Mycologia, 104(5), 2012, pp. 1244–1249. DOI: 10.3852/12-061
© 2012 by The Mycological Society of America, Lawrence, KS 66044-8897

New *Elaphomyces* species (Elaphomycetaceae, Eurotiales, Ascomycota) from Guyana

Michael A. Castellano
USDA, Forest Service, Northern Research Station, 3200 Jefferson Way, Corvallis, Oregon 97331

Terry W. Henkel[1]
Department of Biological Sciences, Humboldt State University, Arcata, California 95521

Steven L. Miller
Department of Botany, University of Wyoming, Laramie, Wyoming 82071

Matthew E. Smith
Department of Plant Pathology, University of Florida, Gainesville, Florida 32611

M. Catherine Aime
Department of Plant Pathology and Crop Physiology, Louisiana State University Agricultural Center, Baton Rouge, Louisiana 70803

Abstract: *Elaphomyces compleximurus* sp. nov. and *E. digitatus* sp. nov. are described from the Pakaraima Mountains of Guyana. Macromorphological, micromorphological, habitat and DNA sequence data are provided for each new species. This is the first report of *Elaphomyces* ascomata associated with ectomycorrhizal members of the Fabaceae and also for the genus from the lowland South American tropics.

Key words: *Dicymbe*, Guiana Shield, hypogeous fungi, sequestrate fungi

Introduction

The hypogeous false truffle genus *Elaphomyces* Fries (Elaphomycetaceae, Eurotiales, Ascomycota) is composed of ectomycorrhizal (ECM) fungi with a gleba of powdery, dark ascospores, a thick peridium and large cleistothecial ascomata relative to other members of the Eurotiales. Most of the ~ 67 species of *Elaphomyces* currently described are from north temperate forests in association with ECM host plants in the Pinaceae, Fagaceae and Betulaceae (Castellano et al. 1989). Cooke (1892) and Rodway (1918) provided the first reports of *Elaphomyces* for the southern hemisphere, but they assigned European names to their species (Castellano et al. 2011). The known *Elaphomyces* diversity in the southern temperate zones was increased substantially by Castellano et al. (2011) who published 13 new species associated with Myrtaceae and *Nothofagus* host plants in Australia. Three new *Elaphomyces* species associated with *Nothofagus* recently were described from New Zealand (Castellano et al. 2012).

While *Elaphomyces* is well represented in the subtropical *Quercus* forests of Florida and records exist from montane *Quercus* forests of Costa Rica (Castellano unpubl), records are exceedingly scarce from the low-latitude, lowland forests of the tropics. Corner and Hawker (1955) described the first *Elaphomyces* species from the lowland tropics in association with Dipterocarpaceae in Singapore, but to date no species have been described from the lowland South American tropics.

Over the past 13 y macrofungal collecting expeditions in western-central Guyana have revealed a wealth of ECM fungi in forests dominated by the ECM Fabaceae canopy tree species *Dicymbe corymbosa* Spruce ex Benth., *Dicymbe altsonii* Sandw. (subfam. Caesalpinioideae; Henkel et al. 2011) and *Aldina insignis* (Benth.) Endl. (subfam. Papilionoideae; Smith et al. 2011). Recent molecular studies of ECM fungal communities in these forests have detected belowground fungal diversity similar to those revealed by long-term sporocarp surveys in the region, but the great majority of taxa are basidiomycetes (Smith et al. 2011). So far, one member of the Elaphomycetaceae, *Pseudotulostoma volvata* O.K. Mill. & T.W. Henkel, has been described from Guyana (Miller et al. 2001). Here we describe two new *Elaphomyces* species discovered in Guyana's *Dicymbe* forests. Morphological, habitat and DNA sequence data are provided for each species. These are the first described species of *Elaphomyces* associated with ECM Fabaceae hosts and the first records for the genus from the lowland South American tropics.

