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Morphological species of *Gloeandromyces* (*Ascomycota*, *Laboulbeniales*) evaluated using single-locus species delimitation methods

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Abstract: In this paper, new species and *formae* of the genus *Gloeandromyces* (*Ascomycota*, *Laboulbeniales*) are described and illustrated. These are: *Gloeandromyces dickii* sp. nov. on *Trichobius joblingi* from Nicaragua and Panama; *G. pageanus* f. *alarum* f. nov. on *Tri. joblingi* from Panama; *G. pageanus* f. *polymorphus* f. nov. on *Tri. dugesioides* and *Tri. joblingi* from Panama and Trinidad; and *G. streblae* f. *sigmomorphus* f. nov. on *Tri. joblingi* from Panama. *Gloeandromyces pageanus* on *Tri. dugesioides* from Panama as described in *Nova Hedwigia* 105 (2017) is referred to as *G. pageanus* f. *pageanus*. Support for these descriptions of species and *formae* comes from phylogenetic reconstruction of the large subunit ribosomal DNA and from the application of species delimitation methods (ABGD, bPTP, GMYC). Host specialization results in phylogenetic segregation by host species in both *G. pageanus* and *G. streblae* and this may represent a case of incipient speciation. A second mechanism driving diversity involves position-induced morphological adaptations, leading to the peculiar morphotypes that are associated to growing on a particular position of the integument (*G. pageanus* f. *alarum*, *G. streblae* f. *sigmomorphus*).

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INTRODUCTION

Laboulbeniales are microscopic fungi (*Ascomycota*, *Laboulbeniomycetes*) that live as obligate epibionts on arthropod hosts. They are developmentally and morphologically unique among fungi that often have mycelia of unlimited growth – in *Laboulbeniales* subsequent divisions of a single two-celled ascospore result in the production of a multicellular unit of determinate growth, or *thallus*. Ascospores are thought to be predominantly directly transmitted through activities of the host (De Kesel 1995), such as mating, grooming, and random physical contacts in overwintering aggregations. Most *Laboulbeniales* are host specific; they are often associated with a single host species or hosts in the same genus. De Kesel (1996) showed that *Laboulbenia slackensis*, specific to *Pogonus chalceus* in nature, can be grown on other hosts under conditions atypical for these hosts. Thus, host specificity is driven by characters of the host, but also by environmental conditions (as selected by that host). A number of species have been reported from multiple hosts. For one of these, *Hesperomyces virescens*, we recently showed using an integrative approach with morphometric, molecular phylogenetic, and ecological data, that it is a complex of several species, segregated by host (Haelewaters *et al*. 2018a). On the other hand, different arthropods can co-occur in a single microhabitat, creating opportunities for transmission of ascospores to "atypical" hosts and host shifting, which may ultimately lead to speciation (e.g. Blum 1924, Rossi 2011, Pfliegler *et al*. 2016).

The majority of described species of *Laboulbeniales*, about 80 %, are associated with beetles (order *Coleoptera*) whereas only 10 % are associated with flies (order *Diptera*) (Weir & Hammond 1997). Four genera are known from bat flies (suborder *Hippoboscoidea*, families *Nycteribiidae* and *Streblidae*): *Arthrorhynchus* (four described species, two additional nominal species placed in synonymy), *Dimeromyces* (two described species), *Gloeandromyces* (three described species), and *Nycteromyces* (two described species) (Peyritsch 1871, 1873, Thaxter 1901, 1917, 1931, Haelewaters *et al*. 2017b, Dogonniuck *et al*. in press). Species in the genera *Arthrorhynchus*, *Gloeandromyces*, and *Nycteromyces* have been reported from bat flies exclusively. The genus *Dimeromyces*, on the other hand, is one of the largest genera of *Laboulbeniales* encompassing 115 species, of which only two are found on bat flies (Rossi *et al*. 2015, 2016, Dogonniuck *et al*. in press). In the last few years, studies on bat fly-associated *Laboulbeniales* have focused on extensive surveying, taxonomy (description of species), host specificity, tripartite association networks, phylogenetic placement of bat fly-specific genera, and morphological *versus* molecular diversity of *Gloeandromyces* (Haelewaters *et al*. 2017a, b, 2018b, Szentiványi *et al*. 2018, Walker *et al*. 2018, Dogonniuck *et al*. in press).

The genus *Gloeandromyces* was described by Thaxter (1931) to accommodate two species he had earlier described as *Stigmatomyces nycteribiidarum* and *S. streblae* (Thaxter 1917). He argued that the fan-like organization of the appendage separates the genus from *Stigmatomyces*, and described a gelatinous disorganization, which "ultimately affects the cells subtending the antheridia and even those below, so that the spreading portion of the appendage is largely obliterated" (Thaxter 1931). *Gloeandromyces nycteribiidarum* was described from a *Megistopoda aranea* bat fly [as *Pterellipsis aranea*] from Grenada; *G. streblae* from a *Strebla wiedemanni* bat fly (as *S. vespertilionis*) from Venezuela. Since their description in 1917, both species were re-collected only a century later, during the studies of D.H. Note that unidentified *Laboulbeniales* have been reported on bat flies from Brazil (Graciolli & Coelho 2001, Bertola *et al*. 2005) and Costa Rica (Fritz 1983). A third species of *Gloeandromyces* was described from *Trichobius dugesioides* bat flies (*Diptera*, *Streblidae*, *Trichobinae*) collected in Gamboa, Panama (Haelewaters *et al*. 2017b). Haelewaters *et al*. (2018b) and Walker *et al*. (2018) reported and illustrated undescribed forms of *Gloeandromyces* but refrained from morphological descriptions. In this paper, we apply sequence-based species delimitation methods to evaluate species limits in the genus *Gloeandromyces*, which thus far has only been reported from neotropical bat flies.

MATERIALS AND METHODS

Collection of bats, bat flies, and *Laboulbeniales*

Protocols to capture bats and to screen for ectoparasitic bat flies are given in the studies by Haelewaters *et al*. (2018b) and Walker *et al*. (2018). All capturing and sampling procedures were licensed and approved by the Smithsonian Tropical Research Institute (IACUC protocol: 2017-0102-2020-A5) and the Government of Panama (Ministerio de Ambiente de Panamá: SE/AH-2-16, SC/AH-117, SE/P-13-17). Specimens of bat flies preserved in 70–99 % ethanol were made available by collaborators C.W. Dick (Ecuador, Nicaragua) and J.J. Camacho (Trinidad). Field sites are shown in Fig. 1. Bat flies were screened for the presence of *Laboulbeniales* thalli using a stereomicroscope at 50× magnification. Thalli were removed from the host using Minuten Pins inserted onto wooden rods. Slide mounts were made following Benjamin (1971), with the help of Hoyer's medium (30 g arabic gum, 200 g chloral hydrate, 16 mL glycerol, 50 mL ddH_20) to dry-fix the thalli to the slide as described in Haelewaters *et al*. (2018a). Mounted fungal material was viewed at 400× to 1000× magnification under an Olympus BX53 compound microscope equipped with an Olympus DP73 digital camera (Waltham, Massachusetts). For detailed morphological study and descriptions, we used an Olympus BX40 microscope with XC50 camera, available at the Farlow Herbarium. Fungal specimens were identified using Thaxter (1917, 1924, 1931) and Haelewaters *et al*. (2017b). Voucher slides are deposited at Farlow Herbarium (FH; Harvard University, Cambridge, Massachusetts) and Herbario de la Universidad Autónoma de Chiriquí (UCH; David, Panamá).

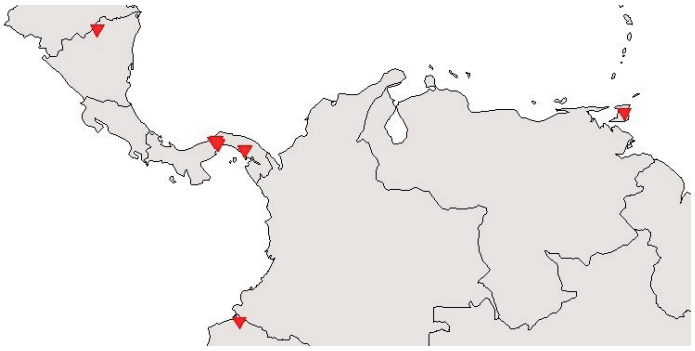


Fig. 1. Field sites where the streblid bat flies (*Diptera*, *Streblidae*) were collected that hosted the *Gloeandromyces* species and *formae* described in this paper. Field sites are located in Nicaragua and Panama in Central America; Ecuador in South America; and Trinidad, the southernmost island in the Caribbean.

DNA extraction, PCR amplification, sequencing

Laboulbeniales DNA was extracted from 3–12 thalli using the Extract-N-Amp Plant PCR Kit (Sigma-Aldrich, St. Louis, Missouri) (Haelewaters *et al*. 2015) or from 1–4 thalli using the REPLI-g Single Cell Kit (Qiagen, Valencia, California) (Haelewaters *et al*. 2018a). Pre-treatments employed with the Extract-N-Amp method included a prolonged incubation period at 56 °C in 20 µL Extraction Solution up to 24 h in a Shake 'N Bake Hybridization Oven (Boekel Scientific model #136400-2, Feasterville, Pennsylvania) and mechanically crushing fungal material in a FastPrep FP120 Cell Disrupter (Thermo Fisher Scientific, Waltham, Massachusetts) at 5.5 m/s for 20 s. For about half of our extractions with the REPLI-g Single Cell Kit, we manually cut thalli in 2 or 3 parts (usually through the perithecium) using a #10 surgical blade on disposable Bard-Parker handle (Aspen Surgical, Caledonia, Michigan) to ensure successful lysis.

