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Molecular phylogenetic analyses and micromorphology reveal placement of the enigmatic tropical discomycete *Polydiscidium* in *Sclerococcum* (Sclerococcales, Eurotiomycetes)

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ABSTRACT

Polydiscidium is an enigmatic, monotypic, and rarely reported genus of Ascomycota of uncertain placement. The morphologically unique *Polydiscidium martynii* grows on dead wood and forms compound ascomata composed of thick, black, gelatinous somatic tissue that branches out from a common base. Multiple apothecia are located on the branches, mostly toward the tips, and are composed of 8-spored asci and paraphyses embedded in a gelatinous matrix that turns blue in Melzer's reagent. The species was previously known from only three collections from Guyana (holotype), Trinidad, and the Democratic Republic of the Congo and no sequences exist. Due to its peculiar morphology, taxonomic affinities of *Polydiscidium* have been debated, with different authors having placed it in Helotiaceae, Leotiaceae, or Leotiomycetes incertae sedis. Recent collections of this species resulting from long-term field work in Guyana and Cameroon led us to revisit the morphology and phylogenetic position of this fungus. Newly generated sequences of *P. martynii* were added to an Ascomycota-wide six-locus data set. The resulting phylogeny showed *Polydiscidium* to be a member of order Sclerococcales (Eurotiomycetes). Next, a four-locus (18S, ITS, 28S, mtSSU) phylogenetic reconstruction revealed that *Polydiscidium* is congeneric with *Sclerococcum*. A new combination is proposed for this species, *Sclerococcum martynii*. Micromorphological features, including the gelatinous hymenium composed of asci with amyloid gel cap and septate brown ascospores, are in agreement with *Sclerococcum*. New combinations are proposed for two additional species: *Sclerococcum chiangraiensis* and *S. fusiformis*. Finally, Dactylosporales is considered a later synonym of Sclerococcales.

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INTRODUCTION

Tropical systems—with their density and diversity of host organisms, high humidity, and a plethora of microhabitats—are hot spots for fungi. This is especially evident when such habitats have been explored through repeated standardized sampling to reveal exceptional fungal diversity (e.g., Aime and Brearley 2012; Haelewaters et al. 2021b; Piepenbring 2015; Rossman et al. 1998). A long-term fungal surveying project in Guyana has thus far yielded over 100 newly described species, and a number of these taxa have altered contemporary understanding of taxonomic concepts. For example, *Craterellus pleurotoides*, with its highly reduced pleurotoid basidiomata that are atypical in the genus, was described from Guyanese material (Henkel et al. 2006; Wilson et al. 2012). Other morphologically divergent examples include *Pseudotulostoma volvatum*, an ectomycorrhizal fungus in Eurotiales (Ascomycota) that was originally thought to be a species of *Tulostoma* (Agaricales) because of its stalked and volvate sporocarps (Miller et al. 2010), and *Guyanagaster necrorhizus*, a sequestrate, insect-dispersed, root-associated relative of the otherwise agaricoid genus *Armillaria* (Henkel et al. 2010; Koch and Aime 2018). Another "strange" (as noted by Roland Thaxter; Cash 1958) fungus described from Guyana and reported again in this study for the first time in over 50 years is *Polydiscidium martynii* Wakef.

Wakefield (1934) erected the monotypic genus *Polydiscidium* and described *P. martynii* as the type species based on material collected in Guyana (as British Guiana) in 1929. Compound ascomata of the species emerge from decaying wood as masses of thick, black, gelatinous branching tissue of up to 5 cm in height and end apically with multiple apothecia (FIGS. 1, 2). The hymenia of these apothecia are composed of 8-spored asci and paraphyses embedded in a gelatinous matrix that turns blue in Melzer's reagent (Dennis 1961).

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Additional reports of *P. martynii* come from Cash (1958), who provided a brief description of a collection made by Thaxter on the southern Caribbean Island of Trinidad in 1913, and Dennis (1961), who reported a collection from the Democratic Republic of the Congo (D.R. Congo) in 1928. Cash (1958) reported slightly larger ascospores from the Trinidad collection than those of the holotype, whereas Dennis (1961) reported slightly smaller ones. Both remarked that the fungus must be relatively rare, as it had been collected on few occasions, even though it is large and conspicuous.

Due to its apothecial ascomata, *Polydiscidium* was originally considered a member of the "Discomycetes" (Wakefield 1934), which we now know is a form group. Dennis (1970) classified it in Helotiales. Korf (1973) placed *Polydiscidium* in Leotiaceae based on its gelatinous ascoma tissue, although the utility of this character for higher-rank classifications has been called into question by others (Moore 1965; Wang et al. 2006). No sequences have been published for *Polydiscidium*, but most modern treatments have included this genus in Helotiales (Dennis 1970; Lumbsch and Huhndorf 2007) or Leotiomycetes incertae sedis (Ekanayaka et al. 2019a; Wijayawardene et al. 2020, 2017) based on apothecial morphology and inoperculate asci.

Our objective in this study was to sort out the systematics of the enigmatic *P. martynii*, capitalizing on new collections from Guyana and Cameroon. Here, we redescribe the species and provide multilocus molecular phylogenetic analyses indicating the placement of the species within Ascomycota. We also propose a number of taxonomic changes based on our studies and previous work.

MATERIALS AND METHODS

Collections.—Ascomata were collected in Cameroon and Guyana as part of two long-term fungal surveying projects. The Cameroonian collection was made at the Dja Biosphere Reserve in the Upper Dja River Basin, within a 2-km radius of a base camp (3°21′29.8″N, 12°43′46.9″E, 650 m above sea level [a.s.l.]), in a forest dominated by *Gilbertiodendron dewevrei*. Most Guyanese collections were made between 2000 and 2022 in the Upper Potaro River Basin in the west-central Pakaraima Mountains, within a 5-km radius of a base camp (5°18′04.8″N, 59°54′40.4″W, ~800 m a.s.l.), in forests dominated by *Dicymbe corymbosa*. Additional Guyanese collections were made from the Upper Demerara River Basin (in 2011) and the Upper Mazaruni River Basin (in 2019). Fresh specimens were photographed in the field camp or in situ in the field (FIG. 1). Ascomata were dried in the field using silica gel. Collections were deposited at the following herbaria: BRG, University of Guyana; HSC, Humboldt State University; PUL, Kriebel Herbarium at Purdue University; and YA, National Herbarium of Cameroon.

Figure 1. Field habit of *Sclerococcum martynii* on decaying hardwood log in the Upper Potaro River Basin, Guyana, collection TH 11122. Total width of compound ascoma = 70 mm. Photo by Noah Siegel.

Morphological study.—Macroscopic characters were documented in the laboratory with an SC30 camera mounted on an SZ61 dissecting microscope (Olympus, Center Valley, Pennsylvania). Microscopic structures were observed using an Olympus BH-2 bright-field microscope and Eclipse E600 microscope (Nikon, Melville, New York) with differential interference contrast (DIC) optics and photographed using the same SC30 camera and with Olympus cellSens 1.18 and Piximètre 5.10 (http://ach.log.free.fr/Piximetre/). Descriptions of microscopic features were made from material rehydrated in tap water. Mounts in Lugol's solution (IKI), 5% KOH, and 1% Congo red were used to visualize structures and color-changing reactions. Measurements in the morphological description are given as (a–)b–c(–d), with b and c representing average minus/plus standard deviation and a and d representing extreme values. In addition, the following abbreviations and notations are used: Me = average, Q = length/width ratio, Qe = average length/width ratio, and {n,m,p} = "n" structures measured from "m" ascomata of "p" collections. Terminology to describe shape, orientation, and presence of cells in gel follows Hengstmengel (2020). Micrographs were edited and plates were assembled in Inkscape 0.92 (https://inkscape.org/) and Adobe Illustrator 25.1 (San Jose, California).

