis*, slide D. Haelew. 1831b (holotype). c. *Gloeandromyces diversiformis* f. *musiformis*, slide D. Haelew. 1854c (holotype). d. *Gloeandromyces diversiformis* f. *vanillicarpiformis*, slide D. Haelew. 1854b (holotype). e. *Gloeandromyces plesiosaurus* f. *asymmetricus*, slide GENT:GENTFL00148. f. *Gloeandromyces plesiosaurus* f. *plesiosaurus*, slide GENT:GENTFL00080 (holotype). g. *Gloeandromyces pseudodickii*, slide D. Haelew. 3143a (holotype). Shown are cells I, II, III, VI, VII, the basal cell of the appendage (bca), the perithecium (per), and an ascospore (sp). Bars = 100 µm.

3194b, 1 subadult and 3 adult thalli from right protibia): 18S = OQ971686, 28S = OQ971590, *TEF1* = OQ969945).

Hosts and distribution: On *Trichobius uniformis* (Diptera, Streblidae) in southern Costa Rica along the Pacific Ocean coast and Cusuco National Park, Honduras.

Notes: Gloeandromyces cusucoensis is described based on 11 adult and 8 subadult thalli. All thalli were removed from *Trichobius uniformis* bat flies, which were all collected from *Glossophaga soricina* bats. Thalli were found all over the host integument; there appears to be no position specificity. *Gloeandromyces cusucoensis* is morphologically different from the other species and formae within the genus by the combination of the following characteristics: the conical shape of the perithecial tip, the pronounced constriction in the perithecial neck, and the upward broadening of the perithecial venter. These morphological differences can be subtle, especially when compared with *G. pageanus* f. *polymorphus* and *G. streblae*. These two species differ from *G. cusucoensis* by having one or two small bumps on their perithecial neck, lacking the constriction in the perithecial neck, and not having the pronounced conical shape of the perithecial tip. Host association can help to identify this species, as it thus far seems strictly associated with *T. uniformis*. In their overview of bat fly–associated Laboulbeniales, de Groot et al. (2020) incorrectly listed *T. uniformis* as a host of *G. streblae*.

Gloeandromyces diversiformis Blondelle, B. Santam. & Haelew., sp. nov. FIG. 2b
Index Fungorum IF900220

Typification: COSTA RICA. PROVINCIA DE PUNTARENAS: Parque National Piedras Blancas, "Laguna perdida" cave, 8.7265278N 83.1843056W, 19 Mar 2012, on male *Strebla wiedemanni* Kolenati 1856 (Diptera, Streblidae) collected from female *Desmodus rotundus* (Chiroptera, Phyllostomidae), *Thomas Hiller 2012CR364* (fly vial code), slide D. Haelew. 1831b (**holotype** at GENT, 4 adult thalli from left procoxa); ibid., slide D. Haelew. 1831a (**isotype** at GENT, 1 adult thallus from left mesocoxa), GenBank (isolate D. Haelew. 1831d, 4 adult thalli from left procoxa): 18S = OQ971688, 28S = OQ971591.

Etymology: Referring to the multiple morphotypes of this phylogenetic species, confirmed by molecular phylogenetic analysis.

Diagnosis: Different from the other species and formae in the genus by its long and straight cell I with almost parallel margins, the elongated basal cell of the appendage, and the indistinct transition from the perithecial venter toward the perithecial neck. Unique molecular autapomorphies and motifs in the 28S nuc rDNA at positions 53 (A), 58 (A), 80 (G), 106 (A), 164 (T), 260 (C), 406 (T) (insertion), 408 (C), 461 (G), 475–476 (GG) (insertion), 479 (C), 499 (G), 502 (A), 522 (G), 559–563 (5′-TTTAT-3′) (insertion), 703–724 (5′-TTTTTCCATTC ATGGAAGAAGA-3′) (insertion), and 732 (A).

Description: Thallus hyaline with a blackened foot; basal cell of the appendage rusty orange. Cell I 3.8× longer than broad, long, straight with almost parallel margins, giving it a trapezoid-like habitus, carrying cells II and VI. Cell II 1.2× broader than long, trapezoidal. Cell III 1.3× longer than broad, trapezoidal. Basal cell of the appendage 1.2× longer than broad, elongated, pentagonal, with margins slightly broadening distally, carrying two suprabasal cells, ending in antheridial cells. Cell VI 1.3× longer than broad, asymmetrical, lens-shaped, obliquely positioned between cells II and VII. Cell VII 1.2× longer than broad, turbinate, proximal end sometimes seemingly in contact with cell I. Perithecium 4.2× longer than broad; venter with subparallel margins; indistinct transition from the perithecial venter toward the perithecial neck; neck distinguished by narrowing, tapering to a rounded tip.

Measurements: Thallus (198.5–)246.7–286.8–342.3(–393.5) µm [16] from foot to perithecial tip. Cell I (72.7–)82.2–106.7–131.2(–168.8) × (16.9–)22.4–28.1–33.8(–39.6) µm [18]. Cell II (8.6–)10.7–13.9–17.1(–19.2) × (10.2–)12.8–16.8–20.8(–23.4) µm [17]. Cell III (8.9–)12.0–15.8–19.6(–21.6) × (8.0–)9.9–12.1–14.3(–14.8) µm [17]. Basal cell of the appendage (10.9–)11.4–13.1–14.8(–16.5) × (9.4–)9.9–10.9–11.9(–13.4) µm [17]. Cell VI (6.2–)8.8–13.2–17.6(–21.7) × (6.3–)7.1–10.6–14.1(–20.1) µm [17]. Cell VII (8.8–)10.0–12.5–15.0(–17.3) × (4.9–)7.5–10.7–13.9(–17.0) µm [15]. Perithecium (100.4–)122.8–153.4–184.0(–203.4) × (17.1–)29.4–35.9–42.4(–49.4) µm [19]. Ascospores not measured.

Additional specimens examined: COSTA RICA. PROVINCIA DE PUNTARENAS: Parque Nacional Piedras Blancas, "Laguna perdida" cave, 8.7265278N 83.1843056W, 19 Mar 2012, on male *S. wiedemanni* collected from female *D. rotundus, Thomas Hiller 2012CR364* (fly vial code), GenBank (isolate D. Haelew. 1833a, 2 adult thalli from left mesocoxa): 18S = OQ971689, 28S = OQ971592; ibid., on male *S. wiedemanni* collected from female *D. rotundus, Thomas Hiller 2012CR364* (fly vial code), slide D. Haelew. 1829a (GENT, 4 adult thalli, left procoxa); ibid., 23 Mar 2012, on male *S. wiedemanni* collected from female *D. rotundus, Thomas Hiller 2012CR439* (fly vial code), slides D. Haelew. 1835a (GENT, 1 adult thallus from left procoxa) and D. Haelew. 1835b (GENT, 2 adult thalli from left mesocoxa); Piedras

Blancas, cave 1, 8.809222N 83.239W, 8 Feb 2012, on male *S. wiedemanni* collected from female *D. rotundus, Thomas Hiller 2012CR155* (fly vial code), slide D. Haelew. 1851a (GENT, 1 adult thallus from left procoxa); ibid., on male *S. wiedemanni* collected from female *D. rotundus, Thomas Hiller 2012CR167* (fly vial code), slides D. Haelew. 1856a (GENT, 1 adult thallus from left procoxa), D. Haelew. 1856b (GENT, 2 adult thalli from left mesocoxa), and D. Haelew. 1856c (GENT, 1 adult thallus from median pleurotrochantinal lobe); ibid., on male *S. wiedemanni* collected from female *D. rotundus, Thomas Hiller 2012CR152* (fly vial code), slides D. Haelew. 1860a (GENT, 2 adult thalli from left procoxa), D. Haelew. 1860b (GENT, 1 adult thallus from median pleurotrochantine), and D. Haelew. 1860c (GENT, 1 adult thallus from head); ibid., on male *S. wiedemanni* collected from male *D. rotundus, Thomas Hiller 2012CR164* (fly vial code), slides D. Haelew. 1870a (GENT, 1 adult thallus from left mesocoxa), D. Haelew. 1870b (GENT, 1 adult thallus from left mesofemur), and D. Haelew. 1870c (GENT, 1 adult thallus from between left metacoxa and -trochanter); ibid., 27 Mar 2012, on female *S. wiedemanni* collected from male *D. rotundus, Thomas Hiller 2012CR486* (fly vial code), slide D. Haelew. 1863d (GENT, 2 adult thalli from left sternopleuron).

Hosts and distribution: On *Strebla wiedemanni* (Diptera, Streblidae) in and around Parque Nacional Piedras Blancas, Costa Rica.

Notes: Gloeandromyces diversiformis f. *diversiformis* is described based on 22 adult thalli. All thalli were removed from *Strebla wiedemanni* bat flies, which were all collected from *Desmodus rotundus* bats. Thalli of this morphotype were only found on the left ventral side (left pro-, meso-, or metacoxa and left mesofemur) of male bat flies. Only one female bat fly (D. Haelew. 1863) was found infected with this morphotype, occurring on the left sternopleuron, in addition to both *G. diversiformis* f. *musiformis* and *G. diversiformis* f. *vanillicarpiformis* on the left wing (see below). *Desmodus rotundus* is often highly infested by multiple bat flies, with a reported mean intensity of up to 9.7 flies per bat (Hiller et al. 2021; Patterson et al. 2007). As a result of this, random nonmating contacts may lead to unexpected infection patterns.

This morphotype is easily identified by the combination of the following characteristics: the long and straight cell I with almost parallel margins; the elongated basal cell of the appendage (BCA), and the indistinct transition from the perithecial venter toward the perithecial neck. It shares the elongated BCA with the two other morphotypes of *G. diversiformis* as well as with the two morphotypes of *G. verbekeniae*, whereas all other known species have a more flattened BCA. *Gloeandromyces diversiformis* f. *diversiformis* can be easily separated from the two morphotypes of *G. verbekeniae* by its overall habitus and longer total length (246.7–342.3 µm vs. 166.7–188.1 µm in *G. verbekeniae* f. *verbekeniae* vs. 169.9–197.8 µm in *G. verbekeniae* f. *inflexus*).

Gloeandromyces diversiformis* f. *musiformis Blondelle, B. Santam. & Haelew., forma nov. FIG. 2c
Index Fungorum IF900221

Typification: COSTA RICA. PROVINCIA DE PUNTARENAS: Piedras Blancas, cave 1, 8.809211N 83.238989W, 8 Feb 2012, on female *Strebla wiedemanni* Kolenati 1856 (Diptera, Streblidae) collected from female *Desmodus rotundus* (Chiroptera, Phyllostomidae), *Thomas Hiller 2012CR167* (fly vial code), slide D. Haelew. 1854c (**holotype** at GENT, 9 adult thalli from left wing M_{1+2} vein).

Etymology: Referring to the genus *Musa* (Musaceae, Zingiberales), because of the banana-like habitus of the thallus.

Diagnosis: Different from the other species and formae in the genus by the consistently banana-like curvature of the stout thalli toward the anterior side, the elongated basal cell of the appendage, and the indistinct transition from the perithecial venter toward the perithecial neck.

Description: Thallus hyaline with a blackened foot; basal cell of the appendage rusty orange. Cell I 2.5× longer than broad, broadening upward, the anterior margin strongly concave and the posterior margin convex, carrying cells II and VI. Cell II as long as broad, turbinate. Cell III 1.2× longer than broad, quadrangular to obovoid. Basal cell of the appendage 1.1 × longer than broad, elongated, cylindrical, carrying two suprabasal cells, ending in antheridial cells. Cell VI 1.4× longer than broad, irregular, lens-shaped, obliquely positioned between cells II and VII. Cell VII 1.9× longer than broad, turbinate, proximal end sometimes seemingly in contact with cell I. Perithecium 3.5× longer than broad; venter with subparallel margins, with the anterior margin straight and the posterior one convex; indistinct transition from the perithecial venter toward the perithecial neck; neck distinguished by narrowing, bent to anterior, tapering to a blunt tip.