The nuclear large ribosomal subunit (LSU) of the ribosomal DNA (rDNA) was amplified for this study. Primer pairs were LR0R (5'–ACCCGCTGAACTTAAGC–3') and LR5 (5'–ATCCTGAGGGAAACTTC–3') or LIC24R (5'–GAAACCAACAGGGATTG–3') and LR3 (5'–GGTCCGTGTTTCAAGAC–3'). Amplification reactions consisted of 13.3 µL of RedExtract *Taq* polymerase (Sigma-Aldrich), 2.5 µL of each 10 µM primer, 5.7 µL of ddH_2O and 1.0 µL of template DNA. Amplifications were done in a 2720 Thermal Cycler (Applied Biosystems, Foster City, California) with initial denaturation at 94 °C for 3 min; followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 50 °C for 45 s, and extension at 72 °C for 90 s; and final extension at 72 °C for 10 min. We used the Q5 Hot Start High-Fidelity DNA Polymerase (New England BioLabs, Ipswich, Massachusetts) for difficult isolates, which had resulted in unsuccessful PCR amplification using the RedExtract *Taq*. PCR was done in 25 µL consisting of 5.0 µL of 5× Q5 Reaction Buffer, 0.5 µL of 10 mM dNTP Mix (Quantabio, Beverly, Massachusetts), 1.25 µL of each 10 µM primer, 0.25 µL of Q5 High-Fidelity DNA Polymerase, 12.75 µL of ddH_2O, and 4.0 µL of template DNA. Thermal conditions were as follows: initial denaturation at 98 °C for 30 s; followed by 35 cycles of denaturation at 98 °C for 10 s, annealing at 58 °C for 30 s (as calculated using the New England BioLabs online Tm Calculator tool, at tmcalculator.neb.com/), and extension at 72 °C for 30 + 5/cycle s; and final extension at 72 °C for 2 min.

PCR purification and sequencing steps were outsourced to Genewiz (South Plainfield, New Jersey). However, when we performed our molecular work routine locally in Panama (at the Molecular Multi-User's Lab at the Naos Marine Laboratories), we purified PCR products using the QIAquick PCR Purification Kit (Qiagen). Subsequently, we prepared 10 µL reactions with the same primers and 3.0 µL of purified PCR product. Sequencing reactions were performed using the BigDye® Terminator v. 3.1 Cycle Sequencing Kit (Life Technologies, Carlsbad, California). Generated sequences were assembled and edited in Sequencher v. 4.10.1 (Gene Codes Corporation, Ann Arbor, Michigan). All sequences are deposited in GenBank (accession numbers in Table 1).

Sequence alignment and phylogenetic analyses

We constructed an LSU rDNA dataset of newly generated sequences and sequences downloaded from NCBI GenBank to evaluate species discrimination in the genus *Gloeandromyces*. Alignments were done using MUSCLE v. 3.7 (Edgar 2004), available on the CIPRES Science Gateway v. 3.3 (Miller *et al*. 2010). Maximum likelihood (ML) analysis was run using PAUP on XSEDE 4.0b (Swofford 1991), which is available on CIPRES. The appropriate nucleotide substitution model was selected by considering the Akaike Information Criterion (AIC) in jModelTest v. 2.1 (Darriba *et al*. 2012). A transitional substitution model (TIM2) with the assumption of a gamma distribution (+G) gave the best-scoring tree (-lnL = 2114.8480). ML was inferred under this model, and bootstrap (BS) values were calculated with 500 replicates. We ran Bayesian analyses using the BEAST on XSEDE tool in CIPRES with a Markov chain Monte Carlo (MCMC) coalescent approach under a strict molecular clock model, assuming a single rate of evolution across the tree. We selected the Yule speciation model (Yule 1925, Gernhard 2008) as tree prior with the TPM2uf+G nucleotide substitution model (considering the Bayesian Information Criterion, jModelTest v. 2.1). Two independent runs were performed from a random starting tree for 40 M generations, with a sampling frequency of 4 000. Resulting log files of the individual runs were imported in Tracer v. 1.6 (Rambaut *et al*. 2014) to check trace plots for convergence and effective sample size (ESS). ESS values were well ≥ 200, and so we applied a standard burn-in of 10 % for both runs. Log files and trees files were combined in LogCombiner v. 1.8.4 (Drummond *et al*. 2012) after removal of burn-in. TreeAnnotator v. 1.8.4 was used to generate consensus trees (with 0 % burn-in) and to infer the Maximum Clade Credibility tree, presenting the highest product of individual clade posterior probabilities. Final trees with bootstrap values (BS) and posterior probabilities (pp) were visualized in FigTree v. 1.4.3 (tree.bio.ed.ac.uk/software/figtree/).

Species delimitation in *Gloeandromyces*

For species delimitation analyses within the genus *Gloeandromyces*, we used the LSU rDNA dataset. This region was put forward by our previous work as a barcode marker for species delimitation in *Laboulbeniomycetes* (Walker *et al*. 2018, Haelewaters *et al*. 2018a). We aimed to validate morphology-based species identifications by employing three species delimitation methods (SDMs): ABGD (Puillandre *et al*. 2012), PTP (Zhang *et al*. 2013), and GMYC (Pons *et al*. 2006). The Automatic Barcode Gap Discovery method (ABGD) partitions sequence data into a maximum number of groups based on nucleotide divergence among isolates (Puillandre *et al*. 2012). We used the following parameters in the online version of ABGD (wwwabi.snv.jussieu.fr/public/abgd/abgdweb.html): Pmin = 0.001, Pmax = 0.01 (*sensu* Puillandre *et al*. 2012), steps = 10, and Nb bins = 20. To assess consistency in the recognition of species hypotheses by ABGD, we evaluated results for both the Jukes-Cantor (JC69) and Kimura 2-parameter (K80) distance metrics (Jukes & Cantor 1969, Kimura 1980) and for four gap width values (X): 0.1, 0.5, 1.0, and 1.5. The Poisson tree processes (PTP) model approach uses the number of nucleotide substitutions to infer speciation rate (Zhang *et al*. 2013). We used the bPTP web server (http://species.h-its.org) with default values for number of MCMC generations, thinning, burn-in, and seed, with the Maximum Clade Credibility tree as constructed above as input. The General Mixed Yule Coalescent (GMYC) approach models processes at the population level (coalescence) and processes at the species level (speciation) based on a fully resolved ultrametric tree (Pons *et al*. 2006). We conducted GMYC in R (R Core Team 2013) using the packages "rncl" (Michonneau *et al*. 2015) and "SPLITS" (Ezard *et al*. 2009). Input tree was the same Maximum Clade Credibility tree generated above.

RESULTS

Nucleotide alignment dataset & phylogenetic inference

Our LSU rDNA dataset comprised 955 characters, of which 817 were constant and 110 were parsimony-informative. A total of 27 isolates were included (Table 1). These are *Stigmatomyces protrudens* (one isolate as outgroup); *Gloeandromyces dickii* (four isolates); *G. nycteribiidarum* (two isolates); *G. pageanus* (13 isolates, including three isolates of f. *pageanus*, three isolates of f. *alarum*, and seven isolates of f. *polymorphus*); and *G. streblae* (seven isolates, including one isolate of f. *sigmomorphus*). *Gloeandromyces* forms six clades in both the ML and Bayesian analyses (Fig. 2). However, the statistical support differs between both approaches. ML support was only found for *G. dickii* (BS = 100), *G. nycteribiidarum* (BS = 99), *G. streblae* clade A+B (BS = 88), *G. pageanus* clade C+D (BS = 98), and *G. pageanus* clade C (BS = 96). In comparison, Bayesian inference also supported the distinction of *G. streblae* in host-specific clades A and B (pp = 0.96 and 0.81, respectively). Clade D has no support from either ML or Bayesian inference.

Species delimitation

Results of the species delimitation methods are summarized in Fig. 2 and Tables 2 and 3. The number of putative species in *Gloeandromyces* varied from 4 to 7 with ABGD analyses, depending on the prior intraspecific divergence (Table 3). The relative gap width and used distance metrics (JC69, K80) had no influence on the results. The bPTP analysis of the LSU topology resulted in four highly supported species (the "b" in bPTP standing for Bayesian support calculated for putative species): *Gloeandromyces dickii*, *G. nycteribiidarum*, *G. pageanus* (clade C+D), and *G. streblae* (clade A+B). The GMYC model led to the same results (four species delimited), but without strong support for *G. pageanus* and *G. streblae*.

Table 1. Overview of *Laboulbeniales* sequences used in this study. For each isolate is listed: current fungal species or *forma* when applicable, host species, location, and GenBank accession number for the LSU rDNA sequence. References for sequence data: Weir & Blackwell (2001), Haelewaters *et al*. (2015, 2018b).

Isolate	Species	Host species	Location	GenBank #
AW-793	*Stigmatomyces protrudens*	*Ephydridae* sp.	USA	AF298234
D. Haelew. 1319b	*Gloeandromyces nycteribiidarum*	*Megistopoda aranea*	Panama, Chucantí	MH040566
D. Haelew. 1334c	*Gloeandromyces nycteribiidarum*	*Megistopoda aranea*	Panama, Chucantí	MH040567
D. Haelew. 1312b	*Gloeandromyces dickii*	*Trichobius joblingi*	Panama, Chucantí	MH040580
D. Haelew. 1312c	*Gloeandromyces dickii*	*Trichobius joblingi*	Panama, Chucantí	MH040581
D. Haelew. 1323b	*Gloeandromyces dickii*	*Trichobius joblingi*	Panama, Chucantí	MH040582
D. Haelew. 1323c	*Gloeandromyces dickii*	*Trichobius joblingi*	Panama, Chucantí	MH040583
D. Haelew. 1090a	*Gloeandromyces streblae*	*Trichobius dugesioides*	Panama, Gamboa	MH040584
D. Haelew. 1306c	*Gloeandromyces streblae*	*Trichobius joblingi*	Panama, Chucantí	MH040585
D. Haelew. 1308b	*Gloeandromyces streblae*	*Trichobius dugesioides*	Panama, Chucantí	MH040586
D. Haelew. 1309a	*Gloeandromyces streblae*	*Trichobius dugesioides*	Panama, Chucantí	MH040587
D. Haelew. 1317a	*Gloeandromyces streblae*	*Trichobius joblingi*	Panama, Chucantí	MH040588
D. Haelew. 1335c	*Gloeandromyces streblae*	*Trichobius joblingi*	Panama, Chucantí	MH040589
D. Haelew. 1320b	*Gloeandromyces streblae* f. *sigmomorphus*	*Trichobius joblingi*	Panama, Chucantí	MH040579
D. Haelew. 1091b	*Gloeandromyces pageanus* f. *pageanus*	*Trichobius dugesioides*	Panama, Gamboa	MG906798
D. Haelew. 1367b	*Gloeandromyces pageanus* f. *pageanus*	*Trichobius dugesioides*	Panama, Parque Nacional Soberanía	MH040568
D. Haelew. 1425a	*Gloeandromyces pageanus* f. *pageanus*	*Trichobius dugesioides*	Panama, Parque Nacional Soberanía	MH040569
D. Haelew. 1306b	*Gloeandromyces pageanus* f. *alarum*	*Trichobius joblingi*	Panama, Chucantí	MH040574
D. Haelew. 1322a	*Gloeandromyces pageanus* f. *alarum*	*Trichobius joblingi*	Panama, Chucantí	MH040577
D. Haelew. 1327a	*Gloeandromyces pageanus* f. *alarum*	*Trichobius joblingi*	Panama, Chucantí	MH040578
D. Haelew. 619a	*Gloeandromyces pageanus* f. *polymorphus*	*Trichobius joblingi*	Trinidad	KT800008
D. Haelew. 1073b	*Gloeandromyces pageanus* f. *polymorphus*	*Trichobius joblingi*	Panama, Península Bohío	MH040570
D. Haelew. 1089a	*Gloeandromyces pageanus* f. *polymorphus*	*Trichobius dugesioides*	Panama, Gamboa	MH040571
D. Haelew. 1100b	*Gloeandromyces pageanus* f. *polymorphus*	*Trichobius joblingi*	Panama, Gamboa	MH040572
D. Haelew. 1272a	*Gloeandromyces pageanus* f. *polymorphus*	*Trichobius dugesioides*	Panama, Parque Nacional Soberanía	MH040573
D. Haelew. 1315a	*Gloeandromyces pageanus* f. *polymorphus*	*Trichobius joblingi*	Panama, Chucantí	MH040575
D. Haelew. 1315b	*Gloeandromyces pageanus* f. *polymorphus*	*Trichobius joblingi*	Panama, Chucantí	MH040576