DNA extraction, PCR amplification, sequencing.—Total genomic DNA was extracted using the QIAamp

Figure 2. *Sclerococcum martynii*, collection MCA 3114. a. Compound ascomata with swellings at the sites of apothecia, staged on a leaf for better contrast in the field. b. Field habit, top view of many-branched compound ascoma thinning toward apices. c. Close-up of overlapping, irregularly lobed apothecia on branch tips. d. Desiccated compound ascoma. e. Cross-section of compound ascoma rehydrated in H_2O, with shiny, gelatinous interior and thin layer of sessile apothecia (top) or sterile outer wall (bottom). f. Close-up of cross-section including gelatinous interior and subsessile apothecium. Bars: a, b = 2 cm; c = 2 mm; d = 1 cm; e, f = 0.5 mm.

DNA Micro Kit (Qiagen, Stanford, California), the E.Z.N.A. HP Fungal DNA Kit (Omega Bio-tek, Norcross, Georgia), and the Wizard Genomic DNA Purification Kit (Promega, Madison, Wisconsin), following the manufacturers' instructions. The following gene regions were amplified: small and large subunit (18S, 28S) ribosomal RNA genes, internal transcribed spacer (ITS) region, the second largest subunit of the RNA polymerase II gene (*rpb2*), and translation elongation factor 1-α (*tef1*). We used the following primer combinations for polymerase chain reaction (PCR) amplifications: NS1/NS4 for 18S (White et al. 1990), ITS1f/ITS4 and ITS1f/ITS4A for ITS (Gardes and Bruns 1993; Larena et al. 1999; White et al. 1990), LR0R/LR5 for the 28S D1–D2 domains (Hopple 1994; Vilgalys and Hester 1990), RPB2-6F/RPB2-7R and RPB2-6F/RPB2-7.1R for *rpb2* (Matheny 2005), and EF1-983F/EF1-2218R for *tef1* (Rehner and Buckley 2005). PCRs were done on a Pro S Mastercycler (Eppendorf, Hauppauge, New York) in 25-µL reactions with 12.5 µL of 2× MyTaq Mix (Bioline, Swedesboro, New Jersey), 9.5 µL of double-distilled water (ddH_2O), 1.0 µL for each 10 mM primer, and 1.0 µL of DNA extract.

Cycling conditions for 18S and 28S followed Liu et al. (2020). For 18S: initial denaturation at 95 C for 5 min; then 40 cycles of denaturation at 95 C for 30s, annealing at 55 C for 45s, and extension at 72 C for 45s; and final extension at 72 C for 1 min. For 28S: initial denaturation at 94 C for 5 min; then 35 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 ITS: initial denaturation at 94 C for 5 min; then 40 cycles of denaturation at 94 C for 30s, annealing at 48 C for 45s, and extension at 72 C for 45s; and final extension at 72 C for 7 min. For *rpb2*: initial denaturation at 95 C for 4 min; then 45 cycles of denaturation at 95 C for 1 min, annealing at 50 C for 1 min with 0.3 C increase per cycle, and extension at 72 C for 1 min; and final extension at 72 C for 10 min. Finally, for *tef1*, cycling conditions followed Gómez-Zapata et al. (2021): initial denaturation at 95 C for 10 min; then 30 cycles of denaturation at 95 C for 1 min, annealing at 62 C for 1 min with 1 C decrease every 3 cycles, and extension at 72 C for 90s; then 30 cycles of denaturation at 95 C for 30s, annealing at 55 C for 30s, and extension at 72 C for 1 min; and final extension at 72 C for 7 min.

PCR products were loaded onto Tris-acetate-EDTA (TAE) 1% agarose gels for electrophoresis at 130 V for 30 min to check for successful amplification. Ultraviolet transillumination on a Gel Doc EZ imager (Bio-Rad, Hercules, California) was used to evaluate product size. Purification and Sanger sequencing were outsourced to GENEWIZ (South Plainfield, New Jersey). Sequence reads were assembled and edited in Sequencher 5.4.6 (Gene Codes, Ann Arbor, Michigan). We submitted edited sequences to the National Center for Biotechnology Information (NCBI) GenBank sequence database (https://www.ncbi.nlm.nih.gov/genbank/).

We attempted DNA extraction of the holotype of *P. martynii* (K(M) 264777) with the QIAamp DNA Micro Kit followed by PCR amplification of the ITS region using different primer combinations: ITS1f/ITS4, ITS1f/ITS4A, ITS9mun/ITS4, ITS1f/ITS2, and ITS3/ITS4 (Egger 1995; White et al. 1990). However, none of these resulted in successful amplicons.

Phylogenetic analyses.—First, we used T-BAS 2.1 (Carbone et al. 2019) and the "Place Unknowns" tool to place newly generated *Polydiscidium* sequences onto the Ascomycota-wide tree from Blackwell et al. (2020), named "Laboulbeniomycetes v2." Four FASTA files with unaligned 18S, 28S, *rpb2*, and *tef1* sequences of *Polydiscidium* were uploaded to the T-BAS interface. We selected the "de novo" option for the RAxML placement, with GTRGAMMA as substitution model, *Rhizopus oryzae* (Mucoromycetes) as outgroup, and 200 bootstrap replicates.

Based on the results of this preliminary phylogenetic analysis, we constructed a four-locus data set (sensu Olariaga et al. 2019) of Sclerococcales and related taxa within Eurotiomycetes to investigate the exact placement of *Polydiscidium*. We selected 31 isolates of Sclerococcales and 18 taxa in closely related orders (Coryneliales, Eurotiales, Onygenales, Mycocaliciales, Pyrenulales, Verrucariales) as the ingroup, and *Geoglossum nigritum* (Geoglossomycetes) as the outgroup taxon (details in TABLE 1). Sequences of each of four loci (18S, ITS, 28S, mtSSU) were aligned using MUSCLE 3.7 (Edgar 2004), available on the CIPRES Science Gateway (Miller et al. 2010). Next, ambiguously aligned regions and uninformative regions were removed using the command-line version of TrimAl 1.3 (Capella-Gutiérrez et al. 2009) with the following parameters: gap threshold (-gt) = 0.60 and minimum coverage (-cons) = 0.50. We concatenated the sequences of the four loci in MEGA7 (Kumar et al. 2016) to create a supermatrix with data for 50 DNA isolates. Appropriate models of nucleotide substitution were selected under the Akaike information criterion (AIC) using ModelFinder on the command line (Kalyaanamoorthy et al. 2017). Maximum likelihood (ML) was inferred under partitioned models using IQ-TREE (Nguyen et al. 2015; Chernomor et al. 2016). Rapid bootstrapping was inferred under 1000 replicates (Hoang et al. 2017). The resulting tree topology with bootstrap

Table 1. Species names, order-level classification, DNA isolate/voucher information, GenBank accession numbers, and reference(s) of taxa included in this study.