Materials and methods

Ascomata were collected during the May–July rainy seasons of 2000–2002, 2004, 2007 and 2009–2010 from the Upper Potaro River Basin within a 15 km radius of a permanent base camp at 5°18′04.8″N, 59°54′40.4″W, 710 m (Henkel 2003). More were collected during May–June 2011 from the Upper Demerara River Basin at Mabura Ecological Reserve within 2 km of a field station at 5°09′19.0″N; 58°41′58.9″W, 100 m. At Potaro ascomata were collected from monodominant forests of *D. corymbosa* and other stands containing *D. corymbosa*, *D. altsonii* and *A. insignis*; at

Submitted 23 Feb 2012; accepted for publication 30 Mar 2012.
[1] Corresponding author. E-mail: twh5@humboldt.edu

Mabura collections were made in *D. altsonii* monodominant stands. Fresh characteristics of ascomata were recorded in the field. Color was compared with plates from Kornerup and Wanscher (1978) and is cited in parenthesis (e.g. 5A4). Specimens were field-dried with silica gel. Dried specimens were rehydrated and examined in 3% KOH, Melzer's reagent and cotton blue. Microscopic descriptions are based on 3% KOH mounts unless specified. Twenty ascospores were measured from the holotype 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. Specimens are deposited in these herbaria: (Holmgren et al. 1990): BRG, University of Guyana; HSU, Humboldt State University; OSC, Oregon State University; LSUM, Louisiana State University Mycology. Ribosomal DNA sequencing for the ITS and LSU regions followed the protocols of Aime et al. (2005) and Smith et al. (2011); GenBank accession numbers are provided in *Specimens examined*.

TAXONOMY

Elaphomyces compleximurus Castellano, T.W. Henkel et S.L. Mill., sp. nov. FIG. 1

MycoBank MB564542

Ascomata 9–15 mm tall × 10–20 mm broad, nearly globose to flattened subovate with a distinct, abruptly tapered base, with roots, sand and dense mycelium adherent to the base and upward over lower half of the ascoma, black when fresh, drying to dark gray (near 10F2–10F3); peridial surface completely covered with dense black mycelium visible with a hand lens, beneath this mycelium verrucose throughout except nearly smooth near base; warts contiguous, separated by narrow lines, angular-sided, up to 1 mm tall and 0.3 mm broad, with rounded apices. Peridium when dried collapsing to form an exterior, subreticulate pattern of ridges and folds, these irregular in length, superimposed over surface warts; in section four-layered; outer first layer 0.03–0.06 mm thick, dark brown, of closely appressed mycelium, underlain by a second carbonaceous layer 0.1–0.15 mm thick, of nearly black, closely appressed cells, underlain by a third layer 0.5–0.6 mm thick, mostly white but a thin, pale tan outer zone, cottony, with embedded, black-mantled ectomycorrhizas scattered throughout but most numerous near the ascoma base; the innermost fourth layer 1.3–2 mm thick, pale gray to pale brownish gray (6F2–6F3), gradually more pale toward the gleba, similar in texture to the third layer but lacking embedded ectomycorrhizas. Gleba grayish black (9F2–9F3) to black, powdery, with fine, pale dissepiments. Odor mildly of raw potato with fruity element; taste none. Outer first layer of peridium composed of thick-walled, brown to dark brown, septate, segmented and irregular (not straight-walled) hyphae 1–2 μm broad, these somewhat tuberculate, loosely interwoven and much branched at nearly 90 degree angles; carbonaceous second layer dense, of compact, dark brown to nearly black cells with walls ~ 2 μm thick, quickly grading downward into the third layer of brown, somewhat puzzle-like textura epidermoidea, grading into the fourth layer where the pale brown tissue changes quickly to textura intricata or textura porrecta forming clusters in cross-hatched formations, this pattern continuing inward with the hyphae grading from brown to hyaline to brown near the gleba; hyphae of fourth layer 5–7 μm wide with walls 1–2 μm thick. Gleba of ascospores and sinuous, hyaline, septate, acutely branched, loosely interwoven hyphae, these 2–3 μm wide, with walls < 0.5 μm thick. Asci not observed. Ascospores globose, brown, 22–25 μm diam (mean = 23.2 μm) including the reticulate-alveolate ornamentation; aveolae well defined, 3.5–5.5 μm broad × 2–3 μm tall, nearly round or with subangular sides with irregular to wavy walls; under SEM the individual alveolar wall consisting of two layers separated by vertical ribs; these nearly equally spaced along the wall, with rib ends at the margin of the wall; the ascospore surface exposed inside the alveolae an irregular, slightly roughened, central disk with radiating repent rods and small depressions extending to the base of the surrounding alveolar wall.

Holotype. *Henkel 8880* (BRG; ISOTYPES HSU; OSC)

Habit, habitat and distribution. Scattered to gregarious on the forest floor under *D. corymbosa* or *D. altsonii* on white sand or brown sand soils; May–July rainy season and also in late August during dry season transition; known from the type locality in the Upper Potaro River Basin of Guyana and ~ 100 km east in the Mabura Hill region.