Taxonomy

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

Type species: *Gloeandromyces streblae* (Thaxt.) Thaxt., *Mem. Amer. Acad. Arts* **16**: 113. 1931.

Gloeandromyces dickii Haelew., ***sp. nov.*** MycoBank MB824616. Figs 3A–C, 6.

Etymology: Referring to Dr. Carl W. Dick, Associate Professor of Biology at Western Kentucky University, who provided 7 792 bat flies from Ecuador, Honduras, Mexico, and Nicaragua for our studies dealing with bat fly-associated *Laboulbeniales*.

Diagnosis: Different from the other species and *formae* in the genus by the single peculiar, slender outgrowth halfway at the perithecial venter and the perithecial neck bent in anterior direction. Its LSU sequence is 91.6–94.2 % similar to other species of *Gloeandromyces*, unique molecular synapomorphies at positions 71, 90, 95, 116, 150, 158, 161, 220, 311, 427, 430, 432, 450, 452 (deletion), 476, 510, 512, 515, 530, 533–535, 537, 540, 541, 544–546, 553, 555, 560, 588, 589, 593, 608, 722, 724–729.

Description: *Thallus* irregularly pale yellowish, darker at perithecial venter and neck; basal cell of appendage bright orange. *Cell I* bent or kinked towards anterior side, with parallel margins, 2.5–2.9× longer than broad, carrying cells II and VI. *Cell II* broadly rhomboidal, isodiametric or slightly longer than broad, separated from cell III by oblique septum. *Cell III* broadly trapezoidal, distally narrowing, slightly longer than broad. *Basal cell of appendage* pentagonal to dome-shaped, with margins slightly broadening distally, carrying two short (up to 32 µm) branches of dichotomously dividing cells, outer suprabasal cell always higher than inner one, final cells antheridial. *Cell VI* strongly oblique, lens-shaped or flattened between cells II and VII, posterior margin (= septum II/VI) convex. *Cell VII* next to cell VI, with convex outer margin, proximal end in contact with cell I or almost so. *Perithecium* broadly ovoid, bearing three very different outgrowths: a short but conspicuous rounded bulge at base, an elongate, finger-like protuberance halfway along venter, directed to anterior side, usually straight or slightly bent upwards, and a single

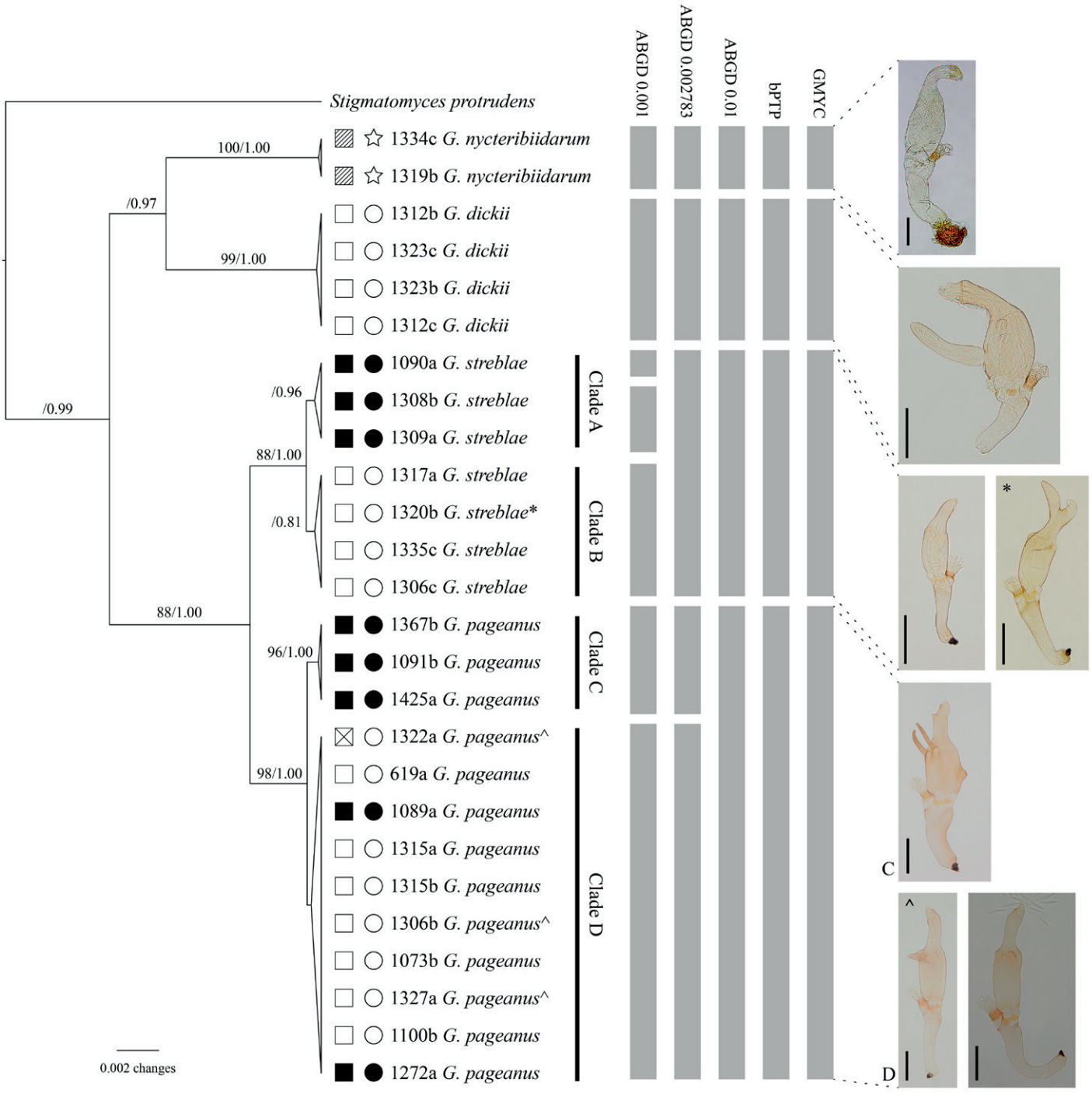


Fig. 2. Maximum clade credibility tree showing species in the genus *Gloeandromyces*, with *Stigmatomyces protrudens* as outgroup (adapted from Haelewaters *et al*. 2018b). The tree is the result of a Bayesian analysis of the LSU dataset. For each node, ML BS (≥ 70)/Bayesian pp (≥ 0.7) are presented above the branch leading to that node. Symbols indicate hosts: (bat flies) ☆ = *Megistopoda aranea*, ● = *Trichobius dugesioides*, ○ = *Tri. joblingi*; (bats) ▨ = *Artibeus jamaicensis*, ☒ = *Carollia brevicauda*, □ = *C. perspicillata*, ■ = *Trachops cirrhosus*. Symbols behind fungus species names designate morphotypes: **Gloeandromyces streblae* f. *sigmomorphus*, ^*G. pageanus* f. *alarum*, all other isolates in clade D: *G. pageanus* f. *polymorphus*. To the right of the terminal labels of the phylogeny, SDM results are summarized, from left to right: ABGD of the aligned LSU data matrix with prior intraspecific divergence (P) = 0.001 (Pmin), ABGD with P = 0.002783, ABGD with P = 0.01 (Pmax), bPTP of the LSU topology and GMYC of the LSU ultrametric tree generated in BEAST. To the right of the SDM results, thalli of *Gloeandromyces* spp. are shown. From top to bottom: *Gloeandromyces nycteribiidarum*; *G. dickii*; *G. streblae* and **G. streblae* f. *sigmomorphus*; *G. pageanus* f. *pageanus* (clade C), and ^*G. pageanus* f. *alarum* and *G. pageanus* f. *polymorphus* (clade D).

bump (rarely two) positioned laterally at distal third of venter; neck abruptly distinguished, strongly bent, with anterior margin concave and posterior margin nearly straight, distally distinctly broader at junction with stout, tapering tip, ending with prominent rounded lips.

Measurements: *Thallus* 183–294 µm in length from foot to perithecial tip. *Cell I* 58–88 × 21–30 µm. *Basal cell of appendage* 9–12 × 10–16 µm. *Perithecium* 123–176 × 40–62 µm. *Finger-like projection* up to 50–86 µm long. *Ascospores* 31–36 × 3–5 µm (up to 10 µm wide including slime sheath).