Order	Isolate/voucher	Genus	Species	18S	ITS	28S	mtSSU	Reference(s)
Coryneliales	CBS 138.64	*Caliciopsis*	*orientalis*	DQ471039	NR_145392	DQ470987	FJ190654	Spatafora et al. (2006); Schoch et al. (2009); Wood et al. (2016)
Coryneliales	CBS 139.64	*Caliciopsis*	*pinea*	DQ678043	KP881691	DQ678097	FJ190653	Schoch et al. (2006, 2009); Wood et al. (2016)
Eurotiales	CBS 658.74	*Aspergillus*	*protuberus*	FJ176842		FJ176897		Schoch et al. (2009)
Eurotiales	DUKE Geiser (culture)	*Eupenicillium*	*javanicum*	EF413620	KT232212	EF413621	FJ225778	Geiser et al. (2006); Gueidan et al. (2008); Carbone et al. (2017)
Eurotiales	CBS 339.97	*Eupenicillium*	*limosum*	EF411061	NR_111496	EF411064		Geiser et al. (2006); Schoch et al. (2014)
Eurotiales	DAOM 216705	*Penicillium*	*freii*	AY640998	KT232211	AY640958	AY584712	Lutzoni et al. (2004); Reeb et al. (2004); Carbone et al. (2017)
Eurotiales	CBS 78893	*Trichocoma*	*paradoxa*	FJ358354		FJ358290	FJ225782	Gueidan et al. (2008)
Geoglossales	OSC 100009	*Geoglossum*	*nigritum*	AY544694	DQ491490	AY544650	AY544740	Lutzoni et al. (2004); James et al. (2006)
Mycocaliciales	DUKE 47956	*Sphinctrina*	*turbinata*	EF413631		EF413632	FJ713611	Geiser et al. (2006); Schoch et al. (2009)
Onygenales	CBS 272.66	*Arthroderma*	*ciferrii*	EF413624		EF413625	FJ225787	Geiser et al. (2006); Gueidan et al. (2008)
Onygenales	CBS 402.96	*Ascosphaera*	*apis*	FJ358343		FJ358275		Gueidan et al. (2008)
Onygenales	CBS 281.48	*Onygena*	*corvina*	FJ358352		AB075355	FJ225792	Sugiyama et al. (2002); Gueidan et al. (2008)
Onygenales	CBS 576.63	*Spiromastix*	*warcupii*	DQ782882	NR_144929	DQ782909	FJ225794	James et al. (2006); Gueidan et al. (2008); Rodríguez-Andrade et al. (2021)
Pyrenulales	CBS 109078	*Pyrenula*	*aspistea*	EF411060		EF411063		Geiser et al. (2006)
Pyrenulales	DUKE 47599	*Pyrenula*	*pseudobufonia*	AY641001	NR_119610	AY640962	AY584720	Lutzoni et al. (2004); Reeb et al. (2004); Schoch et al. (2014)
Pyrenulales	DUKE Lutzoni03.24.03-9	*Pyrgillus*	*javanicus*	DQ823110	DQ826741	DQ823103	FJ225774	James et al. (2006); Gueidan et al. (2008)
Scleroccales	MFLUCC 11-0294	*Cylindroconidiis*	*aquaticus*	MH236580	MH236576	MH236579		Yu et al. (2018)
Scleroccales	CBS 709.88	*Fusichalara*	*minuta*	KX537773	KX537754	KX537758	KX537762	Réblová et al. (2017)
Scleroccales	ARAN Fungi 6619	*Pseudosclerococcum*	*golindoi*	MK759887	MK759885	MK759890	MK759897	Olariaga et al. (2019)
Scleroccales	CBS 129.74	*Rhopalophora*	*clavispora*		KX537751	KX537755	KX537759	Réblová et al. (2017)
Scleroccales	CBS 281.75	*Rhopalophora*	*clavispora*	KX537771	KX537752	KX537756	KX537760	Réblová et al. (2017)
Scleroccales	CBS 637.73	*Rhopalophora*	*clavispora*	KX537772	KX537753	KX537757	KX537761	Réblová et al. (2017)
Scleroccales	H RP23	*Sclerococcum*	*ahtii*		KY661630	KY661659	KY661686	Pino-Bodas et al. (2017)
Scleroccales	H RP127	*Sclerococcum*	*ahtii*		KY661618			Pino-Bodas et al. (2017)
Scleroccales	H RP182	*Sclerococcum*	*ahtii*		KY661622		KY661687	Pino-Bodas et al. (2017)
Scleroccales	H RP235	*Sclerococcum*	*deminutum*		KY661629			Pino-Bodas et al. (2017)
Scleroccales	LE 261065	*Sclerococcum*	*glaucomarioides*		KY661632	KY661660	KY661683	Pino-Bodas et al. (2017)
Scleroccales	JK 5448A	*Sclerococcum*	*haliotrephum*	FJ176802		FJ176855	KJ766382	Schoch et al. (2009); Miadlikowska et al. (2014)
Scleroccales	Diederich 18109	*Sclerococcum*	*lobariellum*			MH698499	MH698503	Diederich et al. (2018)
Scleroccales	Diederich 17708	*Sclerococcum*	*lobariellum*			MH698498	MH698502	Diederich et al. (2018)
Scleroccales	ARAN Fungi 10091	*Sclerococcum*	*lobariellum*			MK759891	MK759898	Olariaga et al. (2019)
Scleroccales	CBS 110444	*Sclerococcum*	*mangrovei*	FJ176836		FJ176890	KJ766383	Schoch et al. (2009); Miadlikowska et al. (2014)
Scleroccales	MCA 7061	*Sclerococcum*	*martynii*	MZ221215	MZ221610	MZ221619		This study
Scleroccales	MCA 1207	*Sclerococcum*	*martynii*		MZ221612	MZ221620		This study
Scleroccales	MCA 3114	*Sclerococcum*	*martynii*		MZ221615	MZ221622		This study
Scleroccales	MCA 5794	*Sclerococcum*	*martynii*		MZ221616	MZ221623		This study
Scleroccales	ARAN Fungi 2724	*Sclerococcum*	*parasiticum*	MK759888		MK759892	MK759899	Olariaga et al. (2019)
Scleroccales	LE 260868	*Sclerococcum*	*parasiticum*		KY661646	KY661666	KY661690	Pino-Bodas et al. (2017)
Scleroccales	S F283586	*Sclerococcum*	*parasiticum*			MK759894	MK759901	Olariaga et al. (2019)
Scleroccales	S F283587	*Sclerococcum*	*parasiticum*			MK759895	MK759902	Olariaga et al. (2019)
Scleroccales	ARAN Fungi A3044025	*Sclerococcum*	*parasiticum*			MK759893	MK759900	Olariaga et al. (2019)
Scleroccales	Diederich 17279	*Sclerococcum*	*sphaerale*			JX081672	JX081677	Diederich et al. (2013)
Scleroccales	BR Ertz 17425	*Sclerococcum*	*sphaerale*			JX081674	JX081676	Diederich et al. (2013)
Scleroccales	ARAN Fungi 3395	*Sclerococcum*	*stygium*	MK759889		MK759896	MK759903	Olariaga et al. (2019)

(*Continued*)

Table 1. (Continued).