Measurements: Thallus (137.3–)170.4–192.3–214.2 (–215.1) µm [11] from foot to perithecial tip. Cell I (51.5–)54.3–56.6–58.9(–59.9) × (20.9–)21.1–23.0–24.9(–26) µm [9]. Cell II (7.6–)8.0–10.3–12.6(–12.8) × (7.5–)8.7–10.6–12.5(–12.5) µm [5]. Cell III (7.8–)7.9–11.2–13.3(–13.7) × (6.8–)7.6–9.3–11(–11.6) µm [5]. Basal cell of the appendage (6.5–)8.1–9.7–11.3(–

11.4) × (6.7–)7.5–8.9–10.3(–10.2) µm [7]. Cell VI (8.7–)8.4–9.2–10.0(–10.1) × 5.8–6.7–7.5 µm [3]. Cell VII 10.6–12.6–14.6 × 8.9–10.3–11.7 µm [2]. Perithecium (75.6–)99.3–115.8–132.3(–123.4) × (19.6–)27.0–32.9–37.5 µm [11]. Ascospores not measured.

Additional specimens examined: COSTA RICA. PROVINCIA DE PUNTARENAS: Piedras Blancas, cave 1, 8.809211N 83.238989W, 8 Feb 2012, on female *S. wiedemanni* collected from female *D. rotundus, Thomas Hiller 2012CR167* (fly vial code), GenBank (isolate D. Haelew. 1854e, 3 adult thalli from middle left wing): 18S = OQ971690, 28S = OQ971593; ibid., 27 Mar 2012, on male *S. wiedemanni* collected from male *D. rotundus, Thomas Hiller 2012CR486* (fly vial code), slide D. Haelew. 1865a (GENT, several blackened foot cells from left wing C vein and around R_{2+3} to M_{1+2} veins); ibid., on female *S. wiedemanni* collected from male *D. rotundus, Thomas Hiller 2012CR486* (fly vial code), slide D. Haelew. 1863a (GENT, 2 adult thalli from left wing R_{2+3} vein).

Hosts and distribution: On *Strebla wiedemanni* (Diptera, Streblidae) in and around Parque Nacional Piedras Blancas, Costa Rica.

Notes: Gloeandromyces diversiformis f. *musiformis* is described based on 11 adult thalli. All thalli were removed from *Strebla wiedemanni* bat flies, which were all collected from *Desmodus rotundus* bats. Thalli of this morphotype have thus far only been found on the center of the left wing; position-induced morphological adaptations are at the basis of this morphotype (Haelewaters and Pfister 2019). One male bat fly (D. Haelew. 1865) was infected with blackened foot cells on the left wing around the R_{2+3} to M_{1+2} veins and at the C vein. These positions match those observed for f. *musiformis* and f. *vanillicarpiformis* (see below), respectively. The overall banana-like habitus, the elongated BCA, and the gradual tapering of the perithecium toward its blunt tip make this morphotype easily recognizable. It shares the elongated BCA with the two other morphotypes of *G. diversiformis* and with the two morphotypes of *G. verbekeniae*.

Gloeandromyces diversiformis* f. *vanillicarpiformis Blondelle, B. Santam. & Haelew., forma nov. FIG. 2d
Index Fungorum IF900222

Typification: COSTA RICA. PROVINCIA DE PUNTARENAS: Piedras Blancas, cave 1, 8.809211N 83.238989W, 8 Feb 2012, on female *Strebla wiedemanni* Kolenati 1856 (Diptera, Streblidae) collected from female *Desmodus rotundus* (Chiroptera, Phyllostomidae), *Thomas Hiller 2012CR167* (fly vial code), slide D. Haelew. 1854b (**holotype** at GENT, 1 adult thallus from left wing R_1 vein); ibid., slide D. Haelew. 1854a (**isotype** at GENT, 1 thallus from left wing C vein).

Etymology: Referring to the genus *Vanilla* (Orchidaceae, Asparagales) and Latin (carpellum + formis), because of the resemblance of the general habitus of the thallus to a vanilla pod.

Diagnosis: Different from the other species and formae in the genus by the elongated, slender habitus; the very long cell I; the straight perithecium; the elongated basal cell of the appendage; and the indistinct transition from the perithecial venter to the perithecial neck.

Description: Thallus hyaline with a blackened foot; basal cell of the appendage rusty orange. Cell I 5.6× longer than broad, slightly broadening upward, carrying cells II and VI. Cell II 1.5× broader than long, triangular. Cell III 1.5× longer than broad, trapezoidal, with the outer wall convex. Basal cell of the appendage 1.7× longer than broad, elongated, pentagonal, with margins slightly broadening distally, carrying two suprabasal cells, ending in antheridial cells. Cell VI 1.1× longer than broad, asymmetrical, lens-shaped, obliquely positioned between cells II and VII. Cell VII 1.4× longer than broad, turbinate. Perithecium 6× longer than broad; venter with subparallel margins; indistinct transition from the perithecial venter to the perithecial neck; neck gradually tapering to a rounded tip.

Measurements: Thallus 366.2–368.9–371.5 µm [2] from foot to perithecial tip. Cell I 168.3–170.8–173.2 × 28.7–30.8–32.8 µm [2]. Cell II 10.5–12.7–14.9 × 18.5–19.7–20.8 µm [2]. Cell III 12.4–14.7–16.9 × 8.5–10.0–11.5 µm [2]. Basal cell appendage 13.2–14.5–15.9 × 6.9–8.5–10.1 µm [2]. Cell VI 8.6–12.8–17.0 × 8.7–11.8–14.9 µm [2]. Cell VII 10.6–13.0–15.3 × 8.2–9.0–9.8 µm [2]. Perithecium 164.7–166.8–168.9 × 21.4–27.8–34.2 µm [2]. Ascospores not measured.

Additional specimens examined: COSTA RICA. PROVINCIA DE PUNTARENAS: Piedras Blancas, cave 1, 8.809211N 83.238989W, 8 Feb 2012, 27 Mar 2012, on male *S. wiedemanni* collected from male *D. rotundus, Thomas Hiller 2012CR486* (fly vial code), slide D. Haelew. 1865a (GENT, several blackened foot cells from left wing C vein and around R_{2+3} to M_{1+2} veins); ibid., 27 Mar 2012, on female *S. wiedemanni* collected from male *D. rotundus, Thomas Hiller 2012CR486* (fly vial code), GenBank (isolate D. Haelew. 1863b, 4 adult thalli from left wing C vein): 18S = OQ971691, 28S = OQ971594, GenBank (isolate D. Haelew. 1863c, 2 adult thalli from left wing C vein): 18S = OQ971692, 28S = OQ971595.

Hosts and distribution: On *Strebla wiedemanni* (Diptera, Streblidae) in and around Parque Nacional Piedras Blancas, Costa Rica.

Notes: Gloeandromyces diversiformis f. *vanillicarpiformis* is described based on 2 adult thalli. All thalli were removed from *Strebla wiedemanni* bat flies, which were all collected from *Desmodus rotundus* bats. Thalli of this morphotype have thus far only been removed from the C vein of the left wing. Again, position-induced morphological adaptations are at the basis of this morphotype (Haelewaters and Pfister 2019). One male bat fly (D. Haelew. 1865) was infected with blackened foot cells on the left wing around the R_{2+3} to M_{1+2} veins and along the C vein. These positions match those observed for the f. *musiformis* and f. *vanillicarpiformis* morphotypes, respectively. We think that the observed foot cells effectively represent those morphotypes. The other bat flies we have found to be infected with *G. diversiformis* f. *musiformis* and f. *vanillicarpiformis* were female; although much is still unknown about mating and mounting behavior in streblid bat flies, we hypothesize that male bat flies may be occasionally infected as a result of same-sex mounting. This would be similar to the explanation Goldmann and Weir (2012) provided for observed infection patterns of *Chitonomyces* spp. on male *Laccophilus maculosus* water beetles. *Gloeandromyces diversiformis* f. *vanillicarpiformis* is easily identifiable by its elongated, slender habitus, in combination with the very long cell I, the straight perithecium, and the elongated BCA.

We note that although the description is based on just 2 adult thalli, 6 more adult thalli were observed. These thalli were found on the same position of the host (left wing C vein) and showed the same slender, vanilla pod-like habitus. These additional 6 thalli were used for DNA extractions toward the beginning of this study before we became aware that this taxon is only scarcely present on *S. wiedemanni*. It not exceptional that Laboulbeniales taxa are described based on minimal material. Other examples include *Bordea gigantea* R.K. Benj. (based on 2 adult thalli and 1 receptacle with appendage), *Camptomyces africanus* W. Rossi & M Leonardi (3 adult thalli and 3 juvenile ones), *Laboulbenia longipilis* Haelew. & W. Rossi (2 adult thalli), and *L. otongaensis* W. Rossi (2 adult thalli and 6 juvenile ones). Decisions to publish these descriptions are often based on obvious morphological characteristics that easily separate the new taxa from previously described ones (Benjamin 2001; Haelewaters and Rossi 2015; Rossi 2011; Rossi and Leonardi 2018); rightfully so, as alpha-taxonomy remains critical to build a more complete picture of the group (Haelewaters et al. 2021c). The difference with our material from *S. wiedemanni* is that in addition to distinguishing morphological features, we were also able to generate molecular data to confirm that it represents a morphotype belonging to the phylogenetic species *G. diversiformis*.

Gloeandromyces plesiosaurus Van Caenegem & Haelew., sp. nov. FIGS. 2f, 4b

Index Fungorum IF900223

Typification: PANAMA. PROVINCIA DE COLÓN: El Giral, Agua Salud Fragment 2, 9.2255N 79.7123889W, 14 Apr 2015, on male *Trichobius yunkeri* Wenzel 1966 (Diptera, Streblidae) collected from adult female *Pteronotus parnellii* (Chiroptera, Mormoopidae), *Thomas Hiller P_3929* (fly vial ID), slide D. Haelew. 1743a (**holotype** GENT:GENTFL00080, 4 adult thalli from right metacoxa).

Etymology: Referring to the resemblance of the thallus to †*Plesiosaurus* (†Plesiosauridae, †Plesiosauria), a genus of extinct aquatic reptiles.

Diagnosis: Different from the other species and formae of the genus by the elongated and curved cell I and the bulging posterior bump on the perithecial venter. Unique molecular autapomorphies in the 28S nuc rDNA at positions 60 (C), 107 (T), and 409 (G).

Description: Thallus hyaline with a blackened foot; basal cell of the appendage rusty orange. Cell I 5.0× longer than broad, slightly broadening upward, lower half curved toward the posterior side, carrying cell II. Cell II 1.2× broader than long, quadrangular or pentagonal. Cell III 1.5× broader than long, irregularly quadrangular, with the outer wall convex. Basal cell of the appendage 1.4× broader than long, flattened, rectangular to pentagonal, carrying two suprabasal cells, ending in antheridial cells. Cell VI 1.9× longer than broad, ellipsoid, obliquely positioned between cells II and VII. Cell VII 1.3× longer than broad, dome-shaped. Perithecium 3.6× longer than broad; venter slightly broadening upward, with four irregular bumps at the distal end, three smaller bumps that are not always well defined, and one larger bump bulging posteriorly; neck narrower than the venter, curved toward posterior, slightly constricted in the middle; tapering to an asymmetrical conical tip.

