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ARTICLE

New species of *Elaphomyces* (*Elaphomycetaceae*, *Eurotiales*, *Ascomycota*) from tropical rainforests of Cameroon and Guyana*

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Abstract: The sequestrate false truffles *Elaphomyces favosus*, *E. iuppitercellus*, and *E. labyrinthinus* spp. nov. are described as new to science from the Dja Biosphere Reserve, Cameroon. *Elaphomyces adamizans* sp. nov. is described as new from the Pakaraima Mountains of Guyana. The Cameroonian species are the first *Elaphomyces* taxa to be formally described from Africa, occurring in lowland Guineo-Congolian tropical rainforests dominated by the ectomycorrhizal (ECM) canopy tree *Gilbertiodendron dewevrei* (*Fabaceae* subfam. *Caesalpinioideae*). The Guyanese species is the third to be discovered in lowland tropical South America, occurring in forests dominated by the ECM trees *Pakaraimaea dipterocarpacea* (*Dipterocarpaceae*) and *Dicymbe jenmanii* (*Fabaceae* subfam. *Caesalpinioideae*). Macromorphological, micromorphological, habitat, and DNA sequence data are provided for each new species. Molecular and morphological data place these fungi in *Elaphomycetaceae* (*Eurotiales*, *Ascomycota*). Unique morphological features are congruent with molecular delimitation of each of the new species based on a phylogenetic analysis of the rDNA ITS and 28S loci across the *Elaphomycetaceae*. The phylogenetic analysis also suggests that a common ancestor is shared between some *Elaphomyces* species from Africa and South America, and that species of the stalked, volvate genus *Pseudotulostoma* may be nested in *Elaphomyces*.

Key words:
biogeography
ectomycorrhizal fungi
Gilbertiodendron
Guiana Shield
Guineo-Congolian rainforest
Pakaraimaea
sequestrate fungi
Uapaca

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INTRODUCTION

Elaphomyces Nees 1820 (*Elaphomycetaceae*, *Eurotiales*, *Ascomycota*) is a sequestrate, ectomycorrhizal (ECM) fungal genus that associates with a broad range of primarily north or south temperate angiosperm and gymnosperm hosts (Trappe *et al.* 2009, Castellano *et al.* 2011, Quandt *et al.* 2015). *Elaphomyces* species generally fruit hypogeously and have relatively large cleistothecial ascomata with a thick peridium, a powdery, hydrophobic gleba, and dark, globose, ornamented ascospores (Trappe 1979). Aside from new tropical Australian species recently described by Castellano *et al.* (2011), there is a paucity of published *Elaphomyces* records from the tropics (e.g. Corner & Hawker 1955, Castellano *et al.* 2012). Unpublished and currently undescribed *Elaphomyces* collections have been reported from Costa Rica, Java, Papua New Guinea, and Thailand (Reynolds 2011, Nampia Sukarno pers. comm., T.F.E., unpubl.data).

Castellano *et al.* (2012) provided the first report of *Elaphomyces* from the lowland South American tropics, describing two new species associated with ECM *Fabaceae* hosts in Guyana. Subsequently, our continued collecting efforts in the tropics of Africa and South America have uncovered four additional new *Elaphomyces* species. Here we describe *Elaphomyces favosus*, *E. iuppitercellus*, and *E. labyrinthinus* spp. nov. from the Dja Biosphere Reserve in Cameroon, and *E. adamizans* sp. nov. from the Pakaraima Mountains of Guyana. The Cameroonian species are the first to be formally described from Africa, although *Elaphomyces* partial ITS root tip sequences have been reported from the African tropics (e.g. Tedersoo *et al.* 2010, 2011) and as yet undescribed *Elaphomyces* ascomata have been collected in Madagascar (Bart Buyck, pers. comm.). The Cameroonian species are currently only known from primary Guineo-Congolian tropical rainforests dominated by the ECM canopy tree *Gilbertiodendron dewevrei* (*Fabaceae* subfam. *Caesalpinioideae*) with additional scattered trees of the ECM genus *Uapaca* (*Phyllanthaceae*). The Guyanese species occurs in primary forests co-dominated by the ECM trees

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Pakaraimaea dipterocarpacea (*Dipterocarpaceae*) and *Dicymbe jenmanii* (*Fabaceae* subfam. *Caesalpinioideae*). Macromorphological, micromorphological, habitat, and DNA sequence data are provided for each new species. A molecular phylogenetic analysis assesses the relationships of the new species within *Elaphomycetaceae*, and suggests that a common ancestor is shared between some species from Africa and South America, and that stalked, volvate species of *Pseudotulostoma* may be nested within *Elaphomyces*.

MATERIALS AND METHODS

Collections

In Guyana, ascomata were collected during the June rainy season of 2012 from Pakaraima Mountains, Upper Mazaruni River Basin, near a base camp at 5°26'21.3" N 60°4'43.1" W, 800 m a.s.l., in savanna fringing forest dominated by *P. dipterocarpacea* and *D. jenmanii* (Smith *et al.* 2013). In Cameroon, ascomata and ECM root tips were collected during the Aug.–Sep. early rainy season of 2014 from the Dja Biosphere Reserve, Northwest Sector near the village of Somalomo, Upper Dja River Basin, within a two km radius of a base camp located at 3°21'29.8" N 12°43'46.9" W, 650 m a.s.l., in forests dominated by *G. dewevrei* (Peh *et al.* 2014).

Descriptions of macromorphological features were made from fresh material in the field. Colours were compared with colour plates from Kornerup & Wanscher (1978) and are cited in parentheses (e.g. 5A4). Fresh collections were dried with silica gel. Preserved specimens were later examined in 3 % KOH, Melzer's reagent, and Cotton blue. Microscopic descriptions are based on 3 % KOH mounts unless specified otherwise. Twenty ascospores were measured from each type collection; dimensions reported include ornamentation. Dried ascospores were mounted on aluminum pegs with double-sided tape and coated with gold for scanning electron microscopy (SEM) with an AmRay 3300 FE field emission scanning electron microscope. Type and additional specimens are deposited in the following herbaria: BRG, University of Guyana; YA, Cameroon National Herbarium; HSC, Humboldt State University; OSC, Oregon State University; K(M), Fungarium, Royal Botanic Gardens, Kew.

DNA extraction, PCR amplification, and sequencing

All DNA work was carried out in the Jodrell Laboratory, Royal Botanic Gardens, Kew. DNA extractions were performed on ascoma tissue from specimens and ECM root tips using the Extract-N-Amp Plant PCR kit (SIGMA-ALDRICH, Saint Louis, MO), followed or not by plate filtration (Dentinger *et al*. 2010), or using a Plant DNeasy mini kit (QIAGEN, Valencia, CA). Full internal transcribed spacers 1 and 2, along with the 5.8S rDNA (ITS), were PCR-amplified with primers ITS1F and ITS4 (White *et al.* 1990, Gardes & Bruns 1993), and the nuclear 28S rDNA D1–D2 domains (28S) were PCR-amplified with LR0R/LR5 (Vilgalys & Hester 1990) following the cycling conditions in Dentinger *et al.* (2010). PCR products were visualized by UV fluorescence after running out 2 µL PCR products in a 1 % agarose gel containing 0.005 % ethidium bromide. Prior to sequencing, amplicons were cleaned of unincorporated dNTPs and excess primers by adding 1 µL ExoSAP-IT (USB, Cleveland, OH) to 5 µL PCR reaction mix and incubating for 15 min at 37 °C followed by 15 min at 80 °C. Unidirectional dye-terminator sequencing used BigDye3.1 (Applied Biosystems, Foster City, CA), by adding 2 µL of cleaned PCR template to 3 µL of solution containing 0.2 µL BigDye, 1 µL sequencing buffer, 0.15 µL 50mM $MgCl_2$, 0.15 µL of 10 µM primer, and 1.5 µL of Milli-Q (Merck Millipore, Darmstadt, Germany) purified water. Sequencing was performed with 60 cycles of 95 °C denaturation for 10 sec, 50 °C annealing for 10 sec, and 60 °C extension for 2 min. Sequencing reactions were cleaned using ethanol precipitation and resuspended in purified water before loading into an ABI 3730 DNA Analyzer (Applied Biosystems, Foster City, CA). Complementary unidirectional sequence reads were aligned and edited in Sequencher v. 4.2 (Gene Codes, Ann Arbor, MI) and deposited in GenBank (Table 1).