Order	Isolate/voucher	Genus	Species	18S	ITS	28S	mtSSU	Reference(s)
Sclerococcales	ARAN Fungi 823	*Sclerococcum*	*stygium*		MK759886		MK759904	Olariaga et al. (2019)
Sclerococcales	FH 00458262 (BHI-F312a)	*Sclerococcum*	*stygium*		MF161218			Haelewaters et al. (2018)
Sclerococcales	NTOU 4002	*Sclerococcum*	*vrijmoediae*	KC692152	NR_138396	KC692153		Pang et al. (2014)
Verrucariales	NYBG 808041	*Agonimia*	sp.	DQ782885	DQ826742	DQ782913	KT232220	James et al. (2006); Carbone et al. (2017)
Verrucariales	DUKE 47501	*Dermatocarpon*	*miniatum*	AY584668	DQ782837	AY584644	AY584616	Lutzoni et al. (2004); James et al. (2006)
Verrucariales	DUKE K. Knudsen 733	*Placidum*	*lacinulatum*			EF643762	FJ225689	Gueidan et al. (2007, 2008)

support values was visualized in FigTree 1.4.3 (http://tree.bio.ed.ac.uk/software/figtree/) and edited using Adobe Illustrator 25.1.

RESULTS

Placement onto the Laboulbeniomycetes v2 reference tree in T-BAS revealed the placement of three isolates of *Polydiscidium* in Eurotiomycetes, in a maximum-supported clade with *Sclerococcum haliotrephum* and *S. mangrovei* (FIG. 3). Our four-locus data set included 50 isolates and 3677 characters, of which 1899 were constant and 1244 were parsimony-informative. The number of total and parsimony-informative characters by locus as well as their evolutionary models as selected by ModelFinder Plus are shown in TABLE 2. The phylogenetic reconstruction of our 18S-ITS-28S-mtSSU data set resulted in the placement of *P. martynii* in Sclerococcales (FIG. 4). Our four isolates of *P. martynii* were nested within the genus *Sclerococcum*, as sister to *Sclerococcum stygium* with maximum support. The single isolate from Cameroon (MCA 7061) was retrieved as sister to three isolates from Guyana (MCA 1207, MCA 3114, MCA 5794).

TAXONOMY

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Dactylosporaceae Bellem. & Hafellner, Cryptogamie Mycologie 3:79. 1982.

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Sclerococcum martynii (Wakef.) Haelew., Stallman & Aime, comb. nov. FIGS. 1, 2, 5

MycoBank MB270620

≡ *Polydiscidium martynii* Wakef., Bulletin of Miscellaneous Information (Royal Botanic Gardens, Kew) 1934(6):256. 1934. Basionym.

Typification: GUYANA. UPPER TAKUTU-UPPER ESSEQUIBO: Essequibo River watershed, Upper Arawau River banks, on rotten wood, May 1929, *Eldred Bridgeman Martyn, Martyn 43* (**holotype** K(M) 264777).

Holotype verbatim: Hab. in ligno putrido, Upper Arawan River, Guiana Anglica, *Martyn 43*, May 1929.

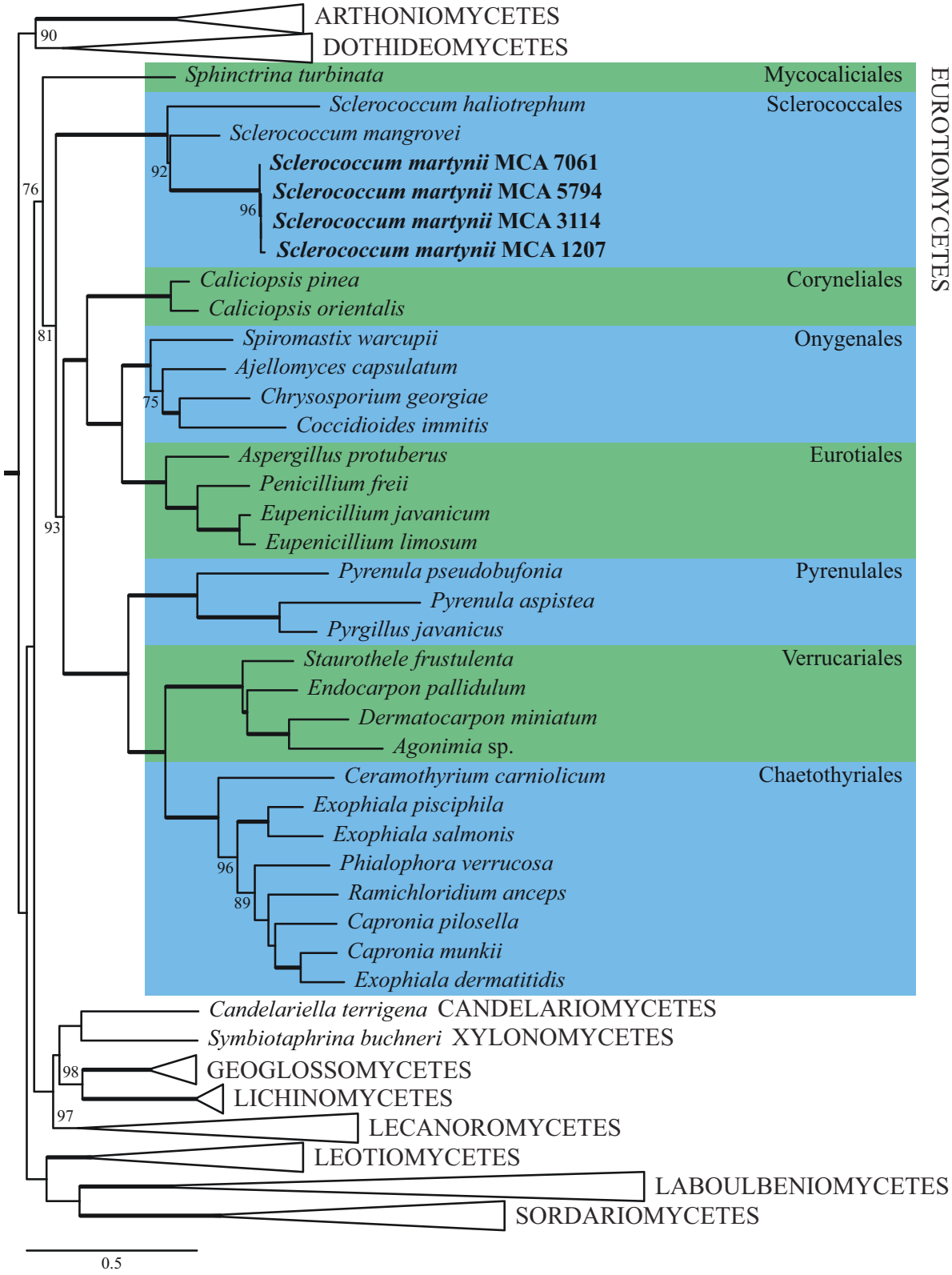

Figure 3. Placement of *Polydiscidium martynii* onto the Ascomycota-wide Laboulbeniomycetes v2 reference tree in T-BAS. The tree is the result of a RAxML analysis with 200 bootstrap replicates. Clades are collapsed to class level except for Eurotiomycetes. For each node, the ML bootstrap support (≥70) is presented above or below the branch leading to that node. Thick branches indicate maximum support. Newly generated sequence data in boldface.