Measurements: Thallus (199.9–)224.1–251.4–278.7(–279.7) µm from foot to perithecial tip [12]. Cell I (72.6–)83.2–96.1–109.0(–112.2) × (14.6–)16.7–19.0–21.2(–22.2) µm [12]. Cell II (10.7–)11.7–13.1–14.5(–15.1) × (9.1–)12.7–15.1–17.5(–17.9) µm [12]. Cell III (6.9–)7.3–8.5–9.6(–10.2) × (9.8–)10.8–12.5–14.2(–15.6) µm [12]. Basal cell of the appendage (7.1–)7.6–9.2–10.7(–12.5) × (11.1–)12.3–12.9–13.5 µm [12]. Cell VI (11.1–)13.5–15.0–16.5(–17.1) × (5.8–)6.5–8.2–9.9(–10.9) µm [12]. Cell VII (11.9–)12.2–13.3–14.4(–14.9) × (8.4–)9.1–

10.5–12.0(–12.8) µm [10]. Perithecium (101.0–)151.6–127.7–139.7(–139.8) × (29.6–)32.4–35.5–38.2(–39.4) µm [12]. Ascospores 19.4–21.5–23.7(–24.9) × 3.2–3.8–4.3(–4.9) µm, up to (4.1–)4.6–5.4–6.2(–6.6) µm wide including slime sheath [7].

Additional specimens examined: PANAMA. PROVINCIA DE COLÓN: Tres Almendras Islands, 9.205363N 79.84853W, 31 Mar 2014, on male *T. yunkeri* collected from male *P. parnellii, Thomas Hiller P_0741* (fly vial code), slide D. Haelew. 3282a (GENT:GENTFL00108, 2 adult thalli from right metacoxa), GenBank (isolate D. Haelew. 3282b, 1 adult thallus from right metacoxa): 18S = OQ971699; PROVINCIA DE VERAGUAS: Parque Nacional Coiba, 7.468805406N 81.75477188W, 8 Feb 2015, on male *T. yunkeri* collected from adult female *P. parnellii, Thomas Hiller P_1942* (fly vial code), slide D. Haelew. 3314a (GENT:GENTFL00150, 1 adult thallus from right metacoxa); ibid., 9 Feb 2015, on male *T. yunkeri* collected from adult male *P. parnellii, Thomas Hiller P_1165* (fly vial code), slide D. Haelew. 3310a (GENT:GENTFL00145, 5 adult thalli from right metafemur), GenBank (isolate D. Haelew. 3310d, 2 adult thalli from right metafemur): 18S = OQ971695, 28S = OQ971600; ibid., 21 Feb 2015, on male *T. yunkeri* collected from adult female *P. parnellii, Thomas Hiller P_3080* (fly vial code), slide D. Haelew. 3281a (GENT:GENTFL00107, 1 adult thallus from right mesofemur).

Hosts and distribution: On *Trichobius yunkeri* (Diptera, Streblidae) in the provinces of Chiriquí and Colón, Panama.

Notes: Gloeandromyces plesiosaurus f. *plesiosaurus* is described based on 13 adult thalli. All thalli were removed from male *Trichobius yunkeri* bat flies, which were all collected from *Pteronotus parnellii* bats. Thalli of this morphotype were only found on the right metacoxae and the right meso- and metafemora of male bat flies and are therefore thought to be position-specific. Adult thalli from slides D. Haelew. 3281a and D. Haelew. 3314a lack a visible larger perithecial bump pointing to posterior. It seems that this particular bump is not always clearly visible—or perhaps it is a variable characteristic. This species is morphologically different from the other species and formae within the genus by the elongated and curved cell I, carrying only cell II, and the posteriorly bulging bump on the perithecial venter.

Gloeandromyces plesiosaurus* f. *asymmetricus Van Caenegem & Haelew., forma nov. FIGS. 2e, 4c, 4d
Index Fungorum IF900224

Typification: PANAMA. PROVINCIA DE VERAGUAS: Parque Nacional Coiba, 7.468805406N 81.75477188W, 8 Feb 2015, on female *Trichobius yunkeri* Wenzel 1966 (Diptera, Streblidae) collected from adult male *Pteronotus parnellii* (Chiroptera, Mormoopidae), *Thomas Hiller P_1940* (parasite_ID), slide D. Haelew. 3311a (**holotype** at GENT, 2 adult thalli from left wing R_4 vein); ibid., slide D. Haelew. 3311b (**isotype** GENT:GENTFL00147, 2 juvenile thalli and 1 subadult thallus from left wing R_5 vein).

Etymology: Referring to the asymmetrical tip of the perithecium.

Diagnosis: Different from the other species and formae in the genus by the stout habitus, the bulging posterior bump on the perithecial venter, and the conspicuously asymmetrical tip of the perithecium.

Description: Thallus hyaline with a blackened foot; basal cell of the appendage rusty orange. Cell I 2.3× longer than broad, broadening upward, slightly curved toward the anterior side; slightly constricted at the proximal end, giving it a bulbous habitus; carrying cells II and VI. Cell II 1.2× broader than long, trapezoidal. Cell III 1.5× broader than long, irregularly quadrangular, with the outer wall convex. Basal cell of the appendage 1.4× broader than long, flattened, rectangular to pentagonal, carrying two suprabasal cells, ending in antheridial cells. Cell VI 1.7× longer than broad, broadly ellipsoid, obliquely positioned between cells II and VII. Cell VII 1.4× longer than broad, asymmetrically dome-shaped. Cell n′ bulging outward, protruding between the basal cells of the perithecium and cell VII. Perithecium 3.3× longer than broad; venter broadening upward, with one bump at the distal end bulging posteriorly; a rather indistinct transition from the perithecial venter toward the perithecial neck; neck distinguished by narrowing, tapering to a conical tip pointing anteriorly.

Measurements: Thallus (179.9–)181.8–191.9–202.1(–209.4) µm from foot to perithecial tip [7]. Cell I (44.5–)46.2–52.1–58.1(–59.2) × (20.0–)20.2–22.3–24.5(–26.0) µm [7]. Cell II (7.5–)9.0–11.2–13.4 × 12.6–13.6–14.6(–15.5) µm [7]. Cell III (7.9–)8.7–9.8–10.8(–11.1) × (10.6–)12.6–14.5–16.5(–16.7) µm [7]. Basal cell of the appendage (6.7–)6.8–8.0–9.2(–10.3) × (10.3–)10.7–11.4–12.0(–12.2) µm [9]. Cell VI (14.5–)15.2–16.3–17.4(–17.8) × (7.2–)8.0–10.2–12.5(–14.1) µm [7]. Cell VII (11.7–)12.3–14.3–16.3(–17.7) × (8.8–)9.2–10.6–11.9(–12.8) µm [6]. Perithecium (115.7–)116.6–120.6–124.6(–127.4) × 34.8–36.9–39.1(–40.0) µm [7]. Ascospores (25.0–)25.8–27.0–28.2(–29.6) × (3.1–)3.4–3.8–4.1(–4.2) µm, up to (4.4–)4.9–5.7–6.5(–6.9) µm wide including the slime sheath [14].

Additional specimens examined: PANAMA. PROVINCIA DE COLÓN: Isla Chicha, 9.195035N 79.86257W, 23 Apr 2014, on female *T. yunkeri* collected from adult female *P. parnellii, Thomas Hiller WP1_0334* (fly vial code), slide D. Haelew. 3317a (GENT:GENTFL00153, multiple thalli from left wing R_4 and M_4

+ Cu_1 veins); PROVINCIA DE PANAMÁ OESTE: Isla Barro Colorado, 9.164459N 79.84811W, 19 Apr 2014, on female *T. yunkeri* collected from adult female *P. parnellii, Thomas Hiller WP1_344* (fly vial code), slides D. Haelew. 1067a (FH, 2 juvenile thalli and 1 adult thallus from left wing), erroneously identified as *G. streblae* in Haelewaters et al. (2017), and D. Haelew. 1067b (GENT: GENTFL00015, 1 subadult thallus from left wing); PROVINCIA DE VERAGUAS: Parque Nacional Coiba, Playa Escondido, 7.468805406N 81.75477188W, 21 Feb 2015, on male *T. yunkeri* collected from adult female *P. parnellii, Thomas Hiller P_3027* (fly vial code), slide D. Haelew. 3296a (GENT:GENTFL00122, 2 juvenile thalli from left wing R_4 vein); ibid., 8 Feb 2015, on *T. yunkeri* collected from adult female *P. parnellii, Thomas Hiller P_1073* (fly vial code), slide D. Haelew. 3299a (GENT: GENTFL00127, 1 adult thallus from right wing R_4 vein); ibid., on female *T. yunkeri* collected from adult female *P. parnellii, Thomas Hiller P_1079* (fly vial code), slide D. Haelew. 3306a (GENT:GENTFL00141, 1 juvenile thallus and 1 subadult thallus from left wing); ibid., on male *T. yunkeri* collected from adult female *Pteronotus parnellii, Thomas Hiller P_1079* (fly vial code), slide D. Haelew. 3307a (GENT:GENTFL00142, 1 subadult thallus from left wing); ibid., on male *T. yunkeri* collected from adult female *P. parnellii, Thomas Hiller P_1079* (fly vial code), slide D. Haelew. 3308a (GENT:GENTFL00143, 1 subadult thallus from right wing); ibid., on male *T. yunkeri* collected from adult female *P. parnellii, Thomas Hiller P_1079* (fly vial code), slide D. Haelew. 3309a (GENT:GENTFL00144, 3 adult thalli from left wing R_5 vein); ibid., on female *T. yunkeri* collected from adult female *P. parnellii, Thomas Hiller P_1942* (fly vial code), slide D. Haelew. 3312a (GENT:GENTFL00148, 2 subadult thalli and 1 adult thallus from right wing R_3 vein); ibid., on female *T. yunkeri* collected from adult female *P. parnellii, Thomas Hiller P_1942* (fly vial code), slide D. Haelew. 3313a (GENT:GENTFL00149, 1 subadult thallus from right wing R_3 vein); ibid., on female *T. yunkeri* collected from adult female *P. parnellii, Thomas Hiller P_1942* (fly vial code), slides D. Haelew. 3315a (GENT:GENTFL00151, 1 juvenile thallus from right wing R_3 vein) and D. Haelew. 3315b (GENT:GENTFL00152, 1 subadult thallus from left wing R_4 vein).

Hosts and distribution: On *Trichobius yunkeri* (Diptera, Streblidae) in the provinces of Chiriquí, Colón, and Panamá Oeste, Panama.

Notes: Gloeandromyces plesiosaurus f. *asymmetricus* is described based on 8 adult and 9 subadult thalli. All thalli were removed from *Trichobius yunkeri* bat flies, which were all collected from *Pteronotus parnellii* bats. Thalli of this morphotype were only found on the wings of both female and male bat flies. This species is morphologically different from the other species and formae of the genus by its stout habitus, the presence of one posterior bump at the distal end of the perithecial venter, and the asymmetrical perithecial tip pointing anteriorly. The perithecial bump is only visible in adult thalli but cannot always be clearly observed. The asymmetrical perithecial tip is the main characteristic of this morphotype; it can already be observed in juvenile thalli. Haelewaters et al. (2017) erroneously reported *G. streblae* on *Trichobius yunkeri* (slide D. Haelew. 1067a), and this record was included in the worldwide overview of bat fly–associated Laboulbeniales by de Groot et al. (2020). After reexamination of the thalli, we observed the distinct asymmetrical perithecial tip and concluded that the correct identification is *G. plesiosaraurus* f. *asymmetricus*. We were unable to successfully sequence this taxon. It remains a hypothesis that this is a morphotype of *G. plesiosaurus*.