Table 2. Summary of results of ML, Bayesian, and species delimitation analyses (ABGD, bPTP, GMYC). Explanation of symbols and values used: — indicates no support; + under ABGD represents supported clades; numbers under bPTP and GMYC are Bayesian support values for delimited species hypotheses. (+) The ABGD analysis found support for two clades within *Gloeandromyces streblae* clade A under prior maximum distance (P) = 0.001, 0.001292, 0.001668 and 0.002154.

Putative species	ML BS	pp	ABGD P 0.001	ABGD P 0.002783	ABGD P 0.01	bPTP	GMYC
nycteribiidarum	100	1.0	+	+	+	0.996	0.85
dickii	99	1.0	+	+	+	0.986	0.81
streblae clade A	68	1.0	(+)	+	+	0.856	0.33
streblae clade B	—	0.9	+				
pageanus clade C	96	1.0	+	+	+	0.906	0.41
pageanus clade D	—	0.3	+	+			

Table 3. Results of the Automatic Barcode Gap Discovery (ABGD) analyses. X, relative gap width; JC69, Jukes-Cantor substitution model; K80, Kimura 2-parameter substitution model.

		Prior intraspecific divergence (P)									
Distance	**X**	0.001	0.001292	0.001668	0.002154	0.002783	0.003594	0.004642	0.005995	0.007743	0.01
JC69	0.1	7	7	7	7	5	4	4	4	4	4
	0.5	7	7	7	7	5	4	4	4	4	4
	1.0	7	7	7	7	5	4	4	4	4	4
	1.5	7	7	7	7	5	4	4	4	4	4
K80	0.1	7	7	7	7	5	4	4	4	4	4
	0.5	7	7	7	7	5	4	4	4	4	4
	1.0	7	7	7	7	5	4	4	4	4	4
	1.5	7	7	7	7	5	4	4	4	4	4

Typus: **Nicaragua**, Jinotega Department, Reserva Natural Bosawás, Mayangna Sauna Bu, Amak, at fork Rio Bocay and Rio Amak, secondary growth forest, 14.2396944 N 85.148 W, 30 May 2003, *M.R. Gannon*, on male *Trichobius joblingi* (*Diptera*, *Streblidae*, *Trichobinae*) (collected from male *Carollia perspicillata*), slide D. Haelew. 1018c (FH 00313692, **holotype**, two juvenile and six mature thalli, abdominal sterna). **Panama**, Colón Province, Forest Fragment near El Giral, 9.2152675 N 79.7301492 W, 11 May 2015, *T. Hiller*, on *Tri. joblingi* (collected from female *C. perspicillata*), slide D. Haelew. 1069a (FH 00313696, **paratype**, one mature thallus, right-hand side abdomen); Darién Province, Reserva Natural Chucantí, field site Waterfall, young secondary succession forest, 8.7865167 N 78.4508333 W, 19 Jun. 2017, *D. Haelewaters et al.*, on *Tri. joblingi* (collected from male *C. perspicillata*), slide D. Haelew. 1312a (FH 00313695, **paratype**, three mature thalli, right-hand side ventral abdomen); Darién Province, Reserva Natural Chucantí, field site Potrerito, old-growth broadleaf forest, 8.7909833 N 78.4510333 W, 22 Jun. 2017, *D. Haelewaters et al.*, on *Tri. joblingi* (from male *C. perspicillata*), slide D. Haelew. 1323a (UCH, **paratype**, three mature thalli, right-hand side ventral abdomen).

Additional materials examined: **Ecuador**, Esmeraldas Province, San Francisco de Bogota, 1.0877 N 78.6915 W, 6 Aug. 2014, *C.W. Dick*, on female *Trichobius longipes* (*Diptera*, *Streblidae*, *Trichobinae*) (collected from female *Phyllostomus hastatus*), slide D. Haelew. 1042a (FH 00313693, seven mature thalli, anterior ventral abdomen); same data, slide D. Haelew. 1043a (FH 00313694, six mature thalli, right-hand side anterior ventral abdomen).

Material sequenced: **Panama**, Darién Province, Reserva Natural Chucantí, field site Waterfall, young secondary succession forest, 8.7865167 N 78.4508333 W, 19 Jun. 2017, *D. Haelewaters et al.*, on *Tri. joblingi* (collected from male *C. perspicillata*), isolate D. Haelew. 1312b (two mature thalli, right-hand side ventral abdomen, SSU: MH040546, LSU: MH040580); same data, isolate D. Haelew. 1312c (two mature thalli, right-hand side ventral abdomen, SSU: MH040547, LSU: MH040581). **Panama**, Darién Province, Reserva Natural Chucantí, field site Potrerito, old-growth broadleaf forest, 8.7909833 N 78.4510333 W, 22 Jun. 2017, *D. Haelewaters et al.*, on *Tri. joblingi* (from male *C. perspicillata*), isolate D. Haelew. 1323b (four mature thalli, right-hand side ventral abdomen, SSU: MG958011, LSU: MH040582); same data, isolate D. Haelew. 1323c (one juvenile & three mature thalli, right-hand side ventral abdomen, SSU: MH040548, LSU: MH040583).

Notes: The perithecia of thalli from slide FH 00313695 look different from the typical form; the venter is slenderer, in combination with a consistently shorter and tapering perithecial projection. The *G. dickii* clade in the LSU phylogeny comprises D. Haelew. 1323b and 1323c ("typical" *G. dickii*) and D. Haelew. 1312b and 1312c. This clade is strongly supported, and our SDMs support *G. dickii* as a single species. All these thalli were removed from the same bat fly host, *Tri. joblingi*. The morphological differences described here seem to represent a range of phenotypic plasticity.

In addition to the Nicaraguan and Panamanian material, we also observed specimens from Ecuador (slides FH 00313693

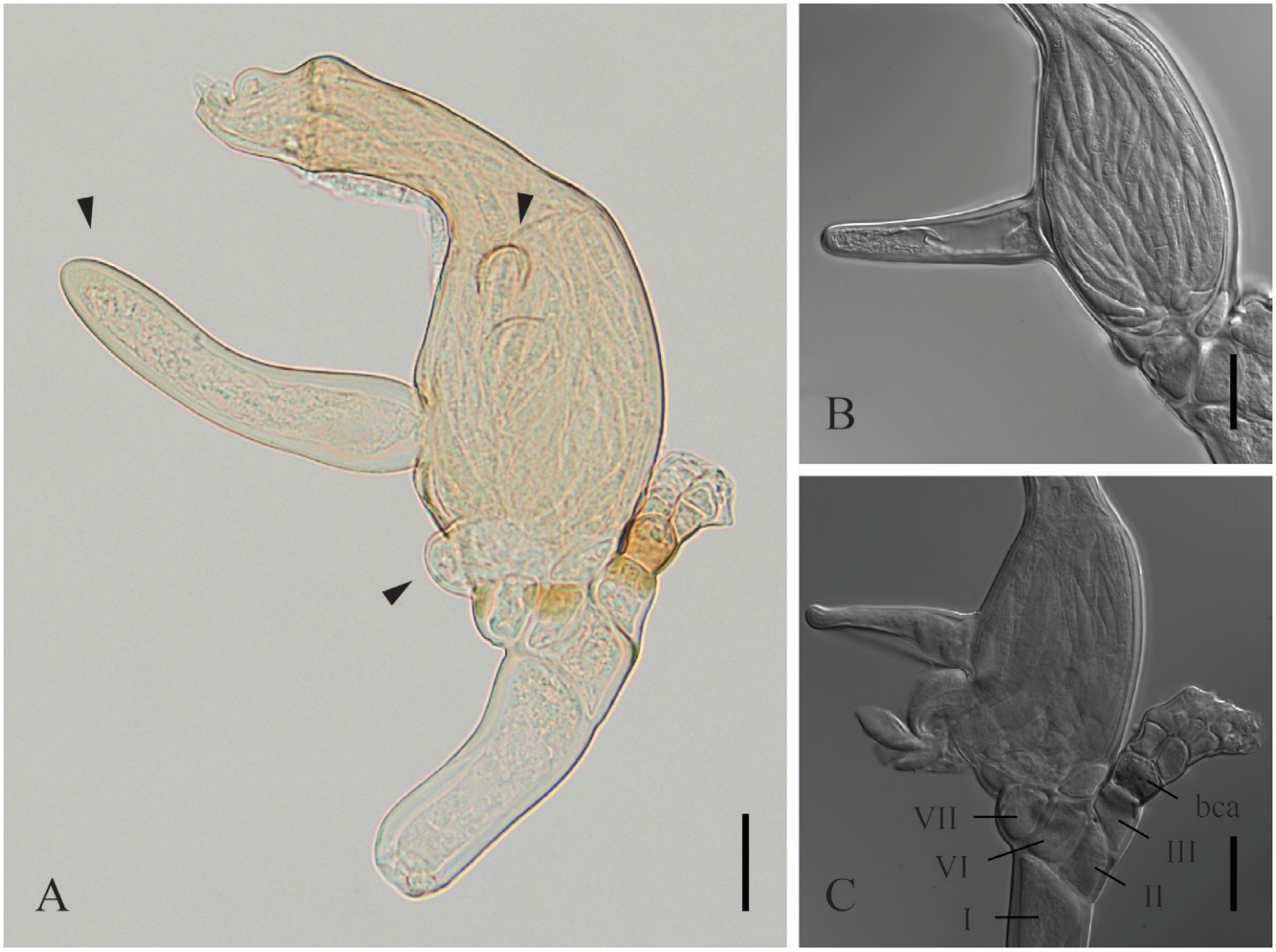


Fig. 3. Thalli of *Gloeandromyces dickii*. **A.** Mature thallus from slide FH 00313692 (holotype), with arrowheads pointing at outgrowths typical for this species. See description for details. **B.** Mature thallus from slide FH 00313694, with the perithecium less ovoidal and the anteriorly directed perithecial outgrowth halfway the venter shorter and more tapered in comparison to the type series. **C.** Mature thallus from slide FH 00313693 (with the perithecial venter ruptured anteriorly). Annotated are cells I, II, III, VI, VII, and the pentagonal-shaped basal cell of the appendage (bca). Scale bars: A = 50 µm, B–C = 20 µm.

and FH 00313694). We did not include them as part of the type series, because they were removed from another host species (*Tri. longipes*). We only performed DNA extractions of thalli taken from *Tri. joblingi*, and consequently, with the data in hand, we cannot rule out the possibility that there is some level of host specialization or (incipient) speciation (*sensu* Haelewaters *et al*. 2018a, b). The Ecuadorian material is also different in the following morphological characters (Fig. 3B–C): cell I can be slightly bent towards anterior side but is straight in the majority of observed thalli, the outer wall of cell VII is not convex/bulbous, the perithecial venter is less ovoidal, the bump at the base of the perithecium is less prominent and the perithecial projection halfway the venter is shorter and more tapered (like in FH 00313695). Other features are in line with those in the description of *G. dickii* above. It is clear that the Ecuadorian thalli and those from Nicaragua and Panama represent taxa that are very closely related if not the same.