Table 2. Overview of number of characters (total, informative, constant) and selected model of nucleotide substitution, by locus.

Locus	Sequences	Sites	Informative	Constant	Model	−lnL
18S	29	1451	313	895	TIM2+F+G4	19 761.872
ITS	30	473	239	170	SYM+I+G4	5533.730
28S	45	1019	339	547	TIM+F+I+G4	16 548.067
mtSSU	37	734	353	287	GTR+F+R3	9050.272

Diagnosis: Different from other species of *Sclerococcum* in its terrestrial-lignicolous habit and large, compound ascomata on which apothecia develop on multiple cespitose branches; microscopically different from its closest relative, *S. stygium*, in having smaller asci and ascospores.

Description: Compound ascomata 0.5–3.5 cm high × 0.5–4.5 cm wide, individual branches 0.5–3.0 × 0.1–0.6 cm (desiccated); 1.5–5.0 cm high × 4–10 cm wide, individual branches 1.5–4.0 × 0.2–0.8(–1.0) cm (rehydrated); consisting of a black somatic "thallus" with 2–8 cespitose branches emerging at or above a common base and dividing upward into further branches; branches irregular, either narrowing, broadening, or remaining the same width with apothecia toward the apices. Ascomata apothecial, <2 mm diam (desiccated), up to 5 mm diam (rehydrated), densely gregarious on distal branches of the ascomata, often overlapping and/or conjoined, dissipating progressively down subbranches toward base, pulvinate to convex, subsessile to sessile; margin irregularly lobed, often in-rolled; hymenium with occasional lobes or breaks in the center. Asci (39.4–)47.6–57.2(–67.0) × (3.4–)4.5–6.3(–8.0) µm {82,7,3}, Me = 52.4 × 5.4 µm, embedded in hyaline to light brown gel, unitunicate, cylindrical to narrowly clavate with rounded apex, with eight ascospores, usually uniseriate but sometimes irregularly arranged, lacking an apical ring reacting in IKI but with external gelatinous cap turning blue in IKI and embedded in hyaline to brown gelatinous hymenium, with upper regions near ascus apices also bluing in IKI; crosiers not observed. Ascospores (4.7–)5.8–7.7(–10.1) × (2.2–)2.7–3.2(–3.7) µm {543,10,7}, Q = (1.6–)1.9–2.8(–3.6), Me = 6.8 × 3.0 µm, Qe = 2.3, fusiform to broadly fusiform, occasionally ellipsoid, uniseptate, bilateral or not bilateral, with one side slightly elongated, more rounded (elliptical), or less commonly offset at an angle, often with constriction of wall at the septum, smooth, hyaline when young, brown with age; lacking visible lipid bodies or other internal structures. Paraphyses 46.0–67.5 × 1–2 µm, septate, filiform, hyaline, unbranched, embedded in hyaline to light brown gel, often with narrowly clavate terminal cell up to 3 µm diam; terminal cell occasionally with brownish pigment. Subhymenium brown, composed of tightly packed, short cylindrical hyphae to ellipsoid cells in a gelatinous matrix. Ectal excipulum a textura globulosa incrassata 40–80 µm thick with globose to ellipsoid cells 7–20 µm diam, with dark brown, crystal-like extraparietal material in outermost region. Medullary excipulum a loose textura intricata imbuta; hyphae irregular, cylindrical, and 2–4 µm diam and up to 100 µm in length, or shorter and "bone-shaped" with capitate swellings up to 13 µm diam at ends where meeting other hyphae; uncommonly branched; gel brown. Asexual morph unknown.

Habitat and distribution: Solitary to scattered on decaying hardwood logs in tropical rainforest. Known from the holotype locality and elsewhere in Guyana, Suriname, Trinidad, Venezuela, Cameroon, and D.R. Congo.

Specimens examined: CAMEROON. EAST REGION: Dja Biosphere Reserve, within 2 km of a base camp located at 3°21′29.8″N, 12°43′46.9″E, 650 m a.s.l., on rotted hardwood, 23 Nov 2016, *M. Catherine Aime MCA 7061* (PUL F27741; YA), GenBank: 18S = MZ221215, ITS = MZ221610, 28S = MZ221619, *rpb2* = MZ229308. GUYANA. CUYUNI-MAZARUNI: Manaka (Forestry Training Center), within 100 m W of base camp, 06°42′37.7″N, 58°52′07.0″W, 82 m a.s.l., on unidentified rotten hardwood in mixed greenheart forest on white sand soils, 21 Jul 2019, *Dillon R. Husbands DRH 238* (PUL F27742; BRG); POTARO-SIPARUNI: Pakaraima Mountains, Upper Potaro River Basin, 20 km E of Mount Ayanganna, within 5 km of base camp located at 5°18′04.8″N, 59°54′40.4″W, ~800 m a.s.l., on rotted hardwood, 12 Jun 2000, *Steven L. Miller MCA 1207* (PUL F27737; BRG), GenBank: ITS = MZ221612, MZ221613, 28S = MZ221620, MZ221621; ibid., on rotted hardwood, 27 Jun 2006, *M. Catherine Aime MCA 3114* (PUL F27736; BRG), GenBank: ITS = MZ221615, 28S = MZ221622; ~15 km E of Mount Ayanganna, Potaro base camp at 5° 18′04.8″N, 59°54′40.4″W, 710–750 m a.s.l., 1 km SE of base camp on Benny's Ridge, on large-diameter partially decomposed hardwood log, 10 Jul 2000, *Terry W. Henkel TH 7487* (HSC G1347; BRG); 4 km SW of base camp in vicinity of *Dicymbe* mushroom plot 3, on dead wood, 5 May 2001, *Terry W. Henkel TH 8039* (HSC G1348; BRG); 3 km SW of base camp in vicinity of *Dicymbe* mushroom plot 3, on large-diameter decaying hardwood log, 16 Jul 2003, *Terry W. Henkel TH 8570*