Taxa used, sequence alignment, and phylogenetic analysis

All *Elaphomycetaceae* (e.g. *Elaphomyces, Pseudotulostoma*) ITS and 28S sequences derived from ascomata and ECM root tips available in GenBank were downloaded. Each gene region was aligned separately with the sequences from our new species using the RNA structure-based algorithm Q-INS-i implemented in MAFFT v7.023b (Katoh *et al.* 2002, Katoh & Toh 2008, Katoh & Standley 2013). After correcting the orientations of four ITS sequences, removing one short sequence (GenBank accession AM087442) and one sequence with substantial numbers of ambiguous bases (GenBank accession AB161194), the uneven ends were trimmed and alignments refined with a second round of alignment in MAFFT, as above, and refined alignments concatenated into a single dataset. Phylogenetic analysis under the maximum likelihood criterion was performed using the Pthreads parallelised version of RAxML v7.0.3 (Stamatakis 2006, Ott *et al.* 2007) with a GTRGAMMA model, allowing model parameters to be estimated for each gene partition separately. Branch support was assessed using nonparametric bootstrapping with the autoMRE option. Geographic sources of sequences used from GenBank were determined primarily from locality information in GenBank records. The alignment and tree have been accessioned in TreeBase at http://purl.org/phylo/treebase/phylows/study/TB2:S18165.

RESULTS

Final alignments consisted of 82 sequences and 832 positions for the ITS (381 parsimony informative, 361 constant, 90 autapomorphic), and of 18 sequences and 887 positions for the 28S (145 parsimony informative, 712 constant, 30 autapomorphic). All 28S sequences had corresponding ITS sequences derived from the same source, except for *Elaphomyces digitatus* where they were derived from two separate conspecific sources and subsequently combined in the dataset. All characters were included in the analysis. RAxML rapid bootstrapping terminated after 550 replicates (WRF average of 100 random splits = 2.319227) and the

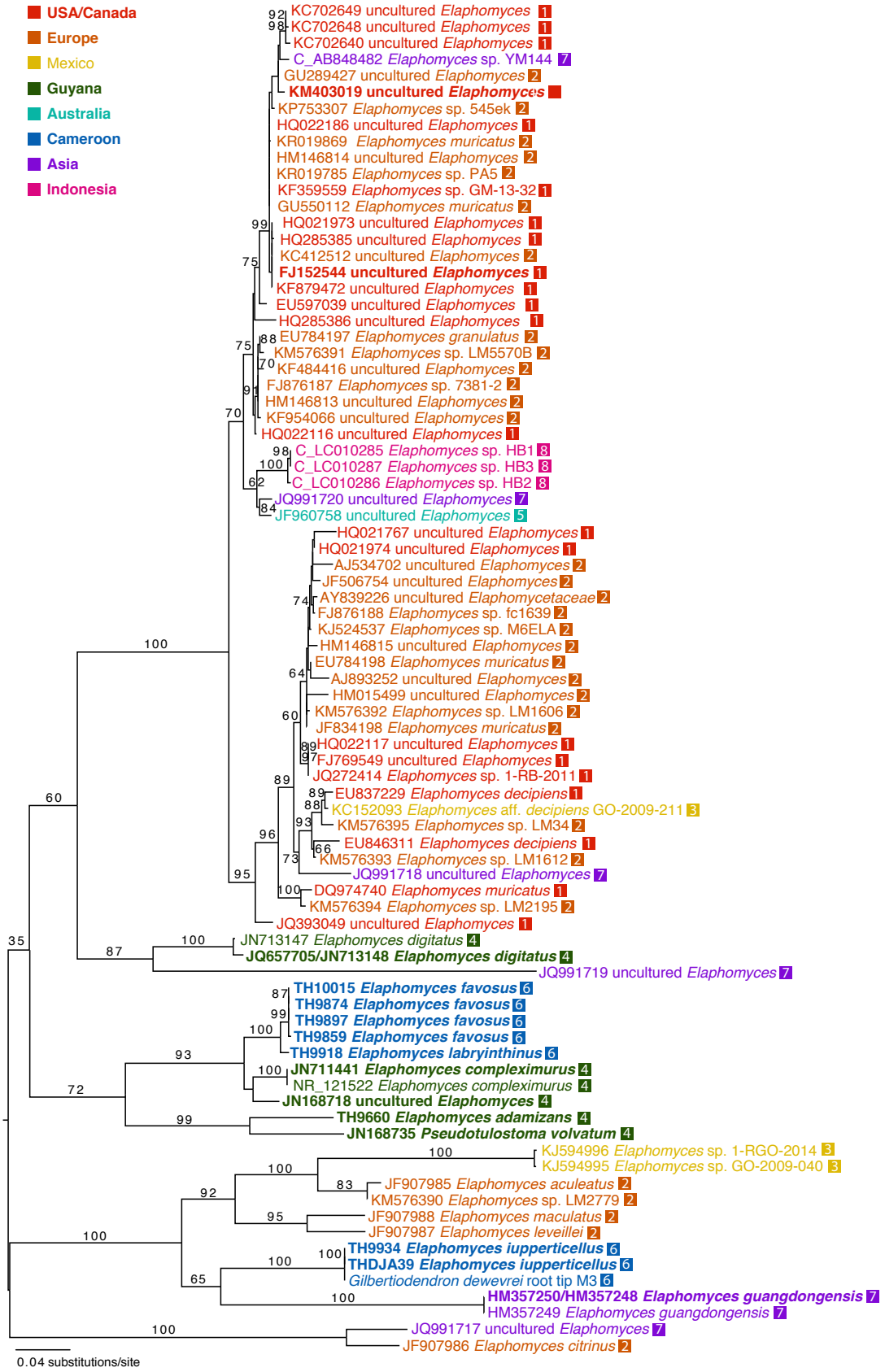

Fig. 1. Best maximum likelihood phylogram (–ln = 10065.206922) of a combined analysis of ITS and 28S sequences of *Elaphomycetaceae* taxa in RAxML using a GTRGAMMA substitution model. Tree is midpoint rooted. Numbers on or next to branches are nonparametric bootstrap supports >70 % from 550 bootstrap replicates. For terminals downloaded from GenBank, labels begin with GenBank accession number and are colour-coded by geographic origin of the sources of the sequences as determined by GenBank records. Terminal labels for taxa generated in this study begin with the collection number. Terminals in bold are represented by both ITS and 28S sequences. Those beginning with a 'C' had their sequence orientation corrected for phylogenetic analysis.

Table 1. *Elaphomycetaceae* taxa, voucher numbers, collection locales, and GenBank accession numbers for ITS and 28S nuc rDNA used in the phylogenetic analysis. Taxa described here and newly generated sequences are in bold at the top. Sequences on the complementary strand are indicated by an asterisk (*).

Taxon	Voucher	Collection locale as indicated in GenBank	ITS	28S
Elaphomyces adamizans	**TH9660 (type)**	**Region 7 Cuyuni-Mazaruni, Guyana**	**KT694133**	**KT694144**
Elaphomyces favosus	**TH10015**	**East Province, Cameroon**	**KT694134**	**KT694145**
Elaphomyces favosus	**TH9859 (type)**	**East Province, Cameroon**	**KT694138**	**KY694149**
Elaphomyces favosus	**TH9874**	**East Province, Cameroon**	**KT694135**	**KT694147**
Elaphomyces favosus	**TH9897**	**East Province, Cameroon**	**KT694136**	**KT694146**
Elaphomyces iupperticellus	**M3 (root tip)**	**East Province, Cameroon**	**KT694140**	
Elaphomyces iupperticellus	**TH9934**	**East Province, Cameroon**	**KT694141**	**KT694142**
Elaphomyces iupperticellus	**THDJA 39 (type)**	**East Province, Cameroon**	**KT694139**	**KT694143**
Elaphomyces labryinthinus	**TH9918 (type)**	**East Province, Cameroon**	**KT694137**	**KT694148**
Elaphomyces aculeatus	16952	Italy	JF907985	
Elaphomyces aff. *decipiens*	GO-2009-211	Mexico	KC152093	
Elaphomyces citrinus	16955	Spain	JF907986	
Elaphomyces compleximurus	TH8880	Guyana	JN711441	JN711441
Elaphomyces compleximurus	TH8880	Guyana	NR_121522	
Elaphomyces decipiens	Trappe 12436	USA	EU837229	
Elaphomyces decipiens	Trappe 28269	USA	EU846311	
Elaphomyces digitatus	MCA1512	Guyana		JN713147
Elaphomyces digitatus	TH8887	Guyana	JQ657705	
Elaphomyces digitatus	MCA1923	Guyana		JN713148
Elaphomyces granulatus	K(M)47712	UK	EU784197	
Elaphomyces guangdongensis	KH-TW09-030	Taiwan	HM357249	
Elaphomyces guangdongensis	KH-TW09-031	Taiwan	HM357250	HM357248
Elaphomyces leveillei	16960	Italy	JF907987	
Elaphomyces maculatus	16961	Italy	JF907988	
Elaphomyces muricatus	src641	USA	DQ974740	
Elaphomyces muricatus	K(M)121442	UK	EU784198	
Elaphomyces muricatus	Hy14 (root tip)	Finland	GU550112	
Elaphomyces muricatus	n.a.	Poland	JF834198	
Elaphomyces muricatus	HA38 (root tip)	Latvia	KR019869	
Elaphomyces sp.	YM144 (root tip)	Japan	AB848482*	
Elaphomyces sp.	HB1	Indonesia	LC010285*	
Elaphomyces sp.	HB3	Indonesia	LC010287*	
Elaphomyces sp.	HB2	Indonesia	LC010286*	
Elaphomyces sp.	7381.2 (root tip)	UK	FJ876187	
Elaphomyces sp.	fc1639 (root tip)	UK	FJ876188	
Elaphomyces sp.	AM3GA3A4	USA	JQ272414	
Elaphomyces sp.	GM 13-32 (root)	USA	KF359559	
Elaphomyces sp.	M6ELA	Poland	KJ524537	
Elaphomyces sp.	GO-2009-040	Mexico	KJ594995	
Elaphomyces sp.	GO-2009-028	Mexico	KJ594996	
Elaphomyces sp.	LM2779 (root tip)	Romania	KM576390	
Elaphomyces sp.	LM5570B (root tip)	Hungary	KM576391	
Elaphomyces sp.	LM1606 (root tip)	UK	KM576392	
Elaphomyces sp.	LM1612 (root tip)	UK	KM576393	
Elaphomyces sp.	LM2195 (root tip)	UK	KM576394	
Elaphomyces sp.	LM34 (root tip)	Spain	KM576395	
Elaphomyces sp.	ITS-545ek (root tip)	Latvia	KP753307	

Table 1. (Continued).