Gloeandromyces pseudodickii Blondelle & Haelew., sp. nov. FIG. 2g

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Typification: ECUADOR. PROVINCIA DE ESMERALDAS: San Francisco de Bogota, settlement 25, 1.0876944N 78.6915W, 74 m a.s.l., 3 Aug 2004, on female *Trichobius longipes* (Rudow 1871) (Diptera, Streblidae) collected from female *Phyllostomus hastatus* (Chiroptera, Phyllostomidae), *Carl W. Dick TK 135586* (fly vial code), slide D. Haelew. 3143a (**holotype** at GENT, 3 adult thalli from abdomen under right metaleg).

Etymology: Referring to the striking resemblance to its sister species *Gloeandromyces dickii*.

Diagnosis: Different from *Gloeandromyces dickii* by its slenderer perithecial venter and the consistently shorter, more tapering perithecial projection as opposed to the finger-like projection of *G. dickii*. Unique molecular autapomorphies in the 28S nuc rDNA at positions 59 (C), 442 (C), and 755 (A). Unique molecular synapomorphy in the *TEF1* at position 396 (T).

Description: Thallus pale orange with a lightly colored foot; distal part of cells III, VI, and VII tinged with orange; basal cell of the appendage rusty orange. Cell I 3.2× longer than broad, slightly broadening upward, slightly curved toward the anterior side, carrying cells II and VI. Cell II as long as broad, quadrangular. Cell III 1.2× longer than broad, trapezoidal, with the outer wall convex. Basal cell of the appendage 1.2× broader than long, pentagonal, with margins slightly broadening upward, carrying two suprabasal cells, ending in antheridial cells. Cell VI 1.6× longer than broad, broadly lens-shaped. Cell VII 1.2× longer than broad, turbinate, with slightly convex outer

margin, proximal end almost in contact with cell I. Perithecium 3.2× longer than broad, bearing a small bulge at its base; venter ovoid, bearing halfway along its length a tapering projection pointing to anterior; neck abruptly distinguished by narrowing, strongly bent to anterior, slightly broadening toward the tip, with two posterior bumps.

Measurements: Thallus (241.5–)268.9–296.9–324.9(–363.7) µm from foot to perithecial tip [24]. Cell I (70.5–)77.1–93.0–108.8(–133.2) × (24.6–)25.9–29.3–32.6(–39.8) µm [24]. Cell II (14.4–)16.2–19.0–21.9(–23.3) × (15.4–)16.9–20.2–23.5(–26.3) µm [23]. Cell III (15.7–)16.4–18.6–20.8(–25.0) × (12.5–)14.1–16.1–18.0(–22.3) µm [23]. Basal cell of the appendage (10.9–)11.6–13.4–15.2(–17.8) × (13.0–)14.1–15.9–17.6(–21.2) µm [21]. Cell VI (17.3–)19.3–21.6–23.9(–25.7) × (10.5–)11.5–13.3–15.1(–18.5) µm [22]. Cell VII (12.8–)16.1–18.2–20.3(–22.1) × (10.3–)13.3–15.8–18.3(–22.6) µm [20]. Perithecium (141.5–)164.8–179.6–194.5(–202.2) [24] × (47.3–)51.3–55.6–59.9(–61.7) µm [23]. Tapering projection (26.9–)47.2–57.3–67.3(–73.9) µm long [23]. Ascospores (26.1–)29.3–31.6–34.0(–35.0) × (3.4–)3.8–4.4–4.9(–5.5) µm, up to (5.4–)6.5–7.5–8.6(–9.4) µm wide including the slime sheath [29].

Measurements, deviant thalli: Thallus (153.3–)167.0–177.3–187.7(–188.3) µm from foot to perithecial tip [11]. Cell I (47.4–)51.5–56.1–60.7(–62.9) × (18.0–)18.5–19.5–20.5(–21.1) µm [11]. Cell II (10.7–)11.0–12.3–13.5(–14.7) × (10.4–)10.8–11.6–12.3(–12.7) µm [11]. Cell III (12.9–)12.8–14.3–15.8(–18.4) × (11.0–)11.3–12.4–13.4(–14.5) µm [11]. Basal cell of the appendage (10.5–)11.1–12.0–12.9(–13.0) × (11.3–)12.0–13.3–14.6(–15.0) µm [7]. Cell VI (12.4–)12.3–13.8–15.3(–16.7) × (5.7–)6.6–7.9–9.2(–10.7) µm [11]. Cell VII (10.0–)10.2–11.7–13.1(–14.5) × (9.4–)9.6–10.6–11.5(–12.4) µm [11]. Perithecium (81.8–)95.1–104.2–113.4(–112.9) × (21.4)–25.6–30.0–34.5(–37.2) µm [11]. Ascospores not measured.

Additional specimens examined: ECUADOR. PROVINCIA DE ESMERALDAS: San Francisco de Bogota, settlement 25, 1.0876944N 78.6915W, 74 m a.s.l., 3 Aug 2004, on female *T. longipes* collected from female *P. hastatus, Carl W. Dick TK 135586* (fly vial code), slide D. Haelew. 3141b (GENT, 1 adult thallus from abdomen under right metaleg); ibid., on female *T. longipes* collected from female *P. hastatus, Carl W. Dick TK 135586* (fly vial code), slides D. Haelew. 3142a (GENT, 5 adult thalli from abdomen under right metaleg) and D. Haelew. 3142b (GENT, 4 adult thalli from abdomen under right metaleg); ibid., 6 Aug 2004, on female *T. longipes* collected from female *P. hastatus, Carl W. Dick TK 135682* (fly vial code), slide D. Haelew. 1042a (FH 00313693, 7 adult thalli from anterior ventral abdomen); ibid., on female *T. longipes* collected from female *P. hastatus, Carl W. Dick TK 135682* (fly vial code), slide D. Haelew. 1043a (FH 00313694, 6 adult thalli from anterior ventral right-hand side of abdomen); ibid., on female *T. longipes* collected from male *P. hastatus, Carl W. Dick TK 135681* (fly vial code), slide D. Haelew. 3145b (GENT, 2 adult thalli from abdomen under right metaleg). PANAMA. PROVINCIA DE PANAMÁ OESTE: Isla Barro Colorado, *P. hastatus* roost, 18 Oct 2013, on female *T. longipes* collected from female *P. hastatus, Thomas Hiller P_0324* (fly vial code), slides D. Haelew. 3417f (destroyed during processing, 2 adult thalli from ventral right-hand side of abdomen), D. Haelew. 3417g (GENT: GENTFL00195, 1 adult thallus from ventral right-hand side of abdomen), and D. Haelew. 3417h (GENT: GENTFL00196, 3 adult thalli from dorsal right-hand side of abdomen), GenBank (isolate D. Haelew. 3417k, 1 adult thallus from dorsal right-hand side of abdomen): 18S = OQ971696, GenBank (isolate D. Haelew. 3417 l, 1 adult thallus from ventral right-hand side of abdomen): 18S = OQ971697, 28S = OQ971601, *TEF1* = OQ117043.

Hosts and distribution: On *Trichobius longipes* (Diptera, Streblidae) in northern Ecuador and on Isla Barro Colorado, Panama.

Notes: Gloeandromyces pseudodickii is described based on 34 adult thalli. All thalli were removed from *Trichobius longipes* bat flies, which were all collected from *Phyllostomus hastatus* bats. Thalli of this species were only found on the right-hand side of the abdomen of female bat flies. *Gloeandromyces dickii* is the only known species in the genus that could be confused with *G. pseudodickii*, hence the name. Morphologically, they are very similar, but *G. pseudodickii* differs from *G. dickii* by the combination of the following characters: the outer margin of cell VII is slightly convex (rather than conspicuously bulging outward as in *G. dickii*), the base of the perithecium carries a small bulge (rather than a conspicuous, rounded bulge), the perithecium is slender (rather than broadly ovoid), and the perithecial projection is slender and tapering (rather than finger-like). Molecular phylogenetic data confirm that *G. dickii* and *G. pseudodickii* are sister species (FIG. 1). Both species are segregated by host species: *Gloeandromyces dickii* is only found on *Trichobius joblingi* bat flies, whereas *G. pseudodickii* is only found on *T. longipes* hosts.

Some thalli from slides D. Haelew. 1043a, D. Haelew. 3417h, and D. Haelew. 3417g had a singular bump positioned at the distal third of the venter, pointing anteriorly. This bump is also observed in *G. dickii* (Haelewaters and Pfister 2019: Fig. 3a), but the bump is more pronounced and always present in the latter species. Thalli from slides D. Haelew. 3141b, D. Haelew. 3142a, and D. Haelew.

Figure 3. New species of *Gloeandromyces*. a. *Gloeandromyces verbekeniae* f. *inflexus*, slide GENT:GENTFL00222. b. *Gloeandromyces verbekeniae* f. *verbekeniae*, slide GENT:GENTFL00078 (holotype). Bars = 100 µm.

3142b were deviant from the holotype, in being remarkably smaller overall and having an underdeveloped perithecial projection. We included their measurements separately. The total length from foot to perithecial tip of these thalli varied between 167.0 and 187.7 µm, making them 1.4–1.9× smaller compared with the majority of observed thalli. These deviating thalli also lack the characteristic bumps on the perithecial tip that are present in the larger thalli, and cell VII appears to be less bulging. Although we were unable to obtain sequences for this material, we think that these deviant thalli are the result of phenotypic plasticity. Evidence for this comes from two sources: (i) Aside from the aforementioned morphological differences, the proportions (i.e., length/width ratios) of the cells and measured structures of both forms are near identical; and (ii) the deviant thalli were found on the exact same position on the same host species and even on the same bat fly specimens. Such phenotypic plasticity was earlier reported for *G. dickii, G. hilleri*, and *G. pageanus* f. *polymorphus* (Haelewaters and Pfister 2019; Liu et al. 2020).

Five hosts (D. Haelew. 1042, D. Haelew. 3141, D. Haelew. 3142, D. Haelew. 3143, D. Haelew. 3145) carried a double infection of *G. pseudodickii* and *Nycteromyces* sp. indet. On one bat fly (D. Haelew. 3417), we observed a triple infection of *G. pseudodickii* on the right-hand side of the abdomen, *G. streblae* f. *streblae* on the wings, and *Nycteromyces* sp. indet. on the right metafemur. The thalli of *Nycteromyces* Thaxt. from *T. longipes* may represent a species other than *N. streblidinus* Thaxt. (W. Van Caenegem and D. Haelewaters, unpubl. data), but species delimitation within the genus *Nycteromyces* will be addressed in another paper. Finally, in the notes section of *G. dickii*, Haelewaters and Pfister (2019) reported morphologically deviant thalli removed from *T. longipes* bat flies (slides FH 00313693 and FH 00313694). We are certain that this material belongs to the newly described *G. pseudodickii* and included the slides in the type series of this species.