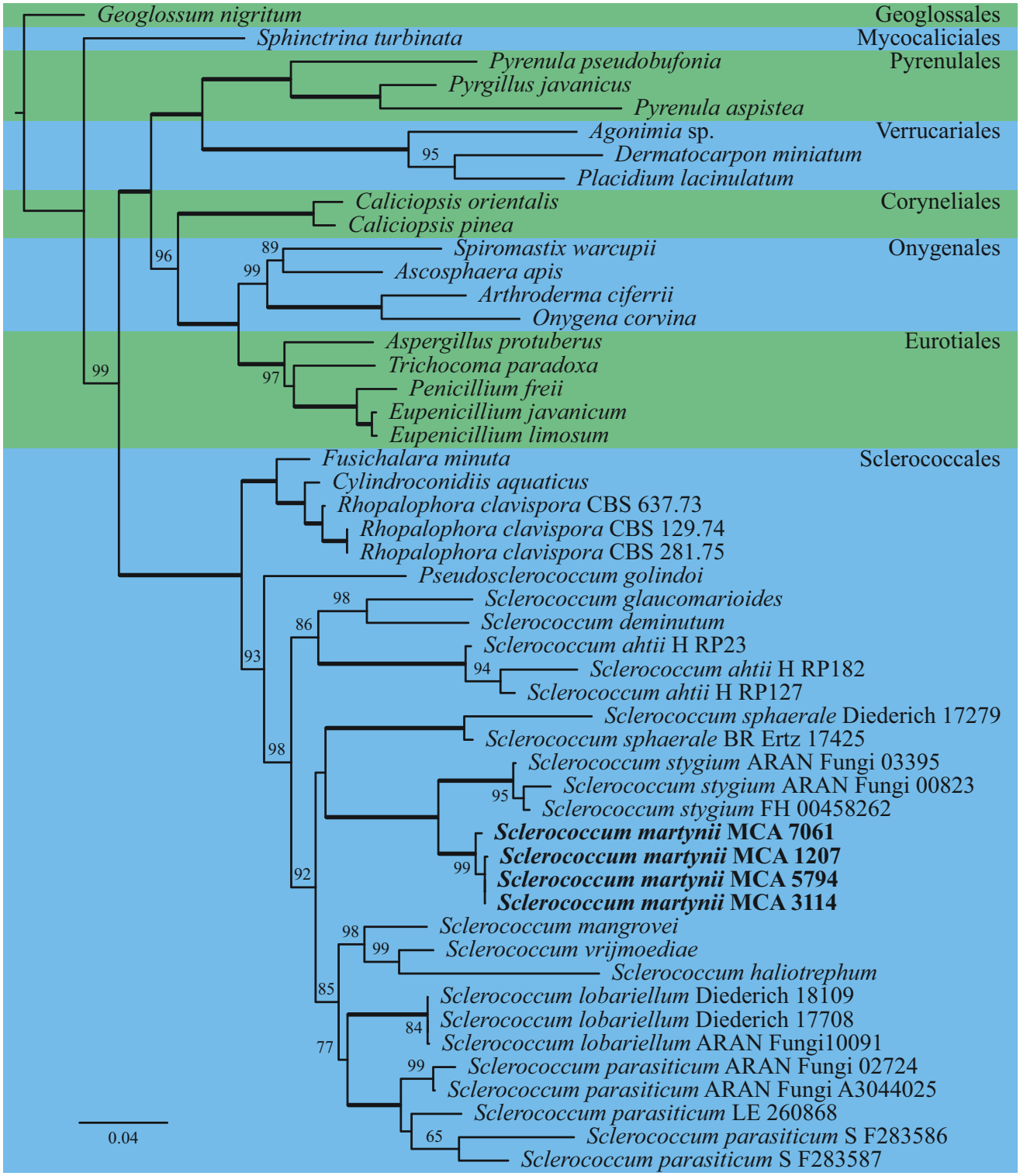

Figure 4. Phylogenetic placement of *Sclerococcum martynii*, comb. nov., in Sclerococcales, reconstructed from a combined data set of 18S, ITS, 28S, and mtSSU sequences. The topology is the result of maximum likelihood inference performed with IQ-TREE. For each node, ML bootstrap support (≥70) is presented above or below the branch leading to that node. Thick branches indicate maximum support. Newly generated sequence data in boldface.

(HSC G1349; BRG); 300 m SE of base camp on Benny's Ridge, on large-diameter decaying hardwood log, 9 Jan 2022, *Terry W. Henkel TH 11122* (HSC G1409; BRG); Pakaraima Mountains, Upper Potaro River Basin, walk to Potaro Falls, on well-decorticated log, 4 Jul 2002, *M. Catherine Aime MCA 2195* (PUL F27738;