Gloeandromyces pageanus Haelew., *Nova Hedwigia* **105**: 272. 2017. MycoBank MB819381. Fig. 4A–B.

Etymology: Referring to Dr. Rachel Page (Smithsonian Tropical Research Institute), mammologist, collaborator, and Principal Investigator at the Bat Lab in Gamboa.

Diagnosis: Different from the other species and *formae* in the genus by its peculiar perithecial bulbous outgrowths and finger-like projections.

Description: *Thallus* irregularly colored reddish, darker at basal cell of appendage, perithecial bulbous outgrowth and finger-like projections; upper part of cell III and cells VI and VII tinged with orange. *Cell I* curved towards anterior side, longer than broad, with divergent margins, carrying cells II and VI. *Cell II* trapezoidal, slightly broader than long. *Cell III* isodiametric, with rounded lower anterior margin. *Basal cell of appendage* pentagonal, with parallel anterior and posterior margins, carrying two very short branches of dichotomously dividing cells, final cells antheridial. *Cell VI* obliquely positioned between cells II and VII,

broadly triangular, lower margin rounded, broader than long. *Perithecium* obclavate, anterior margin bearing a short and bulbous outgrowth at lower third, and two horn-like projections obliquely directed upwards on the posterior side just below base of well-distinguished neck, bearing on upper half of posterior side two very short bulbous outgrowths, the upper one slightly smaller and darkly pigmented; tip undifferentiated, blunt.

Measurements: *Thallus* 195–257 µm in length from foot to perithecial tip. *Cell I* 45–74 × 31–44 µm (distally). *Basal cell of appendage* 7–10 × 11–13 µm. *Perithecium* 113–139 × 43–52 µm (not including bulbous outgrowth). *Perithecial projections* up to 46 µm in length. *Ascospores* 30–35 × 3–5 µm.

Typus: **Panama**, Colón Province, Gamboa, 26 Jun. 2016, *R.A. Page et al*., on female *Trichobius dugesioides* (collected from female *Trachops cirrhosus*), slide D. Haelew. 1093a (FH 00313699, **holotype**, six mature thalli, prescutum and scutum); Colón Province, Gamboa, 24 Jun. 2016, *R.A. Page et al*., on female *Tri. dugesioides* (collected from male *T. cirrhosus*), slide D. Haelew. 1091a (FH 00313697, **paratype**, one mature thallus, right-hand side thorax); same data, slide D. Haelew. 1092a (FH 00313698, **paratype**, one mature thallus, prescutum); Colón Province, Gamboa, 2 Jul. 2016, *R.A. Page et al*., on male *Tri. dugesioides* (collected from *T. cirrhosus*), slide D. Haelew. 1094a (FH 00313700, **paratype**, four mature thalli, right prescutum); Panamá Province, Ocelot Pond, 9.1017 N 79.685 W, 2 Jul. 2016, *R.A. Page et al*., on female *Tri. dugesioides* (collected from *T. cirrhosus*), slide D. Haelew. 1098a (FH 00313701, **paratype**, one mature thallus, thorax).

Additional materials examined: **Panama**, Colón Province, Gamboa, 29 Jan. 2017, *R.A.* Page et al., on *Tri. dugesioides* (collected from female *T. cirrhosus*), slide D. Haelew. 1280b (UCH, 2 mature thalli, left mesoprescutum); Colón Province, Parque Nacional Soberanía, Pipeline Road, Tunnel 17, 28 Jul. 2017, *R.A. Page* et al., on female *Tri. dugesioides* (collected from female *T. cirrhosus*), slide D. Haelew. 1367a (FH 00313702, five mature thalli, left mesoprescutum); Darién Province, Reserva Natural Chucantí, field site Potrerito, old-growth broadleaf forest, 8.7909833 N 78.4510333 W, 22 Jun. 2017, *D. Haelewaters et al*., on male *Tri. dugesioides* (collected from male *T. cirrhosus*), slide D. Haelew. 1329a (UCH, 1 mature thallus, left prescutum).

Material sequenced: **Panama**, Colón Province, Gamboa, 24 Jun. 2016, *R.A. Page et al*., on female *Tri. dugesioides* (collected from male *T. cirrhosus*), isolate D. Haelew. 1091b (six mature thalli, right-hand side thorax, SSU: MH040535, LSU: MG906798); Colón Province, Parque Nacional Soberanía, Pipeline Road, Tunnel 17, 28 Jul. 2017, *R.A. Page et al*., on female *Tri. dugesioides* (collected from female *T. cirrhosus*), isolate D. Haelew. 1367b (six mature thalli, left mesoprescutum, LSU: MH040568); Colón Province, Parque Nacional Soberanía, Pipeline Road, Tunnel 1, 13 Oct. 2016, *I. Geipel*, on *Tri. dugesioides* (collected from male *T. cirrhosus*), isolate D. Haelew. 1425a (four mature thalli, right mesoprescutum, SSU: MH040536, LSU: MH040569).

Notes: Its peculiar perithecial bulbous outgrowths and the two horn-like projections separate this species from the other species in the genus *Gloeandromyces* (Thaxter 1917, 1931, Haelewaters *et al*. 2017b). These characteristics are stable and have been observed in all studied specimens. *Gloeandromyces pageanus* shares with *G. streblae* a simple, blackened foot. The host for *G. pageanus*, *Tri. dugesioides*, is also reported for *G. streblae* in Panama. On most of the host specimens, we found thalli of both parasite species. *Gloeandromyces pageanus* was always found on the thorax, whereas *G. streblae* has no positional restrictions; we have observed this species on the thorax, legs, and wings. On one bat fly (D. Haelew. 1094), both species co-occurred on the right prescutum. Our phylogenetic analyses and SDMs confirm that the two taxa are separate species (Fig. 2).

Molecular synapomorphies diagnostic for clade C+D are found at positions 162, 222, 359, 450, 499, 525, 553 (deletion), 559, 567, 569, 593, 594, 689, 722, 730 (deletion). The phylogenetic reconstruction based on the LSU rDNA region shows divergence by host species into clade C (on *Tri. dugesioides*) and clade D (on *Tri. joblingi*). Because of lack of unique molecular synapomorphies in clade D, this clade is unsupported by both ML and Bayesian inferences. In addition, all SDMs but one do not recognize clades C and D as separate species. As a result, we cannot describe the specimens represented by clade D as a separate species, even though morphologically they are clearly different from the "true" *G. pageanus* (clade C). Based on the available data, we conclude that clade D represents two different morphological types, one that seems restricted to the base of the wings and a second that has no positional restrictions. To avoid confusion regarding these different morphotypes, we will refer to them as *formae*. *Gloeandromyces pageanus* as described above (clade C) will from here on be referred to as f. *pageanus*. The morphotypes from clade D will be referred to as f. *alarum* and f. *polymorphus* and are described formally below.

Gloeandromyces pageanus* f. *alarum Haelew., ***forma nov.*** MycoBank MB827804. Figs 4C–D, 6.

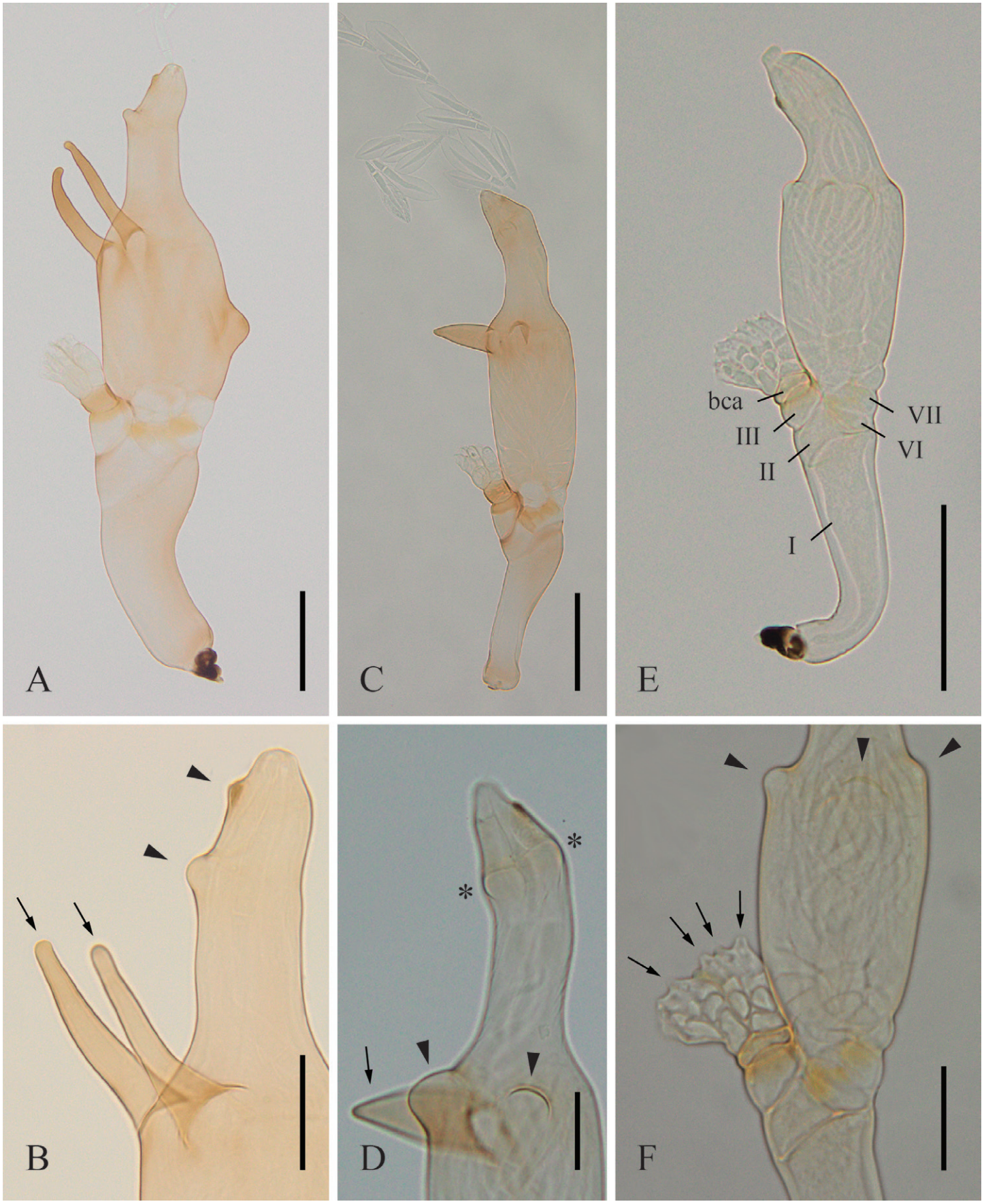
Etymology: From Latin, of the wings.

