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 *Dicymba jemmani* (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, volval 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 hypogeeusally 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 & Hawksw 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, Nampie 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...
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 MgCl₂, 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 Sequerencer 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/phyllows/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
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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 RAxML using a GTRGAMMA substitution model. Tree is midpoint rooted. Numbers on or next to branches are nonparametric bootstrap.

0.04 substitutions/site
| 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 citrus       | 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                                 | LCO10285* |           |
| Elaphomyces sp.          | HB3              | Indonesia                                 | LCO10287* |           |
| Elaphomyces sp.          | HB2              | Indonesia                                 | LCO10286* |           |
| Elaphomyces sp.          | 738.12 (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  |           |
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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*     | 4115-1205 (root tip) | UK                                         | HM146813     |              |
| Uncultured *Elaphomyces*     | 4115-1205 (root tip) | UK                                         | HM146813     |              |
| Uncultured *Elaphomyces*     | 5237-1201 (root tip) | UK                                         | HM146813     |              |
| Uncultured *Elaphomyces*     | 1Bart526S (soil)  | USA                                        | HQQ21767     |              |
| Uncultured *Elaphomyces*     | Bart1760S (soil)  | USA                                        | HQQ21973     |              |
| Uncultured *Elaphomyces*     | 4Bart240R (root tip) | USA                                        | HQQ21974     |              |
| Uncultured *Elaphomyces*     | 4Bart24S (soil)   | USA                                        | HQQ22116     |              |
| Uncultured *Elaphomyces*     | 1Bart34R (root tip) | USA                                        | HQQ22117     |              |
| Uncultured *Elaphomyces*     | 4Bart309S (soil)  | USA                                        | HQQ22186     |              |
| 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                                        | JQ990349     |              |
| 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*| _ji046 (root tip) | Sweden                                     | AY339226     |              |

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-
Fig. 2. Best maximum likelihood phylogram of an analysis of ITS sequences in RAxML using a GTR+GAMMA 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. labyrinthinus, 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 Pseudotolostoma 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, 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 subtertinate 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 epidemoides* 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 sinusous, 5.0–5.5 µm broad with walls 0.5 µm thick. *Gleba* of ascospores and sinuous, hyaline, sepaete, 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 NW 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 NW 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. labyrinthinus* described here, and showed that, within the genus *Elaphomyces*, these two African species share a common ancestor with *E. compleximurus* from Guyana (Fig. 1). Both *Elaphomyces favosus* and *E. labyrinthinus* 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-avellate 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–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

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**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 thick, 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-eruptive 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 the village of Somalomo, Uper 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.

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 macro morphology 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-alovalate.

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, capitulate 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
New species of Elaphomyces

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. labyrinthinus* 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 = 35.2 µm) than those of *E. labyrinthinus* (mean diameter = 15.8 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 peridial structure and ascospore size but differs in having a labyrinthine 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. compleximuris* (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...
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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