Etymology. Complexus (Latin adj. B) = complex + murus (Latin s.m. II) = wall, referring to the distinctive, complex structure of the ascospore ornamentation.

Specimens examined. GUYANA. REGION 8 POTARO-SIPARUNI: Pakaraima Mountains, Upper Potaro River Basin, within a 4 km radius of base camp at 5°18′04.8″N, 59°54′40.4″W, 710–750 m; vicinity of base camp, 4 Jun 2000, *S.L. Miller 10096* (BRG; HSU; OSC) and 23 Jun 2000, *S.L. Miller 10157* (BRG; HSU; OSC); ~ 0.75 km northeast of base camp on white sand soils in mixed *D. corymbosa-Micrandra glabra* forest, 25 Aug 2007, *Henkel 8880* (HOLOTYPE BRG; ISOTYPES HSU, OSC; ITS GenBank JN711441); 1.5 km west of base camp in mixed *D. corymbosa-M. glabra* forest on alluvial sand soils, 3 Jun 2010, *Henkel 9254* (BRG, HSU); REGION 10 UPPER DEMERARA-BERBICE: Mabura Ecological Reserve, field station located at 5°09′19.0″N, 58°41′58.9″W, ~ 100 m; vicinity of field station, under *D. altsonii* on brown sand soils, 28 May 2011, *Aime 4331* (BRG; LSUM).

Commentary. The distinctly reticulate-alveolate ascospores of *E. compleximurus* easily distinguish it from nearly all other described *Elaphomyces* species. The only known *Elaphomyces* species to combine the features of a tapered base of the ascoma, black, verrucose peridium and some degree of reticulate ascospore ornamentation are the European *E. cyanosporus* Tul. & C. Tul. and *E. persoonii* Vittad. Both are easily separated from *E. compleximurus* by their larger