Diagnosis: Different from the other species and *formae* in the genus by its single subulate, almost horizontal projection, positioned posteriorly at the upper venter of the perithecium. Its LSU sequence is 99.7 % similar to *G. pageanus* f. *pageanus*, and 91.9–98.1 % similar to other species of *Gloeandromyces*.

Description: *Thallus* irregularly yellowish-light brown; septum II/III, area around septum between cell III and basal cell of

Fig. 4. Thalli of *Gloeandromyces pageanus*. **A–B.** *Gloeandromyces pageanus* f. *pageanus*. **A.** Mature thallus from slide FH 00313698 (paratype). **B.** Mature thallus from slide FH 00313700 (paratype), showing perithecial details on the posterior side: two horn-like projections (arrows) and two bulbous outgrowths (arrowheads). **C–D.** *Gloeandromyces pageanus* f. *alarum*. **C.** Mature thallus from slide FH 00313707 (paratype), releasing ascospores. **D.** Mature thallus from slide FH 00313709 (paratype), showing upper perithecial venter details: two conspicuous bumps (arrowheads) and a tapering projection directed posteriorly (arrow). Asterisks (*) highlight the two preostiolar bumps at opposite sides. **E–F.** *Gloeandromyces pageanus* f. *polymorphus*, mature thalli from slide FH 00313706 (paratype). **E.** Mature thallus, with annotated cells I, II, III, VI, VII, and the basal cell of the appendage (bca). F. Detail of mature thallus, showing antheridial cells (arrows) and conspicuous bumps at the distal end of the perithecial venter (arrowheads). Scale bars: A, C, E = 50 µm; B, D, F = 20 µm.

appendage, cells VI and VII, and perithecial projection and bumps usually darker. *Cell I* straight, broadening upwards, especially at anterior side, 3.4–4.1× longer than broad, carrying cells II and VI. *Cell II* trapezoidal, slightly broader than long, obliquely positioned. *Cell III* broadly triangular, slightly longer than broad. *Basal cell of appendage* pentagonal, with parallel anterior and posterior margins, carrying two short (up to 25 µm) branches of dichotomously dividing cells, outer suprabasal cell always higher than inner one, final cells antheridial. *Cell VI* broader than long, obliquely positioned, broadly lens-shaped or flattened between



cells II and VII. *Perithecium* with nearly straight, parallel or very slightly diverging margins; venter ending in one to three conspicuous bumps and a subulate, almost horizontal projection directed to posterior side, up to 36 µm in length; venter passing without abrupt transition into neck; the latter with subparallel margins, somewhat curving towards posterior side, tapering to conical tip, with two minute preostiolar bumps at opposite sides.

Measurements: *Thallus* 183–294 µm in length from foot to perithecial tip. *Cell I* 58–102 × 15–26 µm (distally). *Basal cell of appendage* 8–11 × 10–12 µm. *Perithecium* 130–163 × 28–45 µm. *Ascospores* 33–43 × 4–6 µm (with slime sheath up to 12 µm wide).

Typus: **Panama**, Darién Province, Reserva Natural Chucantí, field site Waterfall, young secondary succession forest, 8.7865167 N 78.4508333 W, 18 Jun. 2017, *D. Haelewaters et al*., on *Tri. joblingi* (collected from female *C. perspicillata*), slide D. Haelew. 1306a (FH 00313708, **holotype**, three mature thalli, base of right wing); Colón Province, Gamboa, Harding Avenue past Building 183, 9.115876 N 79.696784 W, 17 Jul. 2016, *D. Haelewaters*, on *Trichobius joblingi* (collected from female *Carollia perspicillata*), slide D. Haelew. 1100a (FH 00313707, **paratype**, one mature thallus, base of left wing); Darién Province, Reserva Natural Chucantí, field site Potrerito, old-growth broadleaf forest, 8.7909833 N 78.4510333 W, 20 Jun. 2017, *D. Haelewaters et al*., on *Tri. joblingi* (collected from female *C. perspicillata*), slide D. Haelew. 1316a (FH 00313709, **paratype**, 1 mature thallus, base of right wing).

Material sequenced: **Panama**, Darién Province, Reserva Natural Chucantí, field site Waterfall, young secondary succession forest, 8.7865167 N 78.4508333 W, 18 Jun. 2017, *D. Haelewaters et al*., on *Tri. joblingi* (collected from female *C. perspicillata*), isolate 1306b (two mature thalli, base of right wing, SSU: MH040541, LSU: MH040574); Darién Province, Reserva Natural Chucantí, field site Camp Site, 8.7996833 N 78.45355 W, 21 Jun. 2017, *D. Haelewaters et al*., on *Tri. joblingi* (collected from female *Carollia brevicauda*), isolate 1322a (one mature thallus, base of right wing R1 vein, SSU: MH040543, LSU: MH040577); Darién Province, Reserva Natural Chucantí, field site Potrerito, old-growth broadleaf forest, 8.7909833 N 78.4510333 W, 22 Jun. 2017, *D. Haelewaters et al*., on male *Tri. joblingi* (collected from male *C. perspicillata*), isolate D. Haelew. 1327a (one mature thallus, base of right wing, SSU: MH040544, LSU: MH040578).

Gloeandromyces pageanus* f. *polymorphus Haelew., ***forma nov.*** MycoBank MB827805. Fig. 4E–F.

Etymology: From Greek (*poly* + *morphus*), existing in multiple forms.

Diagnosis: Recognized by its morphology, with the perithecial venter ending in four conspicuous bumps, in combination with its LSU sequence, which is 100 % similar to *G. pageanus* f. *alarum*, 99.7 % similar to *G. pageanus* f. *pageanus*, and 91.9–98.1 % similar to other species of *Gloeandromyces*.

Description: *Thallus* faintly yellowish, with distinctly darker upper half of cell III, basal cell of appendage, and upper portions of cells VI and VII. *Cell I* 3.3–3.8× longer than broad, curved towards posterior side, broadening upwards, carrying cells II and VI. *Cell II* irregularly trapezoidal, slightly broader than long, septum II/III very oblique. *Cell III* broader than long, usually with convex outer margins. *Basal cell of appendage* pentagonal, with parallel anterior and posterior margins, carrying two short (up to 20 µm) branches of dichotomously dividing cells, final cells antheridial. *Cell VI* broader than long, obliquely positioned between cells II and VII, allantoid to broadly triangular, with rounded lower margin. *Perithecial venter* with slightly diverging margins, anterior nearly straight, posterior slightly convex, ending in four conspicuous bumps; neck abruptly distinguished, with subparallel margins, slightly curving towards anterior side, distinctly inflated at junction with tapering, subconical tip; ending with blunt apex directed upwards.

Measurements: *Thallus* 183–189(–311) µm in length from foot to perithecial tip. *Cell I* 66–69(–120) × 18–26 µm (distally). *Basal cell of appendage* 5–7(–12) × 11–12(–15) µm. *Perithecium* 89–96(–152) × 31–35 µm.

Typus: **Panama**, Colón Province, Península Bohío, 9.2045036 N 79.8299767 W, 3 Jul. 2015, *T. Hiller*, on male *Trichobius joblingi* (collected from female *Carollia perspicillata*), slide D. Haelew. 1073a (FH 00313705, **holotype**, two mature thalli, left-hand side abdomen); Colón Province, Parque Nacional Soberanía, Pipeline Road, Tunnel 10, 2 Jun. 2017, *D. Haelewaters & L.A. Meckler*, on *Tri. dugesioides* (collected from *T. cirrhosus*), slide D. Haelew. 1272b (FH 00313706, **paratype**, three mature thalli, right metatibia).