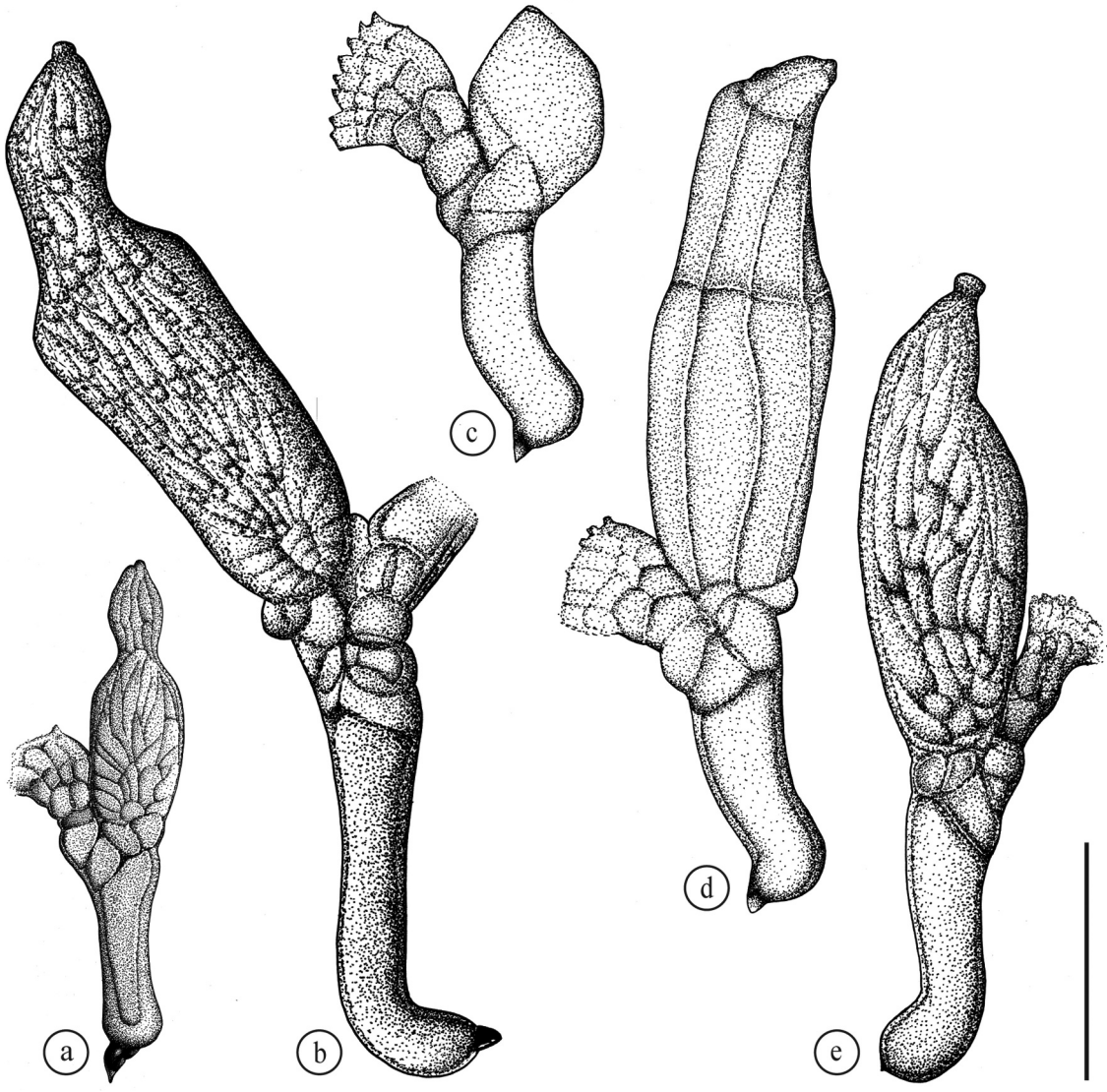

Figure 4. New species of *Gloeandromyces*; del. Jingyu Liu. a. *Gloeandromyces cusucoensis*, subadult thallus, slide D. Haelew. 3194d. b. *Gloeandromyces plesiosaurus* f. *plesiosaurus*, adult thallus with a damaged appendage, slide GENT:GENTFL00108. c. *Gloeandromyces plesiosaurus* f. *asymmetricus*, juvenile thallus, slide GENT:GENTFL00147 (isotype). d. *Gloeandromyces plesiosaurus* f. *asymmetricus*, adult thallus with focus on perithecial wall cells, slide GENT:GENTFL00148. e. *Gloeandromyces verbekeniae* f. *verbekeniae*, slide GENT:GENTFL00078 (holotype). Bars = 50 µm.

Gloeandromyces verbekeniae Van Caenegem & Haelew., sp. nov. FIGS. 3b, 4e
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Typification: PANAMA. PROVINCIA DE COLÓN: Tres Almendras Islands, 9.205363N 79.84853W, 11 Feb 2017, on male *Strebla galindoi* Wenzel 1966 (Diptera, Streblidae) collected from adult male of *Tonatia saurophila* (Chiroptera, Phyllostomidae), *Thomas Hiller P_3596* (fly vial code), slide D. Haelew. 1741a (**holotype** GENT:GENTFL00078, 2 adult thalli from base of right wing).

Etymology: Referring to Prof. Dr. Annemieke Verbeken, professor at Ghent University and head of the Research Group Mycology.

Diagnosis: Different from the other species and formae in the genus by the elongated basal cell of the appendage and the large, tooth-shaped bump at the perithecial tip. Unique molecular autapomorphies and motifs in the 28S nuc rDNA at positions 56 (A) (insertion), 99 (A), 129 (G), 157 (T), 168 (A) (insertion), 181 (C), 260 (T), 329 (G), 397–405 (TTTTTTTTT) (insertion), 427 (A), 474 (G), 500 (A), 514 (A) (insertion), 536 (T), 556–558 (CAT), 565 (G), 598 (A), 701 (G), and 758

(A). Unique molecular synapomorphies in the *TEF1* at positions 112 (C), 129 (C), 137 (G), 142 (A), 195–196 (CT), 198 (G), 225 (C), 255 (C), 273 (C), 288 (C), 346 (A), 354 (A), 363 (C), 432 (A), 456 (A), 558 (C), 573 (C), 592 (C), 597 (T), 603 (C), and 606 (T).

Description: Thallus hyaline with a blackened foot; basal cell of the appendage rusty orange. Cell I 2.8× longer than broad, broadening upward, curved toward the anterior side, carrying cells II, VI, and VII. Cell II 1.4× broader than long, trapezoidal or triangular. Cell III 1.4× longer than broad, trapezoidal. Basal cell of the appendage 1.7× longer than broad, conspicuously elongated; pentagonal, with margins slightly broadening distally; carrying two suprabasal cells, ending in antheridial cells. Cell VI 1.6× longer than broad, asymmetrical, lens-shaped, obliquely positioned between cells II and VII. Cell VII 1.1× longer than broad, slightly turbinate. Perithecium 3.0× longer than broad; venter slightly broadening upward, transitioning indistinctly toward the perithecial neck; neck distinguished by slight narrowing, tapering to a subulate tip carrying a large, tooth-shaped bump.

Measurements: Thallus (164.7–)166.7–177.4–188.1(–188.2) µm [4] from foot to perithecial tip. Cell I (56.1–)55.9–59.2–62.6(–64.0) × 18.9––21.1–23.7(–24.7) µm [5]. Cell II (10.8–)11.0–12.2–13.3(–13.4) × 15.6––17.0–18.6(–18.9) µm [5]. Cell III (13.1–)13.9–15.7–17.4(–17.6) × 10.3–11.6–13.1(–13.9) µm [5]. Basal cell of the appendage (13.4–)14.4–15.8–16.9 × (8.5–)8.7–9.5–10.3(–10.5) µm [5]. Cell VI (11.2–)11.8–12.6–13.4 × (6.4–)6.6–7.9–9.2(–9.8) µm [5]. Cell VII (10.9–)11.2–12.4–13.7 × 10.1–11.1–12.3(–13.0) µm [5]. Perithecium 90.1–97.4–104.6(–107.4) × 31.3–32.9–34.6 µm [4]. Ascospores (34.7–)35.5–37.3–38.9 × 4.2–4.6–4.9 µm, with a slime sheath [5].

Additional specimens examined: PANAMA. PROVINCIA DE COLÓN: Tres Almendras Islands, 9.205363N 79.84853W, 11 Feb 2017, on male *S. galindoi* collected from adult male *T. saurophila, Thomas Hiller P_3596* (fly vial code), GenBank (isolate D. Haelew. 1741b, 2 adult thalli from base of right wing): 18S = OQ971698, 28S = OQ971602, *TEF1* = OQ969950; PROVINCIA DE PANAMÁ OESTE: La Chorrera District, Peña Blanca, 9.153871N 79.88642W, 1 Feb 2017, on male *S. galindoi* collected from adult male *T. saurophila, Thomas Hiller P_3212* (fly vial code), slide D. Haelew. 1739a (GENT:GENTFL00077, 1 subadult thallus and 1 adult thallus from base of right wing); Isla Barro Colorado, Lutz Tower, 16 Nov 2013, on male *S. galindoi* collected from male *T. saurophila, Thomas Hiller P_0342* (fly vial code), slide D. Haelew. 3407a (GENT:GENTFL00181, 1 adult thallus from base of right wing).

Hosts and distribution: On *Strebla galindoi* (Diptera, Streblidae) on and around Isla Barro Colorado, Central Panama.

Notes: Gloeandromyces verbekeniae f. *verbekeniae* is described based on 4 adult thalli and 1 subadult thallus. All thalli were removed from *Strebla galindoi* bat flies, which were all collected from *Tonatia saurophila* bats. Thalli of this morphotype were only found on the base of the right wing of male bat flies. This morphotype is morphologically different from the other species and formae within the genus by its elongated BCA and the large, tooth-shaped bump of the perithecial tip. *Gloeandromyces verbekeniae* f. *verbekeniae* was reported by de Groot et al. (2020) as *Gloeandromyces*, sp. nov.

Gloeandromyces verbekeniae* f. *inflexus Van Caenegem & Haelew., forma nov. FIG. 3a
Index Fungorum IF900227

Typification: ECUADOR. PROVINCIA DE ESMERALDAS: La Chiquita, experimental station, settlement 22, 1.2320N 78.76603W, 5 Aug 2004, on female of *Strebla galindoi* Wenzel 1966 (Diptera, Streblidae) collected from a male *Tonatia saurophila* (Chiroptera, Phyllostomidae), *Carl W. Dick TK 135608* (fly vial code), slide D. Haelew. 3165a (**holotype** at GENT, 3 adult thalli from right wing C vein); ibid., slide D. Haelew. 3165b (**isotype** at GENT, 1 adult thallus from right wing C vein).

Etymology: Referring to the pronounced kink of the perithecial neck.

Diagnosis: Different from the other species and formae in the genus by its elongated basal cell of the appendage, the pronounced kink of the perithecial neck toward posterior, and the flattened perithecial tip.

Description: Thallus hyaline with a blackened foot; basal cell of the appendage rusty orange. Cell I 2.6× longer than broad, broadening upward, carrying cells II, VI, and VII. Cell II 1.2× broader than long, triangular. Cell III 1.4× longer than broad, trapezoidal or triangular. Basal cell of the appendage 1.4× longer than broad, elongated, pentagonal, with margins slightly broadening distally; carrying two suprabasal cells, ending in antheridial cells. Cell VI 1.8× longer than broad, asymmetrical, lens-shaped, obliquely positioned between cells II and VII. Cell VII 1.3× longer than broad, turbinate. Perithecium 3.6× longer than broad; venter with subparallel margins, indistinctly transitioning toward the perithecial neck; neck distinguished by narrowing, strongly curved to posterior, tapering toward a flattened tip.