Taxon	Voucher	Collection locale as indicated in GenBank	ITS	28S
Elaphomyces sp.	PA5 (root tip)	Latvia	KR019785	
Pseudotulostoma volvatum	TH8975	Guyana	JN168735	JN168735
Uncultured *Elaphomyces*	O17 (root tip)	Estonia	AJ534702	
Uncultured *Elaphomyces*	L503Z_E1 (root tip)	Estonia	AJ893252	
Uncultured *Elaphomyces*	UBCOGTR184 (root tip)	Canada	EU597039	
Uncultured *Elaphomyces*	SLUBC46 (environmental sample)	Canada	FJ152544	FJ152544
Uncultured *Elaphomyces*	SDL33 (root tip)	USA	FJ769549	
Uncultured *Elaphomyces*	BJP93T_102 (root tip)	UK	GU289427	
Uncultured *Elaphomyces*	root tip	Poland	HM015499	
Uncultured *Elaphomyces*	4174-1205 (root tip)	UK	HM146813	
Uncultured *Elaphomyces*	4115-1205 (root tip)	UK	HM146814	
Uncultured *Elaphomyces*	5237-1201 (root tip)	UK	HM146815	
Uncultured *Elaphomyces*	1Bart526S (soil)	USA	HQ021767	
Uncultured *Elaphomyces*	Bart1760S (soil)	USA	HQ021973	
Uncultured *Elaphomyces*	4Bart240R (root tip)	USA	HQ021974	
Uncultured *Elaphomyces*	4Bart24S (soil)	USA	HQ022116	
Uncultured *Elaphomyces*	1Bart34R (root tip)	USA	HQ022117	
Uncultured *Elaphomyces*	4Bart309S (soil)	USA	HQ022186	
Uncultured *Elaphomyces*	Ref_306 (root tip)	USA	HQ285385	
Uncultured *Elaphomyces*	Brg_333 (root tip)	USA	HQ285386	
Uncultured *Elaphomyces*	LMAS17c-09 (soil)	France	JF506754	
Uncultured *Elaphomyces*	T566	Tasmania	JF960758	
Uncultured *Elaphomyces*	ecm1108 (root tip)	Guyana	JN168718	JN168718
Uncultured *Elaphomyces*	1_28M5 (root tip)	USA	JQ393049	
Uncultured *Elaphomyces*	ECM92 (root tip)	China	JQ991717	
Uncultured *Elaphomyces*	ECM93 (root tip)	China	JQ991718	
Uncultured *Elaphomyces*	ECM94 (root tip)	China	JQ991719	
Uncultured *Elaphomyces*	ECM95 (root tip)	China	JQ991720	
Uncultured *Elaphomyces*	SJ-LM318 (root tip)	UK	KC412512	
Uncultured *Elaphomyces*	B4pos3.4_35 (clone)	Canada	KC702640	
Uncultured *Elaphomyces*	F4pos1.1_43 (clone)	Canada	KC702648	
Uncultured *Elaphomyces*	F4pos1.2_49 (clone)	Canada	KC702649	
Uncultured *Elaphomyces*	15 (root tip)	Poland	KF484416	
Uncultured *Elaphomyces*	HVM21 (root tip)	USA	KF879472	
Uncultured *Elaphomyces*	ecm62 (root tip)	Latvia	KF954066	
Uncultured *Elaphomyces*	141A (root)	Canada	KM403019	KM403019
Uncultured *Elaphomycetaceae*	jj046 (root tip)	Sweden	AY839226	

best ML tree had a likelihood score of –10065.206922 (Fig. 1). Analysis of a data set consisting only of ITS sequences recovered a best ML tree that differed only in the placement of a few unsupported branches (Fig. 2).

The new species described here were resolved in strongly supported lineages at the 93–100 % bootstrap level within *Elaphomyces* (Fig. 1). Amongst the Cameroonian species, *E. iuppitercellus* was recovered in a strongly supported clade (100 % bootstrap) containing the European *E. aculeatus, E. leveillei*, and *E. maculatus*, an unidentified *Elaphomyces* species, and the east Asian *E. guangdongensis*. Ascoma-derived sequences of *E. iuppitercellus* were identical to those of a sympatric ECM *G. dewevrei* root tip, confirming the ECM status of the species. *Elaphomyces favosus* and *E. labyrinthinus* were strongly supported (100 % bootstrap) as sisters within a well supported (93 % bootstrap) clade that includes *E. compleximuris* and an ECM root tip from Guyana, suggesting that these taxa share a common ancestor within the genus (Fig. 1). The new Guyanese species, *E. adamizans,* was strongly supported (99 % bootstrap) as sister with the stalked, volvate *Pseudotulostoma volvatum* from Guyana; together these sympatric taxa formed a

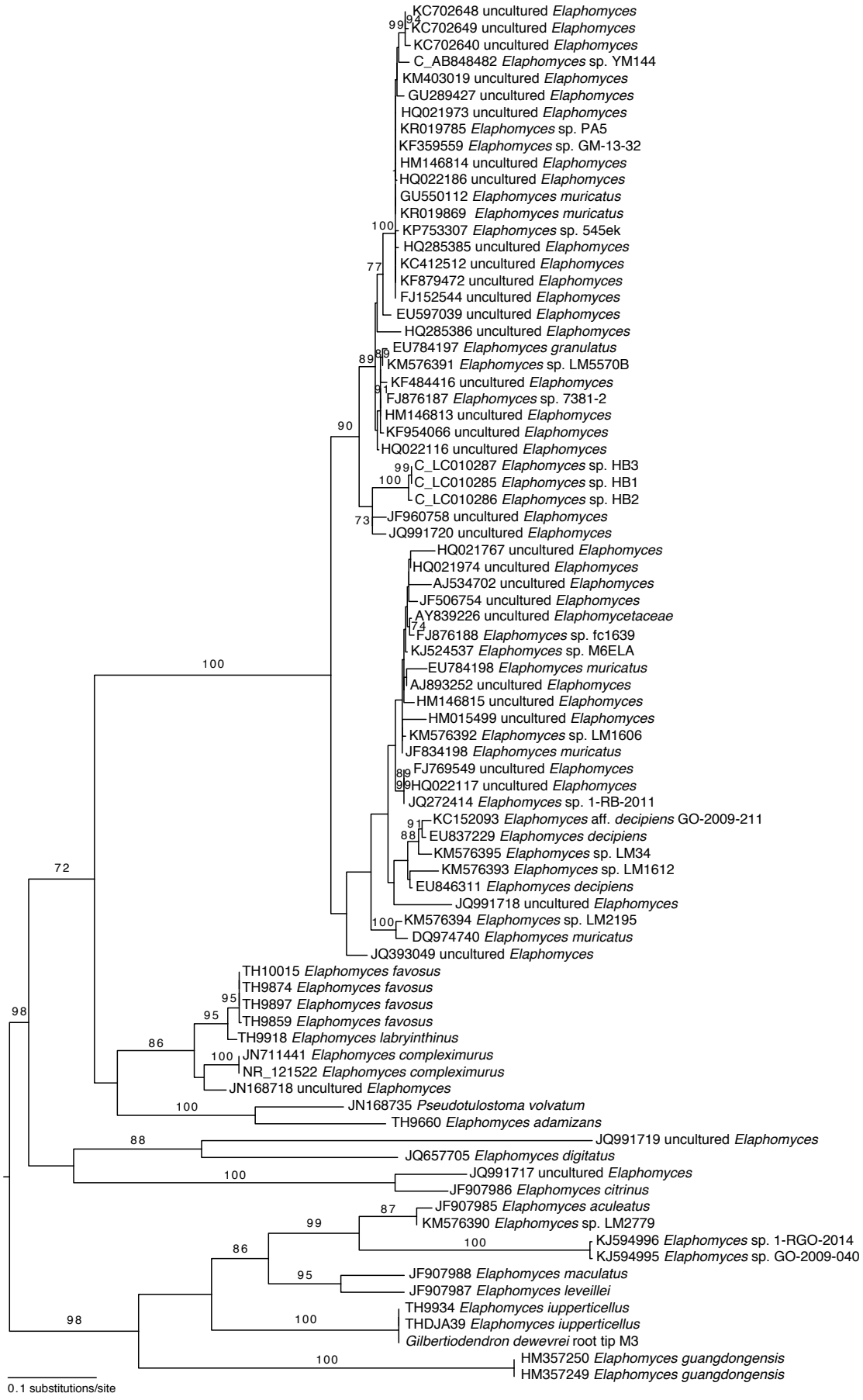

Fig. 2. Best maximum likelihood phylogram of an analysis of ITS sequences in RAxML using a GTRGAMMA substitution model. Tree is midpoint rooted. Numbers on or next to branches are nonparametric bootstrap supports >70 % from 550 bootstrap replicates. Terminal labels for taxa generated in this study begin with the collection number.