Material sequenced: **Panama**, Colón Province, Península Bohío, 9.2045036 N 79.8299767 W, 3 Jul. 2015, *T. Hiller*, on male *Trichobius joblingi* (collected from female *Carollia perspicillata*), isolate D. Haelew. 1073b (three mature thalli, left-hand side abdomen, SSU: MH040538, LSU: MH040570); Colón Province, Gamboa, 25 Apr. 2016, *R.A. Page et al*., on *Trichobius dugesioides* (collected from female *Trachops cirrhosus*), isolate D. Haelew. 1089a (four mature thalli, left-hand side abdomen, SSU: MH040539, LSU: MH040571); Colón Province, Gamboa, Harding Avenue past Building 183, 9.115876 N 79.696784 W, 17 Jul. 2016, *D. Haelewaters*, on *Trichobius joblingi* (collected from female *Carollia perspicillata*), isolate D. Haelew. 1100b (two submature & five mature thalli, right profemur & protibia, SSU: MH040307, LSU: MH040572); Colón Province, Parque Nacional Soberanía, Pipeline Road, Tunnel 10, 2 Jun. 2017, *D. Haelewaters & L.A. Meckler*, on *Tri. dugesioides* (collected from *T. cirrhosus*), isolate D. Haelew. 1272a (two mature thalli, left metafemur, SSU: MH040540, LSU: MH040573); Darién Province, Reserva Natural Chucantí, field site Waterfall, young secondary succession forest, 8.7865167 N 78.4508333 W, 19 Jun. 2017, *D. Haelewaters et al*., on *Tri. joblingi* (collected from male *C. perspicillata*), isolate D. Haelew. 1315a (one mature thallus, right sternopleuron, LSU: MH040575); same data, isolate D. Haelew. 1315b (two mature thalli, right profemur, SSU: MH040542, LSU: MH040576). **Trinidad and Tobago**, Trinidad, Sangre Grande Regional Corporation, 10.4671389 N 61.2025833 W, 9 May 2014, *J.J. Camacho*, on *Tri. joblingi* (collected from female *C. perspicillata*), isolate D. Haelew. 619a (12 mature thalli, different body parts, SSU: MH040537, LSU: KT800008), erroneously identified as *G. nycteribiidarum* in Haelewaters *et al*. (2015).

Notes: The thalli from Península Bohío are slenderer and somewhat darker colored compared to those from Soberanía. This is due to phenotypic plasticity because the DNA of the isolates from these localities is identical. The thalli from slide

D. Haelew. 1308a were preliminarily thought to be identical to those described here, under f. *polymorphus*. Also these thalli show four conspicuous bumps at the distal end of the perithecial venter. However, isolate D. Haelew. 1308b is placed in the A clade, as *G. streblae*. In addition, the host species are different: the bat fly host for *G. streblae* clade A is *Tri. dugesioides*, whereas the (main) host species for *G. pageanus* f. *polymorphus* is *Tri. joblingi*. This might be a case of cryptic diversity in the *Laboulbeniales*. However, it is likely that this form falls under the phenotypic plasticity exhibited by *G. streblae* (see Discussion).

Gloeandromyces streblae (Thaxt.) Thaxt., *Mem. Amer. Acad. Arts* **16**: 113. 1931.
Basionym: *Stigmatomyces streblae* Thaxt., *Proc. Amer. Acad. Arts* **52**: 700. 1917.

Notes: This species was described based on material from a single bat fly *Strebla wiedemanni* [as *S. vespertilionis*] (*Diptera*, *Streblidae*, *Streblinae*) from Venezuela. This poses a problem; our material of *G. streblae* was collected from *Tri. dugesioides* and *Tri. joblingi*. Although not recognized as separate species by our SDMs, we found evidence for two clades within *G. streblae* (clades A and B), both clades correlating with isolates from a single host species. This points to divergence by host species, and because we do not have isolates available of thalli from *S. wiedemanni*, we do not know the "true" *G. streblae*. As a result, we refrain from formally re-describing or emending the description for this species. Molecular synapomorphies diagnostic for clade A+B are found at positions 52, 224, 360, 380, 447 (deletion), 450 (deletion), 518, 557, 566, 594, 725–727 (deletions), 730.

Based on our molecular data, it is evident that the thalli that we had initially identified as a new species based on morphology (*Gloeandromyces* sp. nov. 2 *sensu* Walker *et al*. 2018), are part of the B clade, together with thalli of "typical" *G. streblae*. As is the case with *G. pageanus* f. *alarum*, this morphotype seems restricted to a precise position of the host's integument. We have only observed thalli of this morphotype at the last sternite/tergite. Again, to avoid confusion when referring to these thalli, we will describe them as *G. streblae* f. *sigmomorphus*.

Gloeandromyces streblae* f. *sigmomorphus Haelew., ***forma nov.*** MycoBank MB827806. Figs 5, 6.

Etymology: Referring to the general habitus of the fungus, which is curved like the letter s (sigma in Greek).

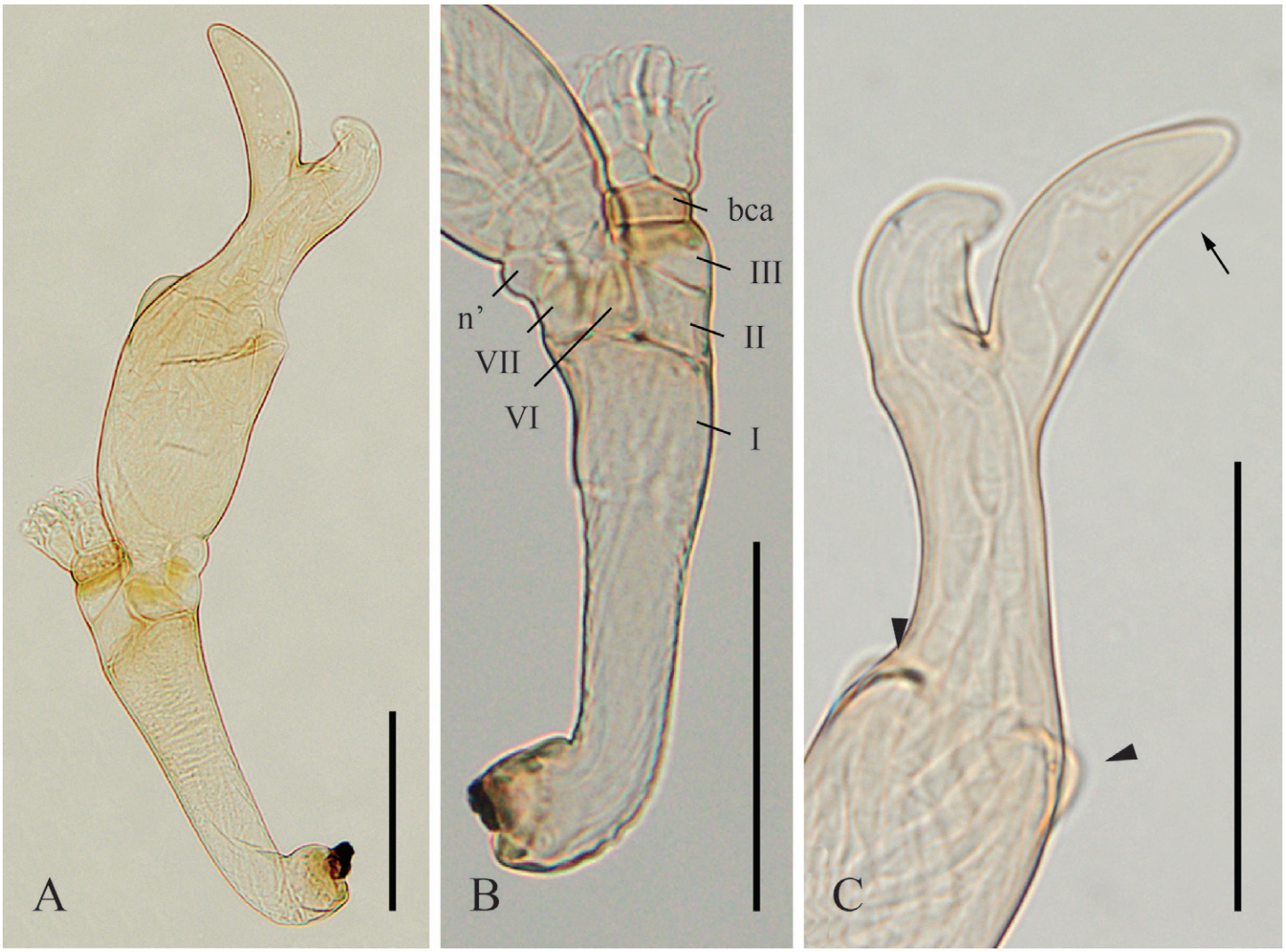


Fig. 5. Thalli of *Gloeandromyces streblae* f. *sigmomorphus*. **A.** Mature thallus from slide FH 00313703 (paratype). **B–C.** Details of mature thallus from slide FH 00313704 (holotype). **B.** Details of receptacle, appendage, and perithecial base, with annotated cells I, II, III, VI, VII, n', and the basal cell of the appendage (bca). C. Details of upper perithecium, with conspicuous rounded bumps at the distal end of the perithecial venter (arrowheads) and the very large preapical outgrowth (arrow). Scale bars = 50 µm.