Measurements: Thallus (159.7–)169.9–183.8–197.8(–200.7) µm [12] from foot to perithecial tip. Cell I (48.9–)50.9–54.6–58.2(–59.6) × (15.2–)18.3–21.1–24.0

(–25.5) µm [12]. Cell II (10.8–)11.4–13.5–15.7(–17.2) × (9.1–)9.8–11.9–13.9(–15.5) µm [12]. Cell III (13.2–)14.8–15.9–16.9(–17.2) × (9.1–)10.0–11.2–12.3(–13.2) µm [12]. Basal cell of the appendage (14.1–)14.6–16.4–18.2(–19.5) × (9.9–)10.2–11.3–12.5(–13.2) µm [12]. Cell VI (9.9–)11.2–12.0–12.9(–13.1) × (4.9–)5.7–6.7–7.6(–8.7) µm [12]. Cell VII (9.3–)10.6–11.8–13.0(–13.5) × (7.1–)7.9–9.1–10.2(–10.5) µm [12]. Perithecium (99.6–)104.8–112.9–121.0(–123.5) × (27.8–)29.0–31.7–34.4(–35.5) µm [12]. Ascospores not measured.

Additional specimens examined: ECUADOR. PROVINCIA DE ESMERALDAS: La Chiquita, experimental station, settlement 22, 1.2320N 78.76603W, 5 Aug 2004, on female *S. galindoi* collected from male *T. saurophila, Carl W. Dick TK 135608* (fly vial code), slide D. Haelew. 3164a (GENT, 2 adult thalli from left wing M4+ Cu vein); Mataje, settlement 23, 1.3559N 78.7243W, 11 Aug 2004, on female *S. galindoi* collected from male *T. saurophila, Carl W. Dick TK 135889* (fly vial code), slide D. Haelew. 3166a (GENT, 1 adult thallus from right wing C vein); surroundings of San Francisco de Bogota, settlement 24, 1.0726N 78.7115W, 7 Aug 2004, on female *S. galindoi* collected from male *T. saurophila, Carl W. Dick TK 135696* (fly vial code), slide D. Haelew. 3167a (GENT, 2 adult thalli from right wing C vein); ibid., 9 Aug 2004, on female *S. galindoi* collected from male *T. saurophila, Carl W. Dick* TK 135859 (fly vial code), slides D. Haelew. 3168a (GENT, 1 adult thallus from right wing C vein) and D. Haelew. 3168b (GENT, 2 adult thalli and 1 subadult thallus from right wing C vein). PANAMA. PROVINCIA DE PANAMÁ OESTE: Isla Barro Colorado, Snyder Molino Trail, 17 Jan 2014, on female *S. galindoi* collected from male *T. saurophila, Thomas Hiller P_0217* (fly vial code), slide D. Haelew. 3406a (GENT:GENTFL00180, 1 adult thallus from right wing C vein); ibid., on female *S. galindoi* collected from male *T. saurophila, Thomas Hiller P_0217* (fly vial code), slide D. Haelew. 3463a (GENT:GENTFL00222, 2 adult thalli from right wing R_1 vein close to C vein).

Hosts and distribution: On *Strebla galindoi* (Diptera, Streblidae) in northern Ecuador and on Isla Barro Colorado, Panama.

Notes: Gloeandromyces verbekeniae f. *inflexus* is described based on 15 adult thalli and 1 subadult thallus. All thalli were removed from *Strebla galindoi* bat flies, which were all collected from *Tonatia saurophila* bats. Thalli of this morphotype were found along the margin of the wings (C vein) of female bat flies and are therefore thought to be female- and position-specific. These thalli are morphologically very similar to the ones of the autonym, *G. verbekeniae* f. *verbekeniae*; they differ in the pronounced kink in the perithecial neck and the flattened perithecial tip. We were unable to sequence this taxon, as very little recent, well-preserved material was available. However, both formae are very similar in morphology and found on the same bat fly species. The phylogenetically most closely related species to *G. verbekeniae* is *G. diversiformis* (FIG. 1). Three morphotypes are described for *G. diversiformis*—confirmed with sequence data. Note that both *G. diversiformis* and *G. verbekeniae* are associated with *Strebla* bat flies.

DISCUSSION

Morphological diversity within phylogenetic species of Gloeandromyces.—

In this paper, we formally describe five new species of *Gloeandromyces* based on morphology, molecular phylogeny, and host association, doubling the known species in this genus. Three of the new species are polymorphic; they have different morphotypes, which we formally described as formae. *Gloeandromyces diversiformis* consists of three morphotypes, all on the same host species (*Strebla wiedemanni*) but each restricted to a certain position and sex of the host. These morphological and ecological findings are confirmed by 28S nuc rDNA sequences of DNA extracts for each of the morphotypes.

Gloeandromyces verbekeniae is described having two formae. *Gloeandromyces verbekeniae* f. *verbekeniae* was found on the base of the right wing of three male *Strebla galindoi* bat flies. *Gloeandromyces verbekeniae* f. *inflexus*, recognized by the characteristically bent perithecial neck, was found on the C vein of the right wing of female *S. galindoi* flies. We obtained sequences for one isolate of *G. verbekeniae* f. *verbekeniae* (D. Haelew. 1741b), confirming the status of this taxon as a separate species. Although we were unable to generate sequences of *G. verbekeniae* f. *inflexus*, we think that the two morphotypes on *Strebla galindoi* represent a case of position-induced morphological variations and thus belong to a single phylogenetic species, *G. verbekeniae*. Both host specialization and position-induced morphological variations were put forward by Haelewaters and Pfister (2019) as drivers of diversity in *Gloeandromyces*. These mechanisms now also support our current observations of *G. diversiformis* and *G. verbekeniae*. More material of *G. verbekeniae* is needed to confirm our conclusions.

Trichobius yunkeri bat flies were observed with thalli that could be distinguished into two forms, each restricted to different positions on the integument. *Gloeandromyces plesiosaurus* f. *plesiosaurus* is found on the right metacoxa and meso- and metafemur of male hosts. *Gloeandromyces plesiosaurus* f. *asymmetricus* is mostly found on the left wings of both male and female hosts. Thalli of the latter morphotype are more compact

and have an asymmetrical perithecial tip that is pointed anteriorly. Again, we were unable to generate sequences for one morphotype, *G. plesiosaurus* f. *asymmetricus*, but nonetheless hypothesize that these formae belong to the same phylogenetic species. This is based on current knowledge of drivers of diversity in the genus (Haelewaters and Pfister 2019). The patterns of infection of *G. plesiosaurus* f. *asymmetricus* and *G. plesiosaurus* f. *plesiosaurus* are similar to those we have observed in *G. diversiformis*, for which we were able to successfully extract DNA from all three of its morphotypes (see above).

Bat fly–associated Laboulbeniales are low in prevalence (4.6% in Haelewaters et al. 2018; 6.9% in Walker et al. 2018). Some of the taxa presented here are rare finds, considering that they are the result of screening thousands of bat flies from over 10 years of fieldwork. Rather than increasing their shelf life (Fontaine et al. 2012), we decided to formally describe both *G. plesiosaurus* and *G. verbekeniae* based on morphological characteristics in combination with ecological data (host association, position of infection). Continued fieldwork will undoubtedly reveal more undescribed species and morphotypes of *Gloeandromyces* and accumulate fresh material necessary to confirm our taxonomic decisions.

A tangential conclusion is that the only haustorial species in the genus, *G. nycteribiidarum*, likely represents a species complex. We included six isolates of *G. nycteribiidarum* in the data set, removed from *Exastinion clovisi* (Pessoa & Guimaraes 1937), *Megistopoda aranea* (Coquillet 1899), and *Trichobius sparsus* Kessel 1925 bat fly hosts (Diptera, Streblidae). Support is lacking for this species in our phylogenetic reconstruction (FIG. 1), but high support is found for a subclade consisting of isolates D. Haelew. 1319b, D. Haelew. 1334c, and D. Haelew. 1661b (bootstrap = 96). All three of these isolates are associated with *M. aranea* bat flies removed from *Artibeus jamaicensis* (Chiroptera, Phyllostomidae) bats in Costa Rica and Panama. Thaxter (1917, 1931) described *G. nycteribiidarum* on *M. aranea* (as *Pterellipsis aranea* Coquillett 1899), which implies that the three-isolate clade may represent *G. nycteribiidarum* sensu stricto. More specimens and sequences are needed, but based on these preliminary results, species in the *G. nycteribiidarum* species complex may be segregated by host, possibly at the level of genus—as is the case within *Hesperomyces virescens* sensu lato (Haelewaters et al. 2018a, 2022b).

Behavior of bat fly hosts in relation to positions of infection.—Based on our observations, all formae of *G. diversiformis, G. plesiosaurus*, and *G. verbekeniae* appear to be restricted to a certain position of the integument. Rossi and Kotrba (2004) found similar positional patterns of infection on *Richardia teevani* Curran 1934 (Diptera, Richardiidae) flies and described a polymorphic species of *Laboulbenia: Laboulbenia richardiana* W. Rossi & Kotrba. The authors hypothesized that morphologically different thalli restricted to different positions on the host's integument were growth forms of a single biological species. Thalli from one morphotype were only found on the wing, whereas the other morphotypes were restricted to the ventral side of the hosts. These observations were explained by primarily mating behavior of the flies, in addition to auto-infection caused by grooming (Rossi and Kotrba 2004). Goldmann and Weir (2012) also investigated positional patterns of infection of *Chitonomyces* Peyr. thalli in relation to the behavior of their host, *Laccophilus maculosus* (Germar 1823). Using molecular data, they verified that 13 morphological species belonged to six phylogenetic species, each with two or three position-specific morphotypes. Some morphotypes were observed on both female and male hosts, whereas others were only found on male hosts. Also in this case, host mating behavior explained the occurrence of morphotypes in restricted positions of the integument. In addition, males mounting other males (same-sex behavior) explained why male *L. maculosus* hosts were infected in all positions observed.

Our observations on the positions of the formae of *Gloeandromyces diversiformis* on *Strebla wiedemanni* and those of *G. plesiosaurus* on *Trichobius yunkeri* may be explained by the mating behavior of their hosts as well. In the study of Fritz (1983) concerning the behavior of streblid bat flies on bats, male flies were described to frequently mount the females to reproduce. This frequent mounting or mating behavior has been anecdotally observed for *Trichobius intermedius* Peterson & Hůrka 1974 bat flies on Isla de Vieques, Puerto Rico (C. W. Dick, pers. obs.). Both fungal species have formae restricted to the wings of females and male bat flies (*G. diversiformis* f. *musiformis* and f. *vanillicarpiformis, G. plesiosaurus* f. *asymmetricus*) and formae limited to the ventral side of the body of male hosts (*G. diversiformis* f. *diversiformis, G. plesiosaurus* f. *plesiosaurus*). This could be an indication of a specific mating position where these body parts would touch frequently enough to transfer ascospores, and it would be in accordance with the infection patterns of morphotypes observed in previous studies (Goldmann and Weir 2012; Rossi and Kotrba 2004; Santamaria and Faille 2009).

Concerning the positions of *G. verbekeniae* f. *verbekeniae* and f. *inflexus* on *Strebla galindoi*, our sample size is currently too limited to make definitive conclusions about patterns of specificity or the

mechanism of ascospore transmission. At this time, thalli of each morphotypes have only been found on specific sections of the wings of either male or female bat flies. This could theoretically be attributed to mating behavior and same-sex mounting behavior (e.g., Goldmann and Weir 2012), although this would require the existence of a third, male-specific morphotype restricted to the ventral side of the body. On one male *S. galindoi* bat fly (D. Haelew. 3401, collection details below), a single juvenile thallus with a similar receptacular morphology and elongated BCA was observed on the left sternopleuron. Based on its position on the integument of the host, this is thought to be the third, male-specific morphotype. However, because we only have one thallus that is juvenile, it cannot be described. *Gloeandromyces verbekeniae* is the sister species of *G. diversiformis*, for which three morphotypes are described, confirmed with molecular data (FIGS. 1, 2). *Gloeandromyces diversiformis* f. *diversiformis* shares the same position of infection as the single juvenile thallus from bat fly D. Haelew. 3401: the ventral side of male bat flies, including the legs.