larger moderately supported (72 % bootstrap) clade with *E. favosus*, *E. labryinthinus*, and *E. compleximurus* (Fig. 1). These phylogenetic results, along with unique morphological features, warrant the description of the Cameroonian and Guyanese species as new to science, and suggest that *Pseudotulostoma* and *Elaphomyces* may not be reciprocally monophyletic.

TAXONOMY

Elaphomyces favosus Castellano & T.W. Henkel, **sp. nov.**
Index Fungorum IF551318
(Fig. 3)

Etymology: *favosus* (L. adj. A) = honey-combed; referring to the distinctive reticulate-alveolate ascospore ornamentation.

Diagnosis: Similar to the neotropical *E. compleximurus* in ascospore ornamentation and colours of the outer peridium and gleba, but differing in its distinctly larger ascospores (mean diameter with ornamentation = 35.7 µm *vs*. 23.2 µm), and grey (*vs*. white) inner peridium.

Type: **Cameroon**: *East Province*: Dja Biosphere Reserve, Northwest Sector near the village of Somalomo, Upper Dja River Basin, within 2 km radius of Dja base camp at 3°21'29.8" N 12°43'46.9" W, ~400 m west of base camp on edge of *Gilbertiodendron dewevrei* monodominant plot 1, 16 Aug 2014, *Henkel 9859* (YA 0063174 – holotype; HSC G1174, OSC 149785, K(M) 200223 – isotypes). GenBank accession numbers ITS: KT694138; 28S: KY694149.

Description: *Ascomata* 6–20 mm tall (without basal attachment) × 7–27 mm broad, subglobose to ovate or somewhat lobed, black overall, with a distinct subturbinate base encompassing dark brown to black ectomycorrhizas, dense extramatrical mycelium, and sand; peridial surface nearly smooth on immature ascomata, on larger, mature ascomata verrucose throughout; warts 0.1–0.2 mm tall and 0.6–0.8 mm broad, polygonal, 4–5–6-sided, with flattened apices. *Peridium* in section subcartilaginous, three-layered; outer layer black, carbonaceous, < 0.25 mm thick, underlain by a greyish tan second layer with occasional reddish tones, to 0.5 mm thick, with embedded, black ectomycorrhizas; inner third layer dark grey to black, to 0.75 mm thick. *Gleba* initially off-white to pale grey, greyish black at maturity, somewhat powdery but mostly arranged in irregular moist masses, with fine, grey hyphae particularly near gleba-peridium interface. *Odour* none. *Taste* mild with a hint of sweetness.

In microscopic section outer first peridium layer carbonaceous, 65–90 µm thick, composed of a palisade-like tier of nearly black, globose to irregularly-shaped cells, these to 9.5 × 17.5 µm; walls 1–2 µm broad; surface with occasional scattered patches of hair-like projecting hyphae, these erect, pale brown to dark brown with obtuse apices, 4.5–6.5 µm broad with walls 2–3 µm thick; underlying second layer 460–500 µm thick, composed of a *textura epidermoidea* of pale brown, irregularly-shaped to elongate, occasionally branched hyphae, to 8.5 µm broad with walls ±1 µm thick, grading into the third layer that is to 750 µm thick, composed of a *textura obita* of bundles of up to 10 hyphae arranged in a cross-hatched arrangement; individual hyphae hyaline, somewhat sinuous, 5.0–5.5 µm broad with walls 0.5 µm thick. *Gleba* of ascospores and sinuous, hyaline, septate, loosely interwoven hyphae, these 2.5–4.5 µm broad with walls < 0.5 µm thick. *Asci* globose, 90–95 µm diam, hyaline, walls 2–2.5 µm thick, eight-spored. *Ascospores* globose, dark brown, (30–)34–38.5(–40.5) µm diam (mean = 35.5 µm) including the reticulate-alveolate ornamentation; alveolae well-defined, 4.5–5.5 µm broad and to 4.5 µm tall, with irregular to wavy walls; under SEM the individual alveolar wall is a composite of densely spaced vertical ribs, these with numerous ends emerging from the wall margin; ascospore surface exposed inside the alveolae with an irregular, extremely roughened, subreticulate texture with occasional ridged projections onto the surrounding alveolar wall.

Habit, habitat, and distribution: Solitary or in small groups, hypogeous in lateritic mineral soil or semi-emergent in leaf litter of forest floor in *Gilbertiodendron dewevrei* monodominant forest with nearby stands of *Uapaca* species; known only from the type locality in the Dja River Basin of southern Cameroon.

Additional specimens examined: **Cameroon**: *East Province*: Dja Biosphere Reserve, Northwest Sector near the village of Somalomo, Upper Dja River Basin, within 2 km radius of Dja base camp located at 3°21'29.8" N 12°43'46.9" W, ~1.4 km WNW of base camp on trail between *Gilbertiodendron* plots 1 & 2, in semi-inundated *G. dewevrei* monodominant forest, 20 Aug. 2014, *Henkel 9874* (YA, HSC G1175, OSC 149786, K(M) 200224; GenBank accession numbers: ITS KT694135; 28S KT694147); 28 Aug. 2014, *Henkel 9897* (YA, HSC G1176, OSC 149788, K(M) 200219; GenBank accession numbers ITS: KT694136; 28S: KT694146); ~2 km WNW of base camp in vicinity of *Gilbertiodendron* plot 3, in *G. dewevrei* monodominant forest, 26 Sep. 2014, *Henkel 10015* (YA, HSC G1177, OSC 149787, K(M) 200220, GenBank accession numbers ITS: KT694134; 28S: KT694145).

Commentary: The molecular phylogenetic analysis strongly supported *E. favosus* as sister to, but distinct from, the sympatric *E. labyrinthus* described here, and showed that, within the genus *Elaphomyces*, these two African species share a common ancestor with *E. compleximuris* from Guyana (Fig. 1). Both *Elaphomyces favosus* and *E. labrynthinus* have a warty, black ascoma with a tapered base, but the peridial warts of *E. favosus* are both taller and broader than those of *E. labyrinthinus*. Additionally, the ascospore ornamentation of *E. favosus* is distinctly reticulate-aveolate while that of *E. labyrinthinus* is labyrinthine. The Guyanese *E. compleximurus* has similar overall ascoma morphology and ascospore ornamentation to those of *E. favosus*, but has smaller ascospores and a white inner peridium (*vs*. grey in *E. favosus*).

Amongst other *Elaphomyces* species worldwide, only two European species, *E. cyanosporus* and *E. persoonii*, combine the features of reticulate ascospores and a black, warty peridium. *Elaphomyces persoonii* has a tapered base like *E. favosus* but its peridial warts are to 1.5 mm broad, twice