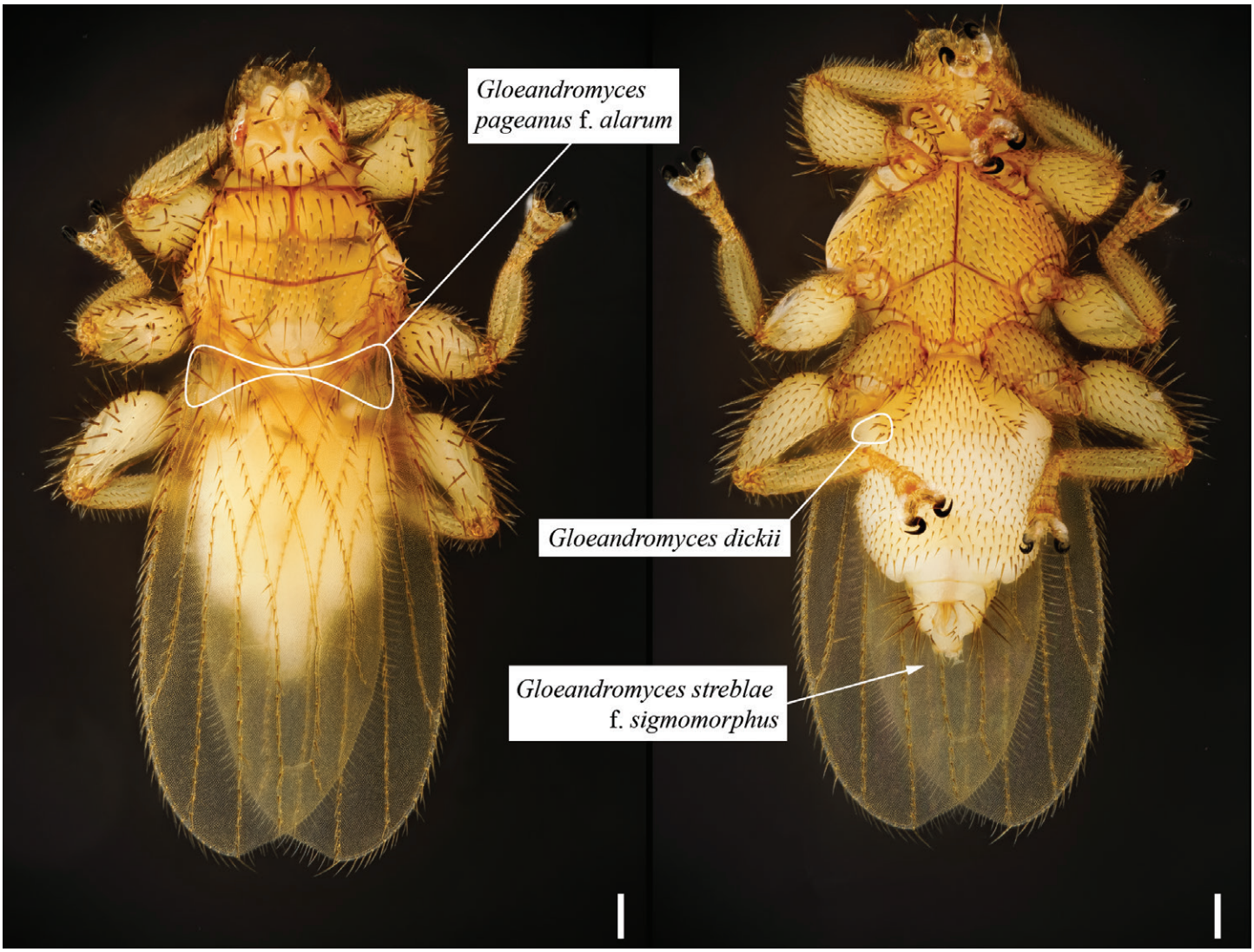


Fig. 6. A specimen of a *Trichobius* bat fly, photographed *in situ* dorsally (left) and ventrally (right). Annotated (encircled or with arrow) are the positions of the bat fly cuticle at which some (morpho-)species of *Gloeandromyces* seem to be restricted to: *G. dickii* on the abdomen, ventrally, at the right side; *G. pageanus* f. *alarum* at the base of both wings; and *G. streblae* f. *sigmomorphus* at the last tergite/sternite. Scale bars = 100 µm. Images provided by André De Kesel.

Diagnosis: Different from the other species and *formae* in the genus by its sigmoid habitus. The LSU sequence is 97.6–98.1 % similar to other species of *Gloeandromyces*.

Description: *Thallus* pale yellowish, upper portion of cell III and basal cell of appendage tinged with darker yellow. *Cell I* 3.0–4.1× longer than broad, basally curved towards anterior side, otherwise straight, gradually broadening upwards, with outer wall longitudinally or radially striped, carrying cells II, VI, and VII. *Cell II* rhomboidal, slightly broader than long, separated from cell III by oblique septum. *Cell III* triangular and broader than long. *Basal cell of appendage* broader than long, pentagonal, with parallel anterior and posterior margins, carrying two short (up to 19 µm) branches of dichotomously dividing cells, outer suprabasal cell always higher than inner one, final cells antheridial. *Cell VI* between cells II and VII, ovoidal to broadly triangular. Cell VII similar to cell VI. Cell n' inflated, outer margin rounded, protruding between cell VII and lower end of perithecium. *Perithecial venter* with margins slightly diverging upwards to conspicuous rounded prominences of wall cells; neck with broad base, short and stout; apex blunt, distinctly bent towards posterior side, subtended by a very large, sickle-shaped outgrowth at posterior side.

Measurements: *Thallus* 201–243 µm in length from foot to perithecial tip. *Cell I* 65–85 × 19–22 µm (distally). *Basal cell of appendage* 6–8 × 11–12 µm. *Perithecium* 115–126 × 27–30 µm. *Horn-like perithecial appendage* 36–44 µm in length.

Typus: **Panama**, Darién Province, Reserva Natural Chucantí, field site Potrerito, old-growth broadleaf forest, 8.7909833 N 78.4510333 W, 20 Jun. 2017, *D. Haelewaters et al*., on *Tri. joblingi* (collected from female *C. perspicillata*), slide D. Haelew. 1320a (FH 00313704, **holotype**, one juvenile & one mature thallus, last sternite/tergite), referred to as *Gloeandromyces* sp. nov. 2 in Walker *et al*. (2018); Colón Province, Gamboa, Harding Avenue past Building 183, 9.115876 N 79.696784 W, 17 Jul. 2016, *D. Haelewaters*, on *Trichobius joblingi* (collected from female *Carollia perspicillata*), slide D. Haelew. 1099b (FH 00313703, **paratype**, five mature thalli, tip of last sternite), referred to as *Gloeandromyces* sp. nov. 2 in Walker *et al*. (2018).

Material sequenced: **Panama**, Darién Province, Reserva Natural Chucantí, field site Potrerito, old-growth broadleaf forest, 8.7909833 N 78.4510333 W, 20 Jun. 2017, *D. Haelewaters et al*., on *Tri. joblingi* (collected from female *C. perspicillata*), isolate D. Haelew. 1320b (one mature thallus, last sternite/tergite, SSU: MH040545, LSU: MH040579).

DISCUSSION

GMYC recognizes *G. streblae* (clade A+B) and *G. pageanus* (clade C+D) as species, but the Bayesian support values are low (pp = 0.33 and 0.41, respectively). De Kesel (1997) argued that populations of *Laboulbeniales*-parasitized insects are similar to islands in the model of island biogeography (MacArthur & Wilson 1967). Divergence of host populations and subsequent speciation will lead to population divergence of the ectoparasites by isolation of gene pools. With regard to bat fly hosts, *Tri. dugesioides* can be exchanged between several bat species and thus co-occur with *Tri. joblingi* (see further). This might lead to intermittent gene flow between *Gloeandromyces* populations, complicating branching rates of gene trees. In addition, Esselstyn *et al*. (2012) mentioned that any given GMYC analysis can accurately estimate the number of species, even though it may not correctly assign individuals to species when taxonomically defined species are not monophyletic, which is the case for *G. pageanus*. The monophyly of clade D is unsupported, causing it to collapse. In other words, the node that describes *G. pageanus* is an unresolved polytomy between the highly supported clade C and the isolates that form clade D.

The ABGD analysis gives different numbers of putative species depending on P, the prior intraspecific divergence. If this parameter is set too high, the entire dataset will be seen as a single species; if set too low, only identical sequences will be retrieved as species (Puillandre *et al*. 2012). These authors also proposed to use P = 0.01 as this setting provided highest congruence with previous studies (meaning that under this setting, ABGD results matched the number of species found by previous studies using other approaches). Indeed, when P = 0.01, the ABGD results in four species of *Gloeandromyces*, congruent with bPTP and GMYC results. These congruent estimates of species diversity within the LSU rDNA dataset provide confidence in our understanding of *Gloeandromyces*, based on the currently available data.

We identified seven "morphospecies" (or morphotypes) of *Gloeandromyces* on the basis of morphological characters but this morphological diversity is not reflected in phylogenetic inference by LSU rDNA barcode sequences. Using SDMs resulted in four species only: *Gloeandromyces dickii*, *G. nycteribiidarum*, *G. pageanus*, and *G. streblae*. In *G. pageanus*, thalli from *Tri. dugesioides* are in line with the original description of the species by Haelewaters *et al*. (2017b). However, thalli on *Tri. joblingi* showed two distinct morphologies. One morphotype, *G. pagenaus* f. *alarum*, was restricted to the base of the wings (Fig. 6), whereas the other, *G. pageanus* f. *polymorphus*, was not restricted to a particular position on the host. In *G. pageanus*, two mechanisms drive diversity: 1) host specialization, resulting in the two clades segregating by host species (*sensu* Haelewaters *et al*. 2018a), and 2) position-induced morphological adaptations, resulting in the wing-restricted f. *alarum* (*sensu* Goldmann & Weir 2012, Goldmann *et al*. 2013).

In their study of species in the genus *Coreomyces* on water boatmen (*Hemiptera*, *Corixidae*), Sundberg *et al*. (2018) found that different species can occupy the same position on the host, without strict position specificity. Each of the four considered species occurs in two or three positions, with one position much more preferred over the others. The authors also pointed out that the considered species did not show strict host specificity. For example, thalli of *C. corixae* (green clade in Sundberg *et al*. 2018) were removed from species in the genera *Callicorixa*, *Hesperocorixa*, and *Sigara* (Sundberg 2018). In other words, contrary to *Hesperomyces* and *Gloeandromyces* in which specialization on different hosts drives divergent evolution (Haelewaters *et al*. 2018a, b, this study), host species does not seem to be a major factor in species delimitation within *Coreomyces*.

Two *G. pageanus* isolates seem aberrant, D. Haelew. 1089a and 1272a; these isolates were removed from *Tri. dugesioides* but are present in clade D, which includes *Tri. joblingi* isolates. We think we can explain this by bat fly behavior and interactions. Bat flies are usually strictly host specific, with non-primary associations being defined as host species with less than 5 % of the total individuals of a parasite species (Dick 2007). When Wenzel *et al*. (1966) described *Tri. dugesioides*, they reported it from *Trachops cirrhosus*, *Chrotopterus auratus*, and *Carollia perspicillata*, all bats in the family *Phyllostomidae*. The main hosts are *T. cirrhosus* and *C. auratus*. Because *C. perspicillata* bats make use of the same roost environments, *Tri. dugesioides* can be "exchanged" between these bat species. Apparently, dynamics are different for *Tri. joblingi*, which is strictly restricted to *Carollia* species.

Finally, even though SDMs only recognize four species of *Gloeandromyces*, it is evident that in *G. pageanus* and *G. streblae*, there is phylogenetic divergence by host species (Haelewaters *et al*. 2018b). This host specialization may represent an important first step in a potential radiation process; our results suggest a case of sympatric speciation into two incipient species, both in *G. pageanus* and *G. streblae*. Rosenblum *et al*. (2012) proposed the "ephemeral speciation model," in which they postulated that speciation is common and rapid, but the new species produced almost never persist. This could be due to extinction or changes in conditions that maintain reproductive isolation.

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