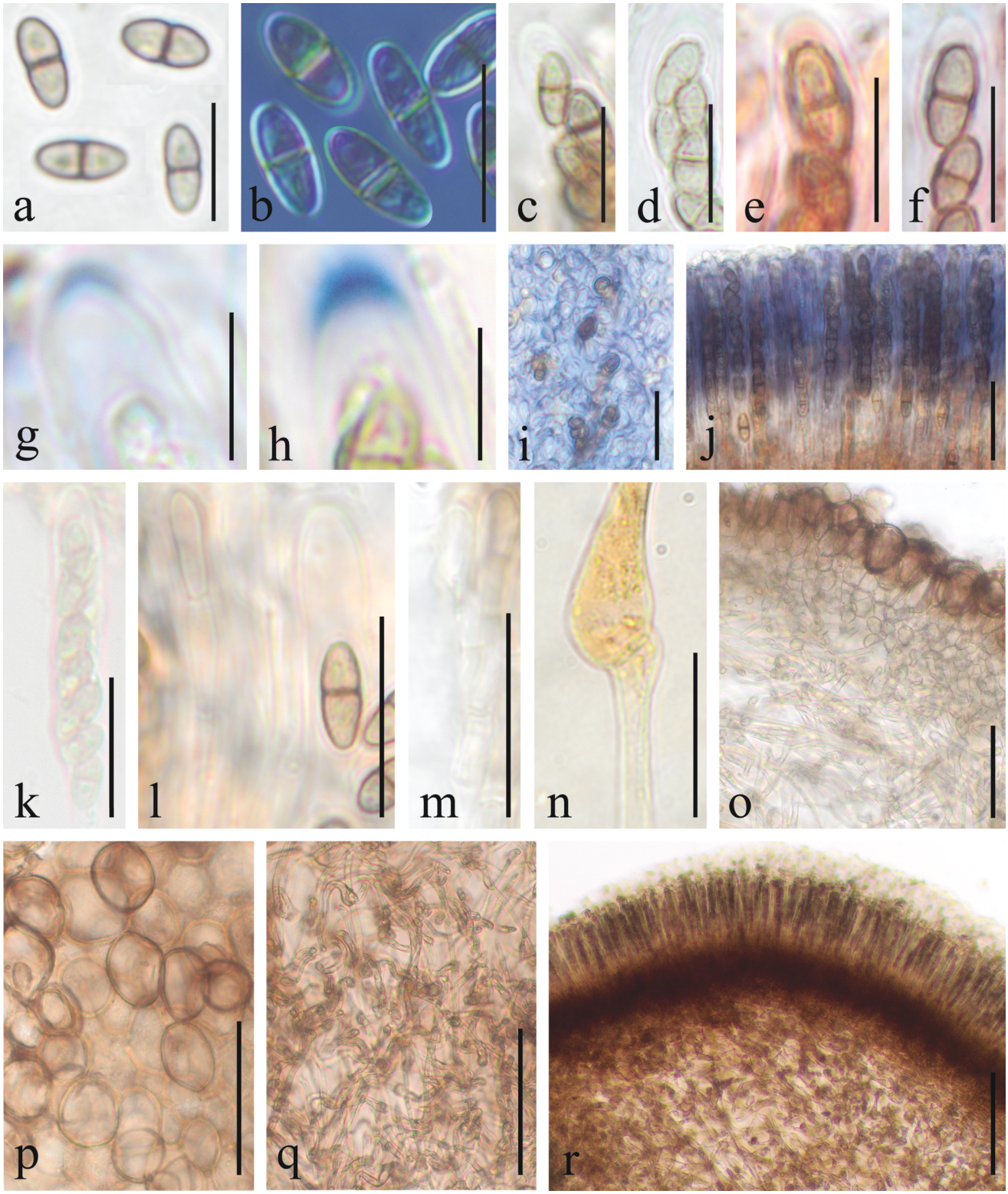

Figure 5. Micromorphological features of *Sclerococcum martynii*, collection MCA 3114. a. Ascospores in KOH. b. Ascospores in H_2O observed with DIC optics. c–d. Ascal apices and spores in H_2O. e–f. Ascus apex in H_2O stained with Congo red. g–h. Amyloid gel at ascus apex in IKI. i. Squash mount of amyloid hymenial gel in IKI. j. Cross-section of hymenium showing immersion in amyloid gel in IKI. k. Ascus with spores in KOH. l. Ascus and paraphysis in KOH. m. Paraphyses in KOH. n. Irregular connection and swelling of hyphal tips in textura intricata imbuta of medullary excipulum in IKI. o. Textura intricata imbuta of medullary excipulum grading into textura globulosa incrassata of outer wall in KOH. p. Close-up of outer wall in KOH. q. Textura intricata imbuta of medullary excipulum in KOH. r. Cross-section of apothecium in H_2O. Bars: a–f = 10 µm; g, h = 5 µm; i–n = 20 µm; o, p = 60 µm; q, r = 100 µm.

BRG), GenBank: ITS = MZ221614, 28S = MZ222283; vicinity of old Ayanganna airstrip, ~720 m a.s.l., on rotted hardwood, 18 Jun 2015, *M. Catherine Aime MCA 5794* (PUL F27739; BRG), GenBank: ITS = MZ221616, 28S = MZ221623, *rpb2* = MZ229309, *tef1* = MZ229307; UPPER TAKUTU-UPPER ESSEQUIBO: Essequibo River watershed, Upper Arawau River banks, on rotten wood, May 1929, *E.B. Martyn 43* (holotype K(M) 264777!); UPPER DEMERARA-BERBICE: Mabura Ecological Reserve, within 2 km of a field station located at 5°09′19.0″N, 58°41′58.9″W, 100 m a.s.l., in monodominant stand of *Dicymbe altsonii*, on rotted hardwood, 22 May 2011, *M. Catherine Aime MCA 4268* (PUL F2774; BRG), GenBank: ITS = MZ221611.

Notes: The collections of *S. martynii* examined here were in general agreement morphologically with the type description provided by Wakefield (1934) and those of Cash (1958) and Dennis (1961). As also noted by Dennis (1961) when comparing African and tropical American material, we found the ascospores of our single Cameroonian collection to be slightly smaller than those of Guyanese specimens but the absolute values overlapped: (4.9–)5.8–7.4(–8.9) × (2.2–)2.7–3.1(–3.4) µm {250,3,1} (Cameroon) vs. (4.7–)5.9–7.9(–10.1) × (2.3–)2.8–3.3(–3.7) µm {293,7,6} (Guyana). Our average ascus length of ~54 µm is closer to that of Dennis (~50 µm) than the ranges reported by both Cash and Wakefield (each 45–50 µm), although these minor differences may have resulted from the difficulty of measuring asci that are firmly embedded in a gelatinous matrix. Wakefield (1934) reported that the ascospores were not constricted at the septum, but in our material a constriction was evident in many ascospores.

Within the genus *Sclerococcum, S. martynii* differs from other species in its large compound ascomata with cespitose branches bearing distal apothecia that are often overlapping or fused. The species most closely related to *S. martynii* in our data set is *S. stygium*, which is easily differentiated micromorphologically by its considerably larger asci (50–75 × 6–10 µm) and larger ascospores (15–20 × 4–6 µm) (Butler 1940 [as *Karschia stygia*]). Olariaga et al. (2019) noted that some *Sclerococcum* species have an easily overlooked hemiamyloid gelatinous hymenium, but this was not observed in *S. martynii*. Other *Sclerococcum* species with known teleomorphs can be differentiated by ascomatal structure, ascospore morphology, and habitat. As opposed to the tropical rainforest hardwood substrata of *S. martynii*, most tropical lignicolous *Sclerococcum* species are found in shallow-water marine environments. Examples are *S. haliotrepha* and *S. mangrovei*, which also differ in their much longer ascospores compared with those of *S. martynii* (Au et al. 1996; Jones et al. 1999), and *S. vrijmoediae* (Pang et al. 2014), which differs in its broader ascospores with hyaline sheaths at the septum. Two recently described terrestrial-lignicolous species of *Dactylospora* from Thailand (formally transferred to *Sclerococcum* below), *D. chiangraiensis* and *D. fusiformis*, are both much smaller than *S. martynii* with longer ascospores (Ekanayaka et al. 2019b).

Sclerococcum chiangraiensis (A.H. Ekanayaka, E.B.G. Jones, Q. Zhao & K.D. Hyde) Haelew., Stallman & Aime, comb. nov.
Index Fungorum IF559694

≡ *Dactylospora chiangraiensis* A.H. Ekanayaka, E.B.G. Jones, Q. Zhao & K.D. Hyde, Cryptogamie Mycologie 40(3):28. 2019. Basionym.

Sclerococcum fusiformis (A.H. Ekanayaka, E.B.G. Jones, Q. Zhao & K.D. Hyde) Haelew., Stallman & Aime, comb. nov.
Index Fungorum IF559695

≡ *Dactylospora fusiformis* A.H. Ekanayaka, E.B.G. Jones, Q. Zhao & K.D. Hyde, Cryptogamie Mycologie 40(3):32. 2019. Basionym.

Notes: These combinations are made based on the placement of the holotype sequences. Both species are phylogenetically deeply nested within *Sclerococcum* (= *Dactylospora*) (Ekanayaka et al. 2019b).

DISCUSSION

Multiple lines of evidence place the enigmatic discomycete *P. martynii* in the genus *Sclerococcum* (Eurotiomycetes, Sclerococcales, Dactylosporaceae). Ekanayaka et al. (2019b) created the order Dactylosporales, which is here synonymized with Sclerococcales (erected by Réblová et al. 2017) based on morphological diagnoses and molecular phylogenetic data. Ekanayaka et al. (2019b) may not have been aware of the order Sclerococcales, as they failed to cite Réblová et al. (2017) in their treatment of *Dactylospora*. Réblová et al. (2017) also introduced the family Sclerococcaceae, which in turn is a synonym of Dactylosporaceae (Diederich et al. 2018). *Dactylospora* is considered a synonym of *Sclerococcum* after *D. parasitica*, the type of *Dactylospora*, was found to be closely related to *S. sphaerale*, the type of *Sclerococcum* (Diederich et al. 2018; Pino-Bodas et al. 2017).

The large, compound ascomata of *S. martynii* are exceptional for *Sclerococcum*, continuing to broaden the concept of this genus. *Sclerococcum* was originally erected for *Spiloma sphaerale* (Fries 1825) but soon fell out of use when Nylander (1856) included *S. sphaerale* in

Table 3. *Sclerococcum martynii* collections studied micromorphologically with ascospore measurements.