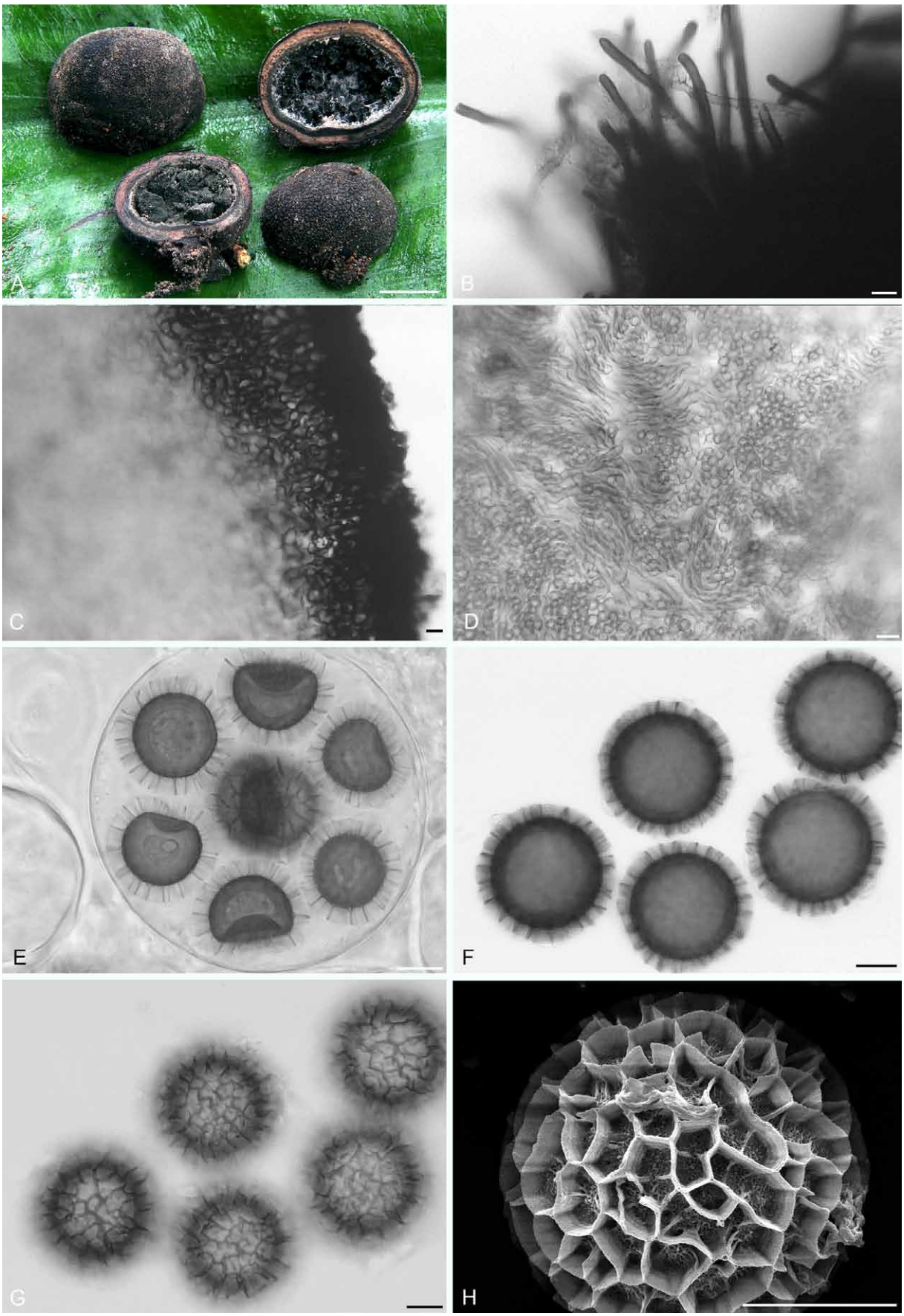

Fig. 3. *Elaphomyces favosus* (holotype; *Henkel 9859*). **A.** Ascomata showing peridial surface, gleba, and peridium in section. **B.** Erect peridial hairs found in patches on peridial surface. **C.** Microscopic view of peridium in section. **D.** Third layer of peridium with cross-hatched, bundled hyphae. **E.** Ascus with eight ascospores. **F.** Ascospores with ornamentation in outline. **G.** Ascospores with ornamentation in surface view. **H.** Scanning electron micrograph of an ascospore. Bars A = 10 mm, B, D–H = 10 µm, C = 20 µm.

as wide as those of *E. favosus*. Also, the globose ascospores of *E. persoonii* are somewhat smaller with a mean diameter of 31.3 µm (*vs*. 35.7 µm for *E. favosus*). The ascospores of *E. cyanosporus*, with a mean diameter of 28.0 µm, are much smaller than those of *E. favosus*.

Elaphomyces iuppitercellus Castellano & T.W. Henkel, **sp. nov.**
Index Fungorum IF551320
(Fig. 4)

Etymology: *iuppiter* (L.) = Jupiter and *–cellus* (L. adj. suf.) = diminutive for small, hence "small Jupiter", in reference to the ascospore ornamentation resembling the swirling atmospheric patterns of the planet Jupiter.

Diagnosis: Similar to the European *E. virgatosporus* in peridium characteristics and ascospore ornamentation but differs in its pinkish brown gleba and larger ascospores (mean diameter = 24.7 µm *vs*. 20.2 µm in *E. virgatosporus*).

Type: **Cameroon**: *East Province*: Dja Biosphere Reserve, Northwest Sector near the village of Somalomo, Upper Dja River Basin, within 2 km radius of Dja base camp at 3° 21' 29.8" N 12° 43' 46.9" W, ~1 km WNW of base camp on trail between *Gilbertiodendron* plots 1 & 2, in semi-inundated *G. dewevrei* monodominant forest, 25 Aug. 2014, *Henkel THDJA 39* (YA 0063175 – holotype; HSC G1178, OSC 149782, K(M) 200226). GenBank accession numbers ITS: KT694139; 28S: KT694143.

Description: *Ascomata* 4–8 mm tall and 6–12 mm broad, subglobose to ovate, without a distinct base, dark brown to black overall under scattered adherent soil, debris, and ectomycorrhizas; peridial surface somewhat smooth macroscopically but close inspection reveals low warts covering the entire surface; warts subcircular, to 150 µm tall and 400 µm broad, blunt at apex. *Peridium* in section subcartilaginous, with two distinct layers; outer first layer ~ 0.15 mm thick, black, carbonaceous; underlying second layer to 0.5 mm thick, greyish to grey-brown, with an apparent "third" inner layer appearing as a thin band of white cottony hyphae emanating from the outer gleba and contiguous at irregular intervals with white glebal veins. *Gleba* white to off-white and arranged in irregular moist masses when immature, at maturity pinkish brown (7C4–7D4) with white veins and

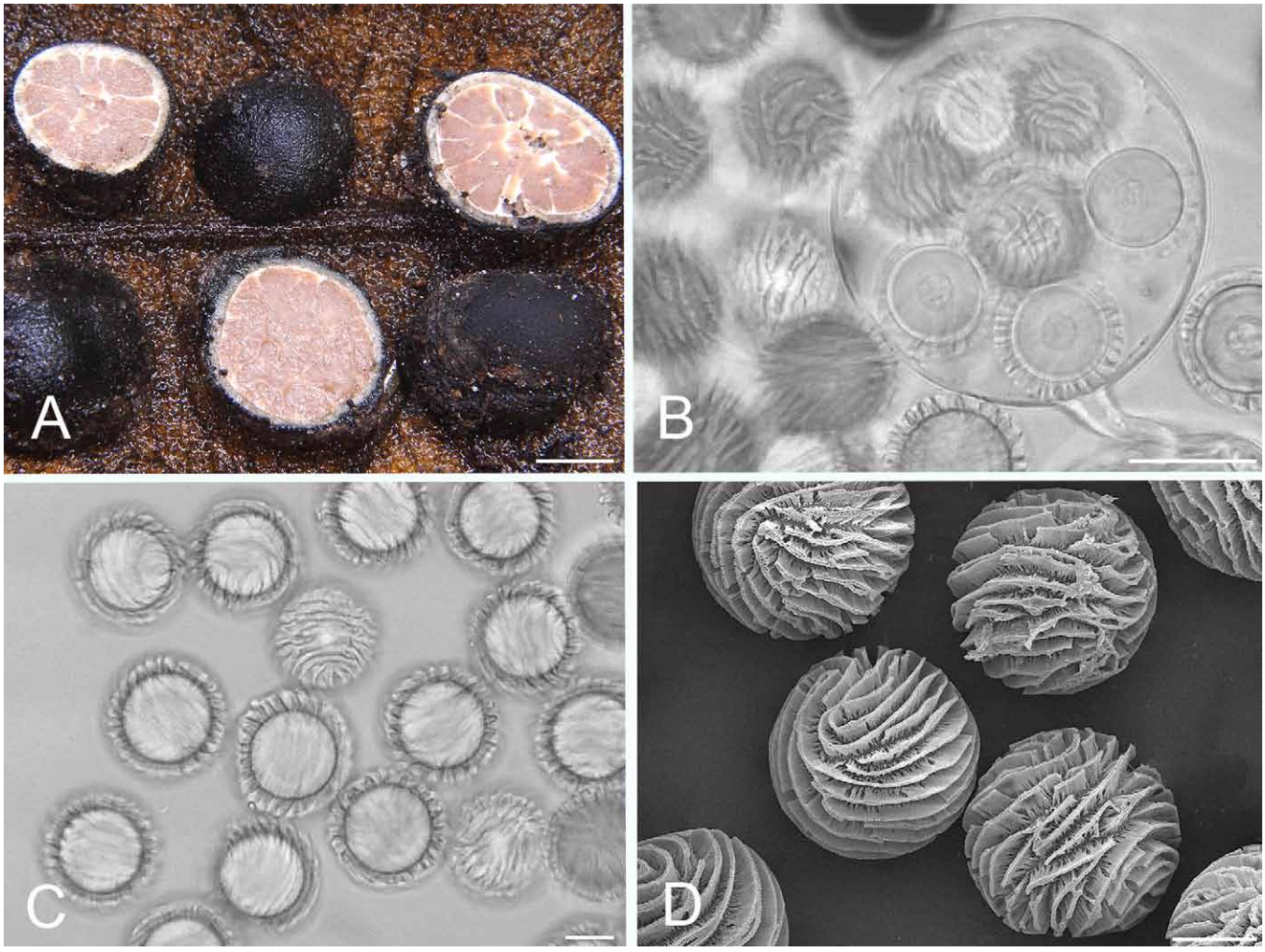

Fig. 4. *Elaphomyces iuppitercellus* (holotype; *Henkel THDJA 39*). **A.** Ascomata showing peridial surface, gleba, and peridium in section. **B.** Ascus showing seven (of eight) ascospores. **C.** Ascospores with ornamentation in outline and surface views. **D.** Scanning electron micrograph of ascospores. Bars A = 5 mm, B = 25 µm, C–D = 10 µm.

eventually powdery, in larger specimens hollow in the center. *Odour* indistinct to mild. *Taste* mild to mealy.