Gloeandromyces verbekeniae, *presumed third morphotype*: PANAMA. PROVINCIA DE PANAMÁ OESTE: Isla Barro Colorado, Lutz Tower, 16 Nov 2013, on male *Strebla galindoi* Wenzel 1966 (Diptera, Streblidae) collected from male *Tonatia saurophila* (Chiroptera, Phyllostomidae), *Thomas Hiller P_0343* (fly vial code), slide D. Haelew. 3401a (GENT:GENTFL00175, 1 juvenile thallus from left sternopleuron).

Final remarks.—In this paper, we described and illustrated five new species of bat fly–associated Laboulbeniales, all in the genus *Gloeandromyces*. Three of the new species consist of multiple morphotypes, each formally described as formae. For *G. diversiformis*, evidence for polymorphism comes from morphology, molecular phylogenetic data, and ecology (host association, patterns of infection). For each of the two other polymorphic species, *G. plesiosaurus* and *G. verbekeniae*, only one morphotype could be successfully sequenced. Evidence for polymorphism in these two species comes from morphology and ecology. The observed positional patterns of infection can be explained by host mating behavior and same-sex mounting behavior. This work helps widen our understanding of morphological and phylogenetic diversity in Laboulbeniales (Goldmann et al. 2013; Haelewaters and Pfister 2019). Further work should aim to finding the presumed third morphotype of *G. verbekeniae* and study the (mating) behavior of bat flies to unequivocally explain observations of their Laboulbeniales ectoparasites. We conclude with a call for more fieldwork in combination with molecular approaches (Cazabonne et al. 2022; Truong et al. 2017) and for an integrative taxonomy approach, combining data as independent lines of evidence, to delimit and describe taxa of Laboulbeniales (Haelewaters et al. 2022a).

KEY TO SPECIES AND FORMAE OF *GLOEANDROMYCES*

1. Large thallus (>300 µm); perithecium without any projections or undulations; attached to the host with a haustorium penetrating the integument, without a blackened foot. Not found on wings .... *Gloeandromyces nycteribiidarum* species complex

1′. Not this combination of characters; attached to the host with a (small) blackened foot..... 2

2. Perithecium with two to three longitudinal rows of undulations and a short cell I. On *Mastoptera guimaraesi* ..... *Gloeandromyces hilleri*

2′. Not this combination of characters 3

3. Perithecium with at least one long (>20 µm) projection 4

3′. Perithecium without any long projections; with or without smaller bumps 8

4. Thallus with a sigmoid habitus; perithecial neck with a large preapical outgrowth (up to 44 µm) pointing posteriorly. On the last abdominal segment of *Trichobius joblingi* .... *Gloeandromyces streblae* f. *sigmomorphus*

4′. Not this combination of characters; perithecial venter with at least one long projection 5

5. Perithecial venter with two horn-like projections (up to 46 µm) directed upward on the posterior side ..... *Gloeandromyces pageanus* f. *pageanus*

5′. Perithecial venter with one long projection 6

6. One almost horizontal subulate projection (up to 36 µm) at the upper perithecial venter, pointing to posterior, surrounded by one to three conspicuous bumps ..... *Gloeandromyces pageanus* f. *alarum*

6′. One elongated projection in the middle of the perithecial venter; thalli found on the right-hand side of the abdomen 7

7. Perithecium broadly ovoid, with a finger-like projection. On *Trichobius joblingi* .... *Gloeandromyces dickii*

7′. Perithecium slenderer, with a tapering projection. On *Trichobius longipes* .... *Gloeandromyces pseudodickii*

8. Basal cell of the appendage elongated, longer than broad. On *Strebla* spp. 9

8′. Basal cell of the appendage flattened, not longer than broad 13

9. Cell I clearly carrying cells II, VI, and VII 10

9′. Cell I carrying cells II and VI; sometimes cell VII is seemingly in close contact with cell I 11

10. Perithecial neck without a kink to posterior; perithecial tip subulate, carrying a large, tooth-shaped bump. On base of right wing of *Strebla galindoi* .... *Gloeandromyces verbekeniae* f. *verbekeniae*

10′. Perithecial neck with a kink to posterior; perithecial tip flattened. Along margin of wings of *Strebla galindoi* .... *Gloeandromyces verbekeniae* f. *inflexus*

11. Thallus with curved, stout habitus; cell I shorter than 70 µm. On wings of *Strebla wiedemanni* .... *Gloeandromyces diversiformis* f. *musiformis*

11′. Thallus long and slender, cell I longer than 70 µm 12

12. Cell I straight, with almost parallel margins, giving it a trapezoid-like habitus. On left-hand ventral side of *Strebla wiedemanni* .... *Gloeandromyces diversiformis* f. *diversiformis*

12′. Cell I very long (up to 170 µm), slender. Along margin of wings of *Strebla wiedemanni* .... *Gloeandromyces diversiformis* f. *vanillicarpiformis*

13. Distal end of perithecial venter with one bulging posterior bump. On *Trichobius yunkeri* 14

13′. Not this combination of characters 15

14. Thallus with slender and curved habitus; cell I elongated, carrying only cell II. On right-hand ventral side .... *Gloeandromyces plesiosaurus* f. *plesiosaurus*

14′. Stout habitus; cell I carrying cells II and VI; perithecial tip asymmetrical, pointing anteriorly. On wings .... *Gloeandromyces plesiosaurus* f. *asymmetricus*

15. Perithecial neck with pronounced constriction; perithecial tip with conical shape. On *Trichobius uniformis* ..... *Gloeandromyces cusucoensis**

15′. Not this combination of characters 16

16. With or without four conspicuous bumps at distal end of perithecial venter; cells III, VI, and VII not colored distinctly darker orange. On all body parts including wings of different species of tropical American Streblidae .... *Gloeandromyces streblae* f. *streblae**

16′. Always with four conspicuous bumps at distal end of perithecial venter; upper half of cell III and upper portions of cells VI and VII colored distinctly darker orange. On different body parts but not the wings of *Trichobius dugesioides* and *T. joblingi* .... *Gloeandromyces pageanus* f. *polymorphus**

Note.—Species and formae with an asterisk have similar, partly overlapping, and variable morphological characteristics. A combination of morphology and ecology (host association, position of infection) may give an identification. Sequence data are needed for confirmation.

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DISCLOSURE STATEMENT

No potential conflict of interest was reported by the authors.

DATA AVAILABILITY STATEMENT

Unedited images, final alignments, and unedited tree are available through GitHub: https://github.com/dannyhaelewaters/teamlaboul/tree/main/gloeandromyces_paper. Newly generated sequences were submitted to the National Center for Biotechnology Information (NCBI) GenBank database (https://www.ncbi.nlm.nih.gov/genbank/), under the following accession numbers: OQ117043, OQ969945–OQ969950, OQ971589–OQ971602, OQ971686–OQ971699.

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LITERATURE CITED

Abramoff M, Magalhães P, Ram SJ. 2004. Image processing with ImageJ. Biophotonics Int. 11:36–42.

Benjamin RK. 2001. *Autophagomyces, Bordea* and a new genus, *Rossiomyces* (Laboulbeniales). Aliso. 19:99–136.

Blackwell M, Haelewaters D, Pfister DH. 2020. Laboulbeniomycetes: evolution, natural history, and Thaxter's final word. Mycologia. 112:1048–1059. doi: 10.1080/00275514.2020.1718442.

Carlson CJ, Hopkins S, Bell KC, Doña J, Godfrey SS, Kwak ML, Lafferty KD, Moir ML, Speer KA, Strona G, et al. 2020. A global parasite conservation plan. Biol Conserv. 250:108596. doi: 10.1016/j.biocon.2020.108596.

Cazabonne J, Bartrop L, Dierickx G, Gafforov Y, Hofmann TA, Martin TE, Piepenbring M, Rivas-Ferreira M, Haelewaters D. 2022. Molecular-based diversity studies and field surveys are not mutually exclusive: on the importance of integrated methodologies in mycological research. Front Fungal Biol. 3:860777. doi: 10.3389/ffunb.2022.860777.

Chernomor O, von Haeseler A, Minh BQ. 2016. Terrace aware data structure for phylogenomic inference from supermatrices. Syst Biol. 65:997–1008. doi: 10.1093/sysbio/syw037.

de Groot MD, Dumolein I, Hiller T, Sándor AD, Szentiványi T, Schilthuizen M, Aime MC, Verbeken A, Haelewaters D. 2020. On the fly: tritrophic associations of bats, bat flies, and fungi. J Fungi. 6:361. doi: 10.3390/jof6040361.

De Kesel A. 1995. Relative importance of direct and indirect infection in the transmission of *Laboulbenia slackensis* (Ascomycetes, Laboulbeniales). Belg J Bot. 128:124–130.

De Weggheleire S. 2019. Biodiversity of the genus *Laboulbenia*: are there cryptic species? [master thesis]. Antwerp (Belgium): University of Antwerp. p. 48.

Dick CW, Patterson BD. 2006. Bat flies: obligate ectoparasites of bats. In: Morand S, Krasnov B, Poulin R, editors. Micromammals and macroparasites: from evolutionary ecology to management. Tokyo: Springer–Verlag. p. 179–194. doi: 10.1007/978-4-431-36025-4_11.

Fontaine B, Perrard A, Bouchet P. 2012. 21 years of shelf life between discovery and description of new species. Curr Biol. 22:R943–R944. doi: 10.1016/j.cub.2012.10.029.

Fritz GN. 1983. Biology and ecology of bat flies (Diptera: Streblidae) on bats in the genus *Carollia*. J Med Entomol. 20:1–10. doi: 10.1093/jmedent/20.1.1.

Goldmann L, Weir A. 2012. Position specificity in *Chitonomyces* (Ascomycota, Laboulbeniomycetes) on *Laccophilus* (Coleoptera, Dytiscidae): a molecular approach resolves a century-old debate. Mycologia. 104:1143–1158. doi: 10.3852/11-358.

Goldmann L, Weir A, Rossi W. 2013. Molecular analysis reveals two new dimorphic species of *Hesperomyces* (Ascomycota, Laboulbeniomycetes) parasitic on the ladybird *Coleomegilla maculata* (Coleoptera, Coccinellidae). Fungal Biol. 117:807–813. doi: 10.1016/j.funbio.2013.10.004.

Haelewaters D, Blackwell M, Pfister DH. 2021a. Laboulbeniomycetes: intimate fungal associates of arthropods. Annu Rev Entomol. 66:257–276. doi: 10.1146/annurev-ento-013020-013553.

Haelewaters D, De Kesel A, Gorczak M, Bao K, Gort G, Zhao SY, Pfister DH. 2019. Laboulbeniales (Ascomycota) of the Boston Harbor Islands II: species parasitizing Carabidae, and the *Laboulbenia flagellata* species complex. Northeast Nat. 25:110–149. doi: 10.1656/045.025.s906.

Haelewaters D, De Kesel A, Pfister DH. 2018a. Integrative taxonomy reveals hidden species within a common fungal parasite of ladybirds. Sci Rep. 8:15966. doi: 10.1038/s41598-018-34319-5.