Collection(s)	Location	Ascospores length × width {n,m,p}*
DRH 238	Guyana	(5.3–)6.2–8.1(–9.1) × (2.4–)2.8–3.3(–3.7) µm {50,1,1}
MCA 1207	Guyana	(4.7–)5.7–7.4(–8.6) × (2.4–)2.7–3.2(–3.6) µm {50,1,1}
MCA 2195	Guyana	(4.9–)6.1–8.4(–10.1) × (2.3–)2.7–3.4(–3.5) µm {50,1,1}
MCA 3114	Guyana	(5.3–)6.0–7.9(–9.4) × (2.4–)2.7–3.2(–3.3) µm {50,2,1}
MCA 4268	Guyana	(4.9–)5.7–7.6(–8.7) × (2.3–)2.7–3.3(–3.6) µm {43,1,1}
MCA 5794	Guyana	(4.9–)5.7–8.0(–9.2) × (2.5–)2.8–3.2(–3.5) µm {50,1,1}
MCA 7061	Cameroon	(4.9–)5.8–7.4(–8.9) × (2.2–)2.7–3.1(–3.4) µm {250,3,1}
All (Guyana)		(4.7–)5.9–7.9(–10.1) × (2.3–)2.8–3.3(–3.7) µm {293,7,6}
All (Cameroon, Guyana)		(4.7–)5.8–7.7(–10.1) × (2.2–)2.7–3.2(–3.7) µm {543,10,7}

*Measurements are shown as (a–)b–c(–d), with b and c representing average minus/plus standard deviation and a and d representing extreme values; {n,m,p} = "n" ascospores measured from "m" ascomata of "p" collections.

his new genus *Spilomium*. Not until Hawksworth (1975) rejected the name *Spilomium* was *Sclerococcum* rescued from synonymy. The genus was only known from the asexual *S. sphaerale* until new taxa sharing the lichenicolous ecology began to be added in the late 20th century (e.g., Diederich 1990). Diederich et al. (2013) revealed a relationship of *Sclerococcum* to marine species of *Dactylospora* based on a multilocus phylogenetic analysis, which later broadened to lichenicolous, terrestrial-lignicolous, and fungicolous *Dactylospora* species, resulting in formal combinations (Diederich et al. 2013, 2018; Olariaga et al. 2019). Currently, *Sclerococcum* is mostly characterized by small (<1 mm) asexual or sexual lichenicolous species, or sexual saprotrophs on wood in marine or terrestrial environments. Recently, more species sharing the terrestrial-lignicolous habit of *S. martynii* have been described from southeastern Asia (Ekanayaka et al. 2019b). This indicates that there may be more diversity within this ecological group of *Sclerococcum*, although thus far none are morphologically as conspicuous as *S. martynii*. Dramatic macromorphological differences notwithstanding, the microscopic features of *S. martynii* of gelatinized hymenium, asci with an amyloid gel cap, and septate brown ascospores are consistent with other sexual taxa in *Sclerococcum* (FIG. 5).

The ITS sequence of the Cameroonian DNA isolate shares 96–97% identity (13–16 bp differences, 3–5 gaps, 528–548 bp total length) with the Guyanese isolates. Despite separation between South America and Africa since the Cretaceous (Guiraud et al. 1992), the only morphological difference among the intercontinental collections is the slightly smaller ascospores in the African material (Dennis 1961; this study). A review of ascospore measurements from all published records, however, revealed that this character is variable and at least partly overlapping when comparing between continents: Guyana, 7–8 × 3–4 µm (Wakefield 1934) and 6.9 × 3 µm (this study); Trinidad, 7–10 × 3–5 µm (Cash 1958); D.R. Congo, 6–8 × 3 µm (Dennis 1961); and Cameroon, 6.6 × 2.9 µm (this study). In addition, when we compared measurements of all new collections (TABLE 3), the ascospores from MCA 7061 (Cameroon) are nearly identical with those from MCA 1207 (Guyana). For a thorough evaluation of the identity of African material, more collections are needed—to compare morphological features and molecular data with tropical American specimens and perform sequence-based species delimitation analyses.

Many examples of enigmatic fungi exist that are only known from the type locality or a few collections in

Table 4. All known 18 collections of *Polydiscidium* in chronological order with date, country, collector information, and reference.

Date	Country	Collector	Collection	Reference
Apr 1913	Trinidad	R. Thaxter	BPI 656439	Cash (1958)
May 1929	Guyana	E.B. Martyn	K(M) 264777, holotype	Wakefield (1934)
Jun 1928	D.R. Congo	M. Goossens-Fontana	M. Goossens-Fontana 774	Dennis (1961)
20 Jul 1971	Venezuela	K.P. Dumont et al.	NY 03422293	MyCoPortal (2021)
20 Jul 1971	Venezuela	K.P. Dumont et al.	NY 03422294	MyCoPortal (2021)
28 Mar 1987	Guyana	G.J. Samuels & L.W. Kam	NY 03422097	MyCoPortal (2021)
Feb–Mar 1987	Guyana	G.J. Samuels et al.	CUP 062511	MyCoPortal (2021)
10 Jun 2000	Guyana	T.W. Henkel	TH 7487	This paper
12 Jun 2000	Guyana	S.L. Miller	MCA 1207	This paper
5 May 2001	Guyana	T.W. Henkel	TH 8039	This paper
4 Jul 2002	Guyana	M.C. Aime	MCA 2195	This paper
16 Jul 2003	Guyana	T.W. Henkel	TH 8570	This paper
27 Jun 2006	Guyana	M.C. Aime	MCA 3114	This paper
22 May 2011	Guyana	M.C. Aime	MCA 4268	This paper
18 Jun 2015	Guyana	M.C. Aime	MCA 5794	This paper
23 Nov 2016	Cameroon	M.C. Aime	MCA 7061	This paper
22 Jul 2019	Guyana	D.R. Husbands	DRH 238	This paper
9 Jan 2022	Guyana	T.W. Henkel	TH 11122	This paper

a restricted geographical area, for example in Ceraceosorales (Ustilaginomycetes), Helotiales (Leotiomycetes), Pyxidiophorales (Laboulbeniomycetes), and Xylariales (Sordariomycetes) (e.g., Haelewaters et al. 2021a; Kijpornyongpan and Aime 2016; Stadler et al. 2020). In the case of *S. martynii*, more than 20 years of collecting in Guyana (Aime et al. 2010; Henkel et al. 2012) has only yielded 10 physical collections, but the fungus was also observed, though not collected, in different localities in Guyana within a radius of 200 km. In addition, *S. martynii* was observed in Suriname (D. J. Lodge, pers. comm.) in July 2008. These different records imply that *S. martynii* may be widely distributed, at least in tropical America. In Cameroon, 5 years of collecting (e.g., Jumbam et al. 2019; Mighell et al. 2021) has resulted in a single collection and one additional observation. We searched for additional, unpublished records through citizen science websites (iNaturalist.org, MushroomerObserver.org) and the Mycology Collections data Portal (MyCoPortal). Four unpublished collections are present in fungarium collections (MyCoPortal 2021): two collections from Guyana (CUP, NY) and two from Venezuela (NY). We also performed BLAST searches against the NCBI GenBank and UNITE (Abarenkov et al. 2010) sequence databases in search of environmental sequences but found no close matches. The new collections of *S. martynii* reported in this paper represent over half of all known collections of this species made globally (TABLE 4).

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