Peridium in microscopic section two-layered; outer layer carbonaceous, 130–140 µm thick, composed of dark brown, polygonal cells, to 2.0–4.5 µm broad with walls 1 µm thick; surface with adhering debris but lacking erect hyphae; underlying second layer to 450 µm thick and composed of a *textura intricata* of hyaline hyphae, to 9 µm broad with walls 2 µm thick; hyphae closest to gleba with clavate ends to 15.5 µm broad. *Gleba* of ascospores and sinuous, hyaline, septate, loosely interwoven hyphae, these 3.5–4.5 µm broad with walls < 0.5 µm thick. *Asci* globose, 75–80 µm diam, hyaline, walls 1 µm broad, with an elongate, stipe-like base, eight-spored. *Ascospores* globose, hyaline, 23.5–25.5(–26.5) µm diam (mean = 24.7 µm) including the striate ornamentation that is to 2.5 µm tall; ornamentation irregular to wavy; under SEM the individual walls consist of a lattice-work of anastomosed rods and spines, with individual ridges varying somewhat in thickness.

Habit, habitat and distribution: Solitary or in small groups, hypogeous or semi-emergent in leaf litter of forest floor in *Gilbertiodendron dewevrei* monodominant forest with nearby stands of *Uapaca* spp.; known only from the type locality in the Dja River Basin of southern Cameroon.

Additional specimens examined: **Cameroon**: *East Province*: Dja Biosphere Reserve, Northwest Sector near Somalomo Village, Upper Dja River Basin, within 2 km radius of Dja base camp located at 3°21'29.8" N 12°43'46.9" W, ~1.5 km WNW of base camp in *Gilbertiodendron* plot 2, in *G. dewevrei* monodominant forest, 4 Sep. 2014, *Henkel 9934* (YA, HSC G1179, OSC 149783, K(M) 200221; GenBank accession numbers ITS: KT694141; 28S: KT694142).

Commentary: The striate ascospore ornamentation seen in *E. iuppitercellus* is uncommon within *Elaphomyces*, found previously only in the European *E. spirosporus*, *E. striatosporus*, and *E. virgatosporus*, and in the Asian *E. guangdongensis*. The phylogenetic relationships, if any, between these species could not be assessed here as ITS and 28S sequence data for *E. spirosporus*, *E. striatosporus*, and *E. virgatosporus* are lacking in GenBank. *Elaphomyces iuppitercellus* has larger ascospores (mean diameter = 24.7 µm) than *E. guangdongensis* (mean diameter 17.8 µm), *E. spirosporus* (mean diameter 20.5 µm), *E. striatosporus* (mean diameter = 17.5 µm), and *E. virgatosporus* (mean diameter = 20.2 µm); additionally, each of the European species has a grey-toned gleba, which contrasts with the pinkish brown gleba of *E. iuppitercellus*. Also, the striate ornamentation walls of *E. iuppitercellus* are much thinner than those of all other striate-spored *Elaphomyces* species. *Elaphomyces iuppitercellus* ascomata had an identical ITS sequence with that obtained from a *G. dewevrei* ECM root tip collected at the Dja site, confirming its ECM status (Fig. 1).

Elaphomyces labyrinthinus Castellano & T.W. Henkel, **sp. nov.**
Index Fungorum IF551319
(Fig. 5)

Etymology: *labyrinthinus* (L. adj. A) = labyrinthine, referring to the labyrinthine structure of the ascospore ornamentation.

Diagnosis: Similar to the Cameroonian *E. favosus* in overall macromorphology but differing in having peridial warts that are shorter and narrower than those of *E. favosus*, and ascospore ornamentation that is labyrinthine while that of *E. favosus* is reticulate-alveolate.

Type: **Cameroon**: *East Province*: Dja Biosphere Reserve, Northwest Sector near the village of Somalomo, Upper Dja River Basin, within 2 km radius of Dja base camp located at 3°21'29.8 " N 12°43'46.9" W, ~1.5 km WNW of base camp in *Gilbertiodendron* plot 2, in *G. dewevrei* monodominant forest, 1 Sep. 2014, *Henkel 9918* (YA 0063176 – holotype; HSC G1180, OSC 149781, K(M) 200225 – isotypes). GenBank accession numbers ITS: KT694137; 28S: KT694148.

Description: *Ascomata* to 13 mm tall and 20 mm broad, broadly ovate to depressed ovate, with a distinct, slightly tapered base composed of ectomycorrhizas, sand, and dense, dark brown to nearly black mycelium; peridium slightly thickened in this area; peridial surface verrucose beneath a turf of erect, dark brown hyphae; warts black, polygonal, 4–6-sided, with uneven side lengths, 100 µm tall and ± 300 µm broad, flattened, on close inspection appearing finely ridged. *Peridium* in section subcartilaginous, five-layered; outer layer ± 0.05 mm thick, dark brown; second layer ± 0.1 mm thick, black, carbonaceous; third layer 0.35–0.60 mm thick, pale tan, with scattered embedded, black-mantled ectomycorrhizas across the lower portion of the ascoma, these more dense near the basal attachment; fourth layer ± 0.1 mm thick, dark brown; fifth layer ± 0.20 mm thick, grey; all layers most distinct in young specimens; inner layers obscured with age. *Gleba* off-white to pale grey, cottony when immature, becoming greyish black, powdery, with fine, off-white to grey hyphae concentrated near the peridium. *Odour* and *taste* not recorded.

Peridium in microscopic section five-layered; outer layer ± 50 µm thick, composed of a turf of erect, dark brown, septate, capitate hyphae, 6–7(–9.5) µm broad to 17.5 µm long with walls 1.5–2.0 µm thick; surface with scattered patches of erect hair-like hyphae, these pale brown to dark brown with obtuse apices, 4.5–6.5 µm broad with walls 2–3 µm thick; underlying second layer ± 100 µm thick, a *textura epidermoidea* of dark brown, tangled, irregularly-shaped hyphae that are densely packed near the surface and less so towards the gleba; hyphal cells to 7 × 13.5 µm with walls ±1 µm thick; third layer 350–600 µm thick, a compact *textura intricata* of pale brown hyphae, ± 5.5 µm broad with walls 1.0–2.0 µm thick, with amorphous dark brown particles scattered throughout; fourth layer ± 100 µm thick, a *textura intricata* of hyaline, loosely interwoven hyphae, 2.5–5.0 µm broad with walls 1.0–1.5 µm thick; innermost fifth layer ± 200 µm thick, a *textura intricata* of pale brown, interwoven hyphae, somewhat swollen, 5.5–15.0 µm broad, with occasional dark particles scattered through the layer. *Gleba* consisting of ascospores and sinuous, hyaline, septate, loosely tangled hyphae, ± 3.5 µm broad with walls < 0.5 µm thick, with a collar-like thickening at the septa. *Asci* globose, 66–88 µm diam, hyaline, walls 1.5–2.0 µm thick, eight-spored. *Ascospores* globose, dark