Haelewaters D, Dick CW, Cocherán Pittí KP, Dittmar K, Patterson BD. 2021b. Chapter 21. Bats, bat flies, and fungi: exploring uncharted waters. In: Lim BK, Fenton MB, Brigham RM, Mistry S, Kurta A, Gillam EH, Russell A, Ortega J, editors. 50 years of bat research. Fascinating life sciences. Cham: Springer. p. 349–371. doi: 10.1007/978-3-030-54727-1_21.

Haelewaters D, Dima B, Abdel-Hafiz BII, Abdel-Wahab MA, Abul-Ezz SR, Acar I, Aguirre-Acosta E, Aime MC, Aldemir S, Ali M, et al. 2020. Fungal systematics and evolution: FUSE 6. Sydowia. 72:231–356. doi: 10.12905/0380.sydowia72-2020-0231.

Haelewaters D, Gorczak M, Kaishian P, De Kesel A, Blackwell M. 2021c. Laboulbeniomycetes, enigmatic fungi with a turbulent taxonomic history. In: Zaragoza Ó, Casadevall A, editors. Encyclopedia of mycology. Vol. 1. Oxford: Elsevier. p. 263–283. doi: 10.1016/B978-0-12-819990-9.00049-4.

Haelewaters D, Gorczak M, Pfliegler WP, Tartally A, Tischer M, Wrzosek M, Pfister DH. 2015. Bringing Laboulbeniales to the 21st century: enhanced techniques for extraction and PCR amplification of DNA from minute ectoparasitic fungi. IMA Fungus. 6:363–372. doi: 10.5598/imafungus.2015.06.02.08.

Haelewaters D, Lubbers M, De Kesel A. 2022a. The haustorium as a driving force for speciation in thallus-forming Laboulbeniomycetes. IMA Fungus. 13:1. doi: 10.1186/s43008-021-00087-7.

Haelewaters D, Matthews TJ, Wayman JP, Cazabonne J, Heyman F, Quandt CA, Martin TE. resubmitted. Biological knowledge shortfalls impede conservation efforts in poorly studied taxa – a case study of Laboulbeniomycetes. J Biogeogr.

Haelewaters D, Page RA, Pfister DH. 2018. Laboulbeniales hyperparasites (Fungi, Ascomycota) of bat flies: independent origins and host associations. Ecol Evol. 8:8396–8418. doi: 10.1002/ece3.4359.

Haelewaters D, Pfister DH. 2019. Morphological species of *Gloeandromyces* (Ascomycota, Laboulbeniales) evaluated using single-locus species delimitation methods. Fungal Syst Evol. 3:19–34. doi: 10.3114/fuse.2019.03.03.

Haelewaters D, Rossi W. 2015. Three new species of *Laboulbenia* from Roland Thaxter's backlog of slides and a brief review of Laboulbeniales associated with Chrysomelidae. Mycologia. 107:142–148. doi: 10.3852/14-022.

Haelewaters D, Van Caenegem W, De Kesel A. 2022b. *Hesperomyces harmoniae*, a new name for a common ectoparasitic fungus on the invasive alien ladybird *Harmonia axyridis*. Sydowia. 75:53–74. doi: 10.12905/0380.sydowia75-2023-0053.

Haelewaters D, Verhaeghen SJ, Ríos Gonzáles TA, Bernal Vega JA, Villarreal Saucedo RV. 2017. New and interesting Laboulbeniales from Panama and neighboring areas. Nova Hedwigia. 105:267–299. doi: 10.1127/nova_hedwigia/2017/0410.

Hajibabaei M, Singer GAC, Hebert PDN, Hickey DA. 2007. DNA barcoding: how it complements taxonomy, molecular phylogenetics and population genetics. Trends in Genet. 23:167–172. doi: 10.1016/j.tig.2007.02.001.

Hall TA. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser. 41:95–98.

Hiller T, Vollstädt MGR, Brändel SD, Page RA, Tschapka M. 2021. Bat–bat fly interactions in Central Panama: host traits relate to modularity in a highly specialised network. Insect Conserv Divers. 14:686–699. doi: 10.1111/icad.12508.

Hoang DT, Chernomor O, Von Haeseler A, Minh BQ, Vinh LS. 2018. Ufboot2: improving the ultrafast bootstrap approximation. Mol Biol Evol. 35:518–522. doi: 10.1093/molbev/msx281.

Hopple JS. 1994. Phylogenetic investigations in the genus Coprinus based on morphological and molecular characters [doctoral dissertation]. Durham (NC): Duke University. p. 402.

Jensen KM, Rodrigues L, Pape T, Garm A, Santamaria S, Reboleira ASPS. 2019. Hyperparasitism in caves: bats, bat flies and ectoparasitic fungus interaction. J Invertebr Pathol. 166:107206. doi: 10.1016/j.jip.2019.107206.

Kalyaanamoorthy S, Minh BQ, Wong TKF, von Haeseler A, Jermiin LS. 2017. ModelFinder: fast model selection for accurate phylogenetic estimates. Nat Methods. 14:587–589. doi: 10.1038/nmeth.4285.

Katoh K, Rozewicki J, Yamada KD. 2019. MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. Brief Bioinform. 20:1160–1166. doi: 10.1093/bib/bbx108.

Kuraku S, Zmasek CM, Nishimura O, Katoh K. 2013. aLeaves facilitates on-demand exploration of metazoan gene family trees on MAFFT sequence alignment server with enhanced interactivity. Nucleic Acids Res. 41:W22–W28. doi: 10.1093/nar/gkt389.

Kurtzman CP, Robnett CJ. 1997. Identification of clinically important ascomycetous yeasts based on nucleotide divergence in the 5' end of the large-subunit (26S) ribosomal DNA gene. J Clin Microbiol. 35:1216–1223. doi: 10.1128/jcm.35.5.1216-1223.1997.

Liu J, Haelewaters D, Pfliegler WP, Page RA, Dick CW, Aime MC. 2020. A new species of *Gloeandromyces* from Ecuador and Panama revealed by morphology and phylogenetic reconstruction, with a discussion of secondary barcodes in Laboulbeniomycetes taxonomy. Mycologia. 112:1192–1202. doi: 10.1080/00275514.2020.1781496.

Martin TE, Jones SEI, Creedy TJ, Hoskins HMJ, McCann NP, Batke SP, Kelly DL, Kolby JE, Downing R, Zelaya SMS, et al. 2021. A review of the ecological value of Cusuco National Park: an urgent call for conservation action in a highly threatened Mesoamerican cloud forest. J Mesoamerican Biol. 1:6–50.

Mueller GM, Cunha KM, May TW, Allen JL, Westrip JRS, Canteiro C, Costa-Rezende DH, Drechsler-Santos ER, Vasco-Palacios AM, Ainsworth AM, et al. 2022. What do the first 597 global fungal red list assessments tell us about the threat status of fungi? Diversity. 14:736. doi: 10.3390/d14090736.

Nalepa CA, Weir A. 2007. Infection of *Harmonia axyridis* (Coleoptera: Coccinellidae) by *Hesperomyces virescens* (Ascomycetes: Laboulbeniales): role of mating status and aggregation behavior. J Invertebr Pathol. 94:196–203. doi: 10.1016/j.jip.2006.11.002.

Nguyen LT, Schmidt HA, von Haeseler A, Minh BQ. 2015. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Mol Biol Evol. 32:268–274. doi: 10.1093/molbev/msu300.

Patterson BD, Dick CW, Dittmar K. 2007. Roosting habits of bats affect their parasitism by bat flies (Diptera: Streblidae). J Trop Ecology. 23:177–189. doi: 10.1017/S0266467406003816.

Rossi W. 2011. New species of *Laboulbenia* from Ecuador, with evidence for host switch in the Laboulbeniales. Mycologia. 103:184–194. doi: 10.3852/10-117.

Rossi W, Kotrba M. 2004. A new polymorphic species of *Laboulbenia* parasitic on a South American fly. Mycol Res. 108:1315–1319. doi: 10.1017/S0953756204001285.

Rossi W, Leonardi M. 2018. New species and new records of Laboulbeniales (Ascomycota) from Sierra Leone. Phytotaxa. 358:91–116. doi: 10.11646/phytotaxa.358.2.1.

Sánchez-García M, Henkel TW, Aime MC, Smith ME, Matheny PB. 2016. *Guyanagarika*, a new ectomycorrhizal genus of Agaricales from the Neotropics. Fungal Biol. 120:1540–1553. doi: 10.1016/j.funbio.2016.08.005.

Santamaria S, Faille A. 2009. New species of *Laboulbenia* and *Rhachomyces* (Laboulbeniales, Ascomycota), some of them polymorphic, parasitic on termiticolous ground beetles from tropical Africa. Nova Hedwigia. 89:97–120. doi: 10.1127/0029-5035/2009/0089-0097.

Sikes RS, Gannon WL. 2011. Guidelines of the American Society of Mammalogists for the use of wild mammals in research. J Mammal. 92:235–253. doi: 10.1644/10-MAMM-F-355.1.

Stielow JB, Lévesque CA, Seifert KA, Meyer W, Iriny L, Smits D, Renfurm R, Verkley GJM, Groenewald M, Chaduli D, et al. 2015. One fungus, which genes? Development and assessment of universal primers for potential secondary fungal DNA barcodes. Persoonia. 35:242–263. doi: 10.3767/003158515X689135.

Sundberg H, Ekman S, Kruys Å. 2018. A crush on small fungi: an efficient and quick method for obtaining DNA from minute ascomycetes. Methods Ecol Evol. 9:148–158. doi: 10.1111/2041-210X.12850.

Szentiványi T, Haelewaters D, Pfliegler WP, Clément L, Christe P, Glaizot O. 2018. Laboulbeniales (Fungi: Ascomycota) infection of bat flies (Diptera: Nycteribiidae) from *Miniopterus schreibersii* across Europe. Parasit Vectors. 11:395. doi: 10.1186/s13071-018-2921-6.

Thaxter R. 1917. New Laboulbeniales, chiefly dipterophilous American species. Proc Am Acad Arts Sci. 52:649–721. doi: 10.2307/20025703.

Thaxter R. 1931. Contribution towards a Mmonograph of the Laboulbeniaceae. Part V. Mem Am Acad Arts Sci. 16:1–435. doi: 10.2307/25058136.

Truong C, Mujic AB, Healy R, Kuhar F, Furci G, Torres D, Niskanen T, Sandoval-Leiva PA, Fernández N, Escobar JM, et al. 2017. How to know the fungi: combining field inventories and DNA-barcoding to document fungal diversity. New Phytol. 214:913–919. doi: 10.1111/nph.14509.

Vaidya G, Lohman DJ, Meier R. 2011. SequenceMatrix: concatenation software for the fast assembly of multi-gene datasets with character set and codon information. Cladistics. 27:171–180. doi: 10.1111/j.1096-0031.2010.00329.x.

Vilgalys R, Hester M. 1990. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. J Bacteriol. 172:4238–4246. doi: 10.1128/jb.172.8.4238-4246.1990.

Walker MJ, Dorrestein A, Camacho JJ, Hiller T, Meckler LA, Silas KA, Haelewaters D. 2018. A tripartite survey of hyperparasitic fungi associated with ectoparasitic flies on bats (Mammalia: Chiroptera) in a neotropical cloud forest in Panama. Parasite. 25:19. doi: 10.1051/parasite/2018017.

Weir A, Blackwell M. 2001. Extraction and PCR amplification of DNA from minute ectoparasitic fungi. Mycologia. 93:802–806. doi: 10.1080/00275514.2001.12063212.

Weir A, Hammond PM. 1997. Laboulbeniales on beetles: host utilization patterns and species richness of the parasites. Biodivers Conserv. 6:701–719. doi: 10.1023/A:1018318320019.