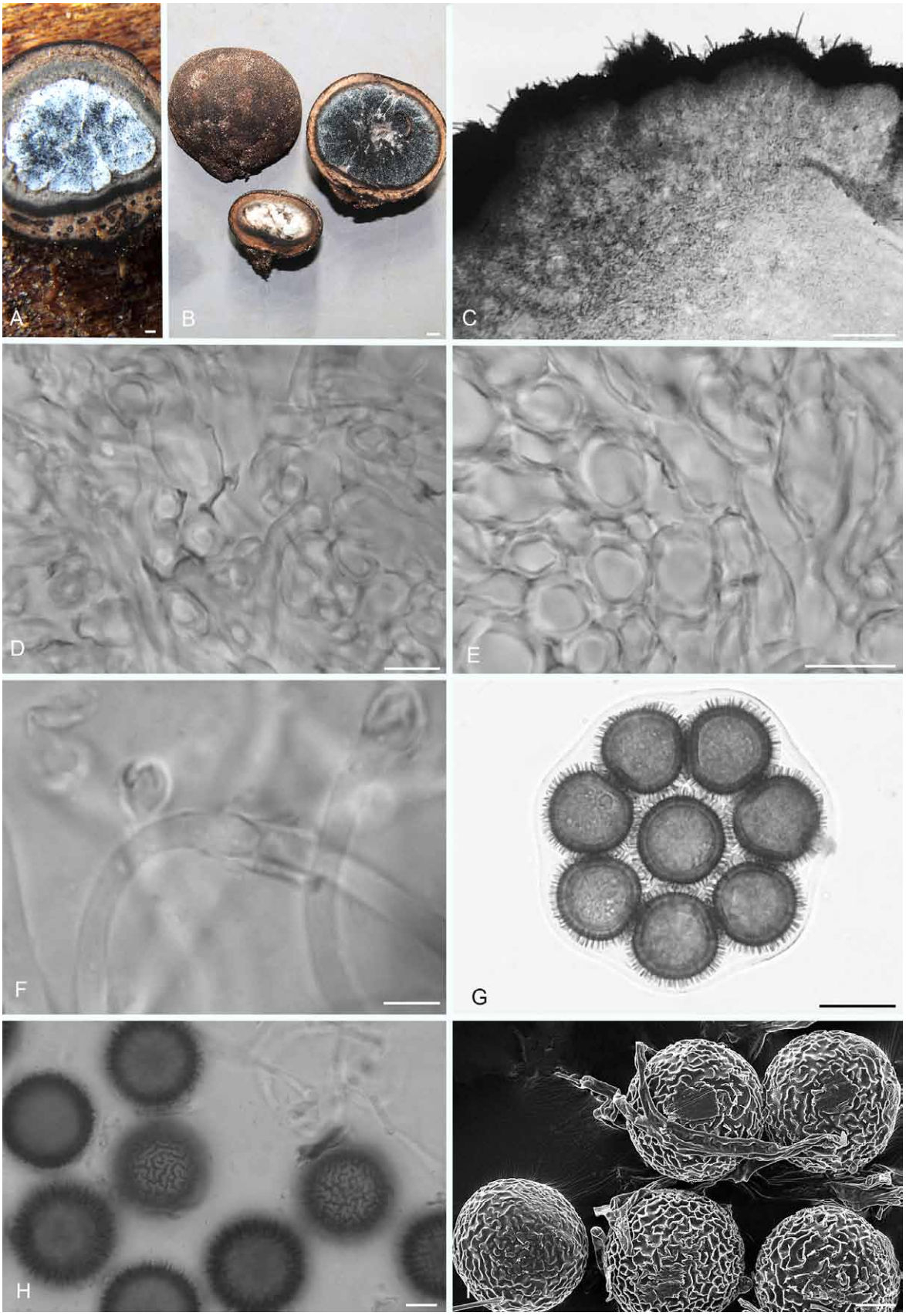

Fig. 5. *Elaphomyces labrynthinus* (holotype; *Henkel 9918*). **A.** Sectioned ascoma showing the embedded black-mantled ectomycorrhizas within the inner peridial layer. **B.** Ascomata showing peridial surface, gleba, and peridium in section. **C.** Microscopic view of sectioned peridium. **D.** Thick-walled, interwoven hyphae from the fourth peridial layer. **E.** Thick-walled, somewhat swollen, interwoven hyphae from the fifth peridial layer. **F.** Collared septa on hyphae within the gleba. **G.** Ascus with eight ascospores in transverse planar view. **H.** Ascospores with ornamentation in outline and surface views. **I.** Scanning electron micrograph of ascospores. Bars A = 0.5 mm, B = 1 mm, C = 100 µm, D = 5 µm, E = 15 µm, F = 5 µm, G = 20 µm, H–I = 10 µm.

brown, 33.5–37.5 µm diam (mean = 35.2 µm), including the labyrinthine-like ornamentation ± 3.5 µm tall; ornamentation with irregular to angular walls, appearing as short, variously shaped, unconnected lines in surface view, spiny in outline view; under SEM individual walls slightly variable in thickness and formed into semi-circles, lines, or irregular shapes.

Habit, habitat, and distribution: In small groups semi-emergent in leaf litter of the forest floor in *Gilbertiodendron dewevrei* monodominant forest, with nearby stands of *Uapaca* spp.; known only from the type locality in the Dja River Basin of southern Cameroon.

Commentary: See above for differences of *E. labrynthinus* from the morphologically and phylogenetically similar *E. favosus*, and the close phylogenetic relationship between these two species and *E. compleximuris* from Guyana. The labyrinthine ascospore ornamentation of *E. labyrinthinus* is similar to that of *E. digitatus* from Guyana, but the distinctly orange peridial surface and much smaller ascospores allow easy separation of the latter species from *E. labyrinthinus*. The European *E. citrinus* has labyrinthine ascospore ornamentation but its ascospores are half the size (mean diameter = 15.8 µm) than those of *E. labyrinthinus* (mean diameter = 35.2 µm).

Elaphomyces adamizans Castellano & T.W. Henkel, **sp. nov.**
Index Fungorum IF551682
(Fig. 6)

Etymology: *adamas* (L.) = diamond and *–izans* (L. adj. suf.) = "becoming like" or "resembling"; in reference to the alluvial diamonds originally found in the Upper Mazaruni River Basin of Guyana, the type locality of the fungus.

Diagnosis: Similar to the Australian *E. rugosisporus* in peridium structure and ascospore size but differs in having a labryinthine ascospore ornamentation (*vs*. finely reticulate for *E. rugosisporus*) and lack of a carbonaceous outer peridial layer.

Type: **Guyana**: *Region 9 Cuyuni-Mazaruni*: Pakaraima Mountains, Upper Mazaruni River Basin, ~10 km west of Mt Ayanganna, within 0.5 km of a base camp at 5° 26' 21.3" N 60° 04' 43.1" W, 100 m north of base camp in savanna fringing forest dominated by *Pakaraimaea dipterocarpacea* and *Dicymbe jenmanii*, 2 Jun. 2012, *Henkel 9660* (BRG 41125 – holotype; HSC G1181, OSC 149784, K(M) 200222 – isotypes). GenBank accession numbers ITS: KT694133; 28S: KT694144.

Description: *Ascomata* 7–14 mm tall and 10–22 mm broad, ovoid-flattened, with a dense layer of ectomycorrhizal roots, mycelium, humic particles, and soil covering the lower quarter, earthen brown (5E7–6E7), unchanging; peridial surface a tightly appressed, brown, tomentose mat, occasionally organized into cord-like fibrils. *Peridium* in section cartilaginous, three-layered; outer layer 0.18–0.22 mm thick, brown; underlying second layer p to 0.13 mm thick, off-white to pale orange-tan; inner third layer overall 1.5–2.0 mm thick but this varying across entire section, off-white, with numerous orange-brown, embedded ectomycorrhizas along the lower half of the ascoma, and there darkening to pale brown to grey-brown near the gleba. *Gleba* hollow, grey when immature, at maturity of ascospores that are dark olivaceous blue-grey (4F2–5F2) in mass, powdery, with scattered narrow hyphae. *Odour* indistinct to slightly earthy. *Taste* slightly sweet.

Peridium in microscopic section three-layered; outer layer 175–220 µm thick, composed of a pale brown, somewhat loose *textura intricata*, not carbonaceous; hyphae 3.5–4.5 µm broad with walls 1 µm thick, with numerous adhering dark small granules; second layer ± 130 µm thick, similarly structured as the first but hyphae lacking adherent granules; inner third layer 1500-2000 µm thick, composed of compact, agglutinated, hyaline hyphae, 3.5–4.5 µm broad, arranged in bundles that are occasionally cross-hatched. *Gleba* consisting of ascospores and sinuous, hyaline, septate, irregularly swollen hyphae, 2.0–3.5 µm broad with walls < 0.5 µm thick. *Asci* irregularly globose, 26–27 µm broad, hyaline, with walls to 2.0 µm thick, eight-spored. *Ascospores* globose, pale brown to brown, 10.5–12.0(–12.5) µm diam (mean = 11.1 µm) including the labyrinthine ornamentation that is 1.5–2.0 µm tall; ornamentation of irregular to wavy walls, appearing as short, variously shaped, unconnected lines; under SEM the individual walls are slightly variable in thickness and formed into semi-circles, lines, or irregular shapes, often with small pits at the tips.

Habit, habitat, and distribution: In group of two, semi-emergent in leaf litter in forest dominated by *Pakaraimaea dipterocarpacea* and *Dicymbe jenmanii*; known only from the type locality in the Upper Mazaruni River Basin of Guyana.

Commentary: In the field, the brown, tomentose peridial surface of *E. adamizans* allows it to be easily distinguished from the two other *Elaphomyces* known from Guyana (Castellano *et al.* 2012). The very small ascospores (mean diameter = 11.1 µm) contrast with the larger ascospores of the Guyanese *E. compleximurus* (mean = 23.2 µm broad) and *E. digitatus* (mean = 21.9 µm broad). There are a number of recently described Australian *Elaphomyces* species with mean ascospore diameter ranging from 9–12 µm, including *E. chlorocarpus*, *E. symeae*, *E. timgroveii*, *E. cooloolanus*, *E. pedicellaris*, and *E. rugosisporus* (Castellano *et al.* 2011). Each of these species differs from *E. adamizans* in peridial characteristics and ascospore ornamentation, and all are associated with ECM *Myrtaceae* hosts (Castellano *et al.* 2011).

Molecular phylogenetic analysis places *E. adamizans* as sister to the stalked, volvate *Pseudotulostoma volvatum*. While *E. adamizans* and *P. volvatum* have highly dissimilar macromorphologies at maturity, the ascospore morphologies *E. adamizans* and *P. volvatum* are very similar. Ascospores in each are between 7–12.5 µm diam with a labyrinthine ornamentation. The stalked, epigeous habit with an exposed ascospore mass in *P. volvatum* allows the species to be easily separated from the fully sequestrate *E. adamizans*. Additionally, SEM images reveal differences in fine detail of the ascospore ornamentation of these taxa that under light microscopy appear similar (Miller *et al.* 2001, Asai *et al.* 2004).

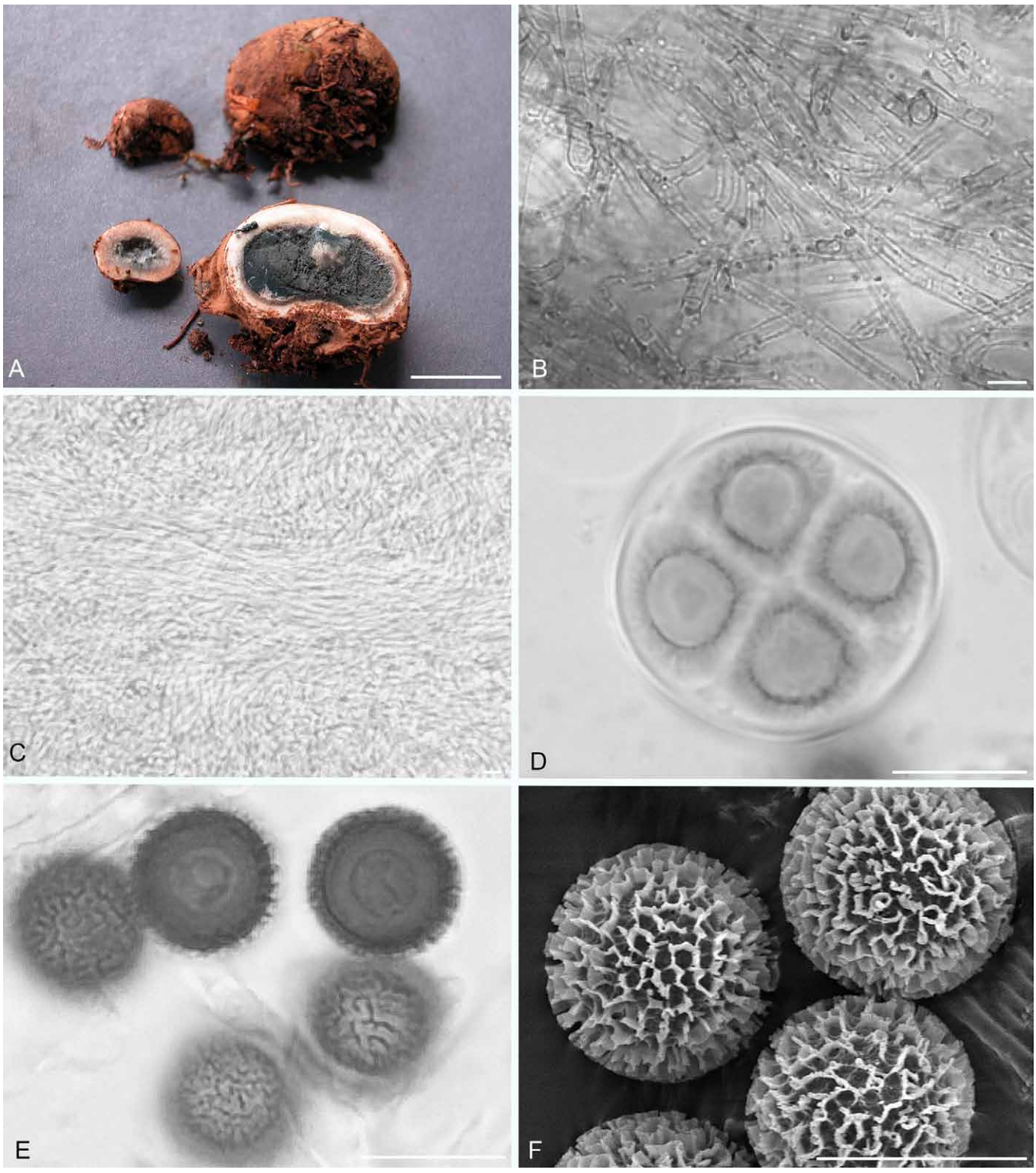

Fig. 6. *Elaphomyces adamizans* (holotype; *Henkel 9660*). **A.** Ascomata showing peridial surface, gleba, and peridium with embedded ectomycorrhizas in section. **B.** Interwoven hyphae with numerous, adherent, dark granules in the first peridial layer. **C.** Cross-hatched, interwoven, bundled hyphae in the third peridial layer. **D.** Immature ascus in focal plane showing four (of eight) developing ascospores. **E.** Ascospores with ornamentation in outline and surface views. **F.** Scanning electron micrograph of ascospores. Bars A = 10 mm, B–F = 10 µm.

DISCUSSION

In addition to supporting the recognition of the new species of *Elaphomyces* reported here, the phylogenetic analysis suggests that the stalked, volvate *Pseudotulostoma volvatum* may be nested within the genus *Elaphomyces*. *Pseudotulostoma volvatum* was described as a new taxon by Miller *et al.* (2001; as *"volvata"*) from Guyana with a macromorphology resembling a basidiomycete stalked puffball but micromorphology consistent with *Elaphomyces*. At maturity this fungus exhibits a powdery ascospore and pseudocapillitium mass exposed on the apex of a woody

Fig. 7. Ascomata of *Pseudotulostoma volvatum* (*Henkel 9786)* showing developmental stages. Bar = 10 mm.

stalk, having expanded upward through the peridium, which remains as a volva-like basal structure (Fig. 7). The 18S rDNA phylogenetic analysis presented by Miller *et al.* (2001) placed *P. volvatum* within the *Eurotiales* and sister to *Elaphomyces* within *Elaphomycetaceae*. *Pseudotulostoma* was therefore recognized as a new genus related to, but outside of *Elaphomyces*, supported morphologically by the radically different form of the mature ascoma. The close relationship of *P. volvatum* to *Elaphomyces* was corroborated by its thick, tough, multi-layered peridium with embedded ectomycorrhizas, and gleba of hydrophobic, thick-walled, ornamented ascospores with *Elaphomyces*-like ultrastructure. The ECM nutritional status typical of *Elaphomyces* species was also demonstrated for *P. volvatum* based on morphological and molecular analysis of ECM roots of *Dicymbe corymbosa* found in proximity to the ascomata (Henkel *et al.* 2006).

Masuya & Asai (2004) subsequently placed *P. volvatum* and the Japanese *P. japonicum* (as *"japonica*") in *Elaphomycetaceae* as sister to *Elaphomyces* based on a SSU rDNA phylogenetic analysis. It is clear from the detailed descriptions and illustrations of *P. japonicum* from Kawamura (1954), Otani (1960), and Asai *et al.* (2004) that the species shares the unusual macromorphological structure with *P. volvatum*, and both species have key micromorphological features shared with *Elaphomyces*. Masuya & Asai (2004) stated "…the fact that unopened ascomata of *P. japonica* are highly similar to the fruit-body found in the genus *Elaphomyces* suggests that this species, which we believe should be treated as *Pseudotulostoma*, may also exist as a species of *Elaphomyces*". It should be noted that during the time of both Miller *et al.* (2001) and Masuya & Asai (2004) very few *Elaphomyces* sequences were available in GenBank, so taxon sampling for the genus was low in both studies. Subsequently, Reynolds (2011) provided unpublished data suggesting a congeneric relationship of *Pseudotulostoma* and *Elaphomyces*, a relationship also suggested by our phylogenetic analysis (Fig. 1). Although our results suggest that *Pseudotulostoma* and *Elaphomyces* are not reciprocally monophyletic and may need to be treated as a single genus, more taxon-extensive sampling with multi-locus DNA sequence data is needed to better understand the relationship between them before formal taxonomic changes can be proposed.

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