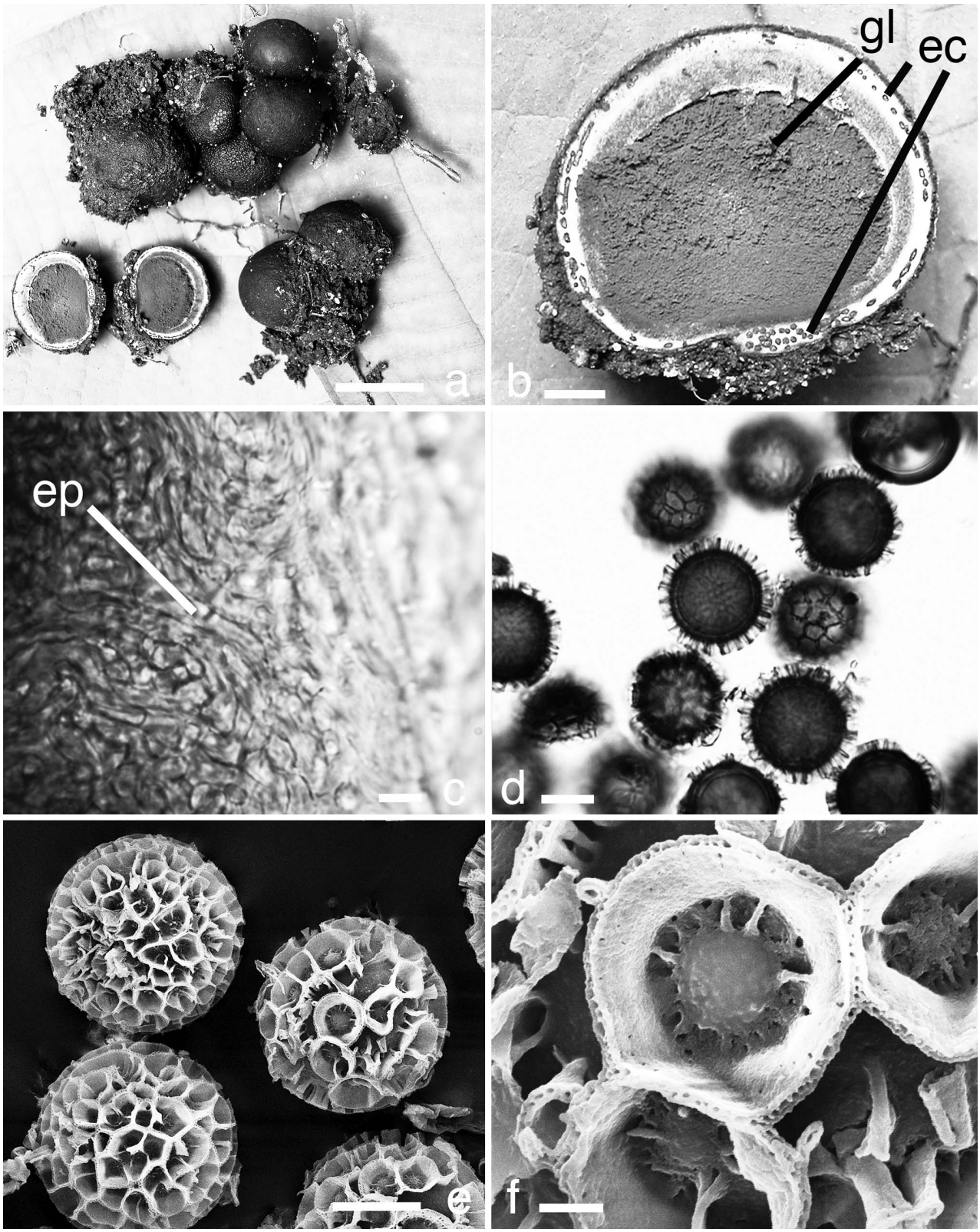

FIG. 1. *Elaphomyces compleximurus* (HOLOTYPE; *Henkel 8880*). a. Ascomata in clusters and in longitudinal section. b. Longitudinal section of ascoma showing the powdery gleba (gl) and the embedded ectomycorrhizas (ec) within the peridium. c. Peridial layer 2 with carbonaceous cells (ep). d. Mature ascospores under bright field microscopy showing ornamentation in outline and surface view of aveolae. e. Ascospores under scanning electron microscopy (SEM) showing the irregularly sided aveolae. f. Aveolae under SEM showing complex ornamentation of ribs between alveolar wall layers. Bars: a = 15 mm; b = 2 mm; c, d, e =10 µm; f = 1 µm.

ascospores; in *E. cyanosporus* the ascospore diameter ranges from 27–30 μm (mean = 28 μm), for *E. persoonii* 29–33 μm (mean = 31.1 μm), vs. 22–25 μm (mean = 23.2 μm) for *E. compleximurus*. Both *E. compleximurus* and *E. persoonii* have ectomycorrhizas embedded within the outer layers of the peridium, a condition otherwise highly unusual in sequestrate fungi.

This is the first report of *Elaphomyces* ascomata associated with fabaceous hosts. However, an ITS rDNA sequence of another *Elaphomyces* species (ECM1108, GenBank JN168718) as yet undiscovered as ascomata recently was reported from ectomycorrhizas of *D. corymbosa* (Smith et al. 2011) and also found on *D. jenmanii* (Smith and Henkel unpubl), indicating that taxa beyond those described here exist in the region. In Guyana *Elaphomyces compleximurus* has been encountered less frequently than *E. digitatus* (described below), but the former is harder to find in the field because of its dark color. *Pseudotulostoma volvata*, another sympatric taxon from the Elaphomycetaceae, is common in *Dicymbe*-dominated forests both as ascomata and on ECM roots and also has the unusual peridium-embedded ectomycorrhizas (Miller et al. 2001; Henkel et al. 2006, 2011).

Elaphomyces digitatus Castellano, T.W. Henkel et S.L. Mill., sp. nov. FIG. 2

MycoBank MB564543

Ascomata 4–11 mm tall × 4–31 mm broad, nearly globose to flattened or ellipsoidal, occasionally furrowed, when immature cream (5A2–5A3) to light orange (5A5), light yellow (4A5) or grayish orange (5B3), at maturity brownish orange (5B3–5B8–5C8) to occasionally darker brown (6E8–6F8), with fine surface tomentum throughout that is occasionally absent at maturity; ectomycorrhizas with adherent soil irregularly covering the lower one-quarter to one-half of the ascoma, forming a loose exterior agglomeration, not embedded within the peridial structure, occasionally attached to more extensive fine roots extending into soil; peridial surface beneath the tomentum finely roughened macroscopically, under hand lens verrucose, consisting of fine, contiguous concolorous warts except where damaged; warts ~ 0.13 mm tall and 0.25 mm broad, pyramidal with obtuse apices, with 4–6 sides of irregular dimensions. Peridium when fresh leathery and tough, 0.9–1.8 mm thick overall; in dried section 0.5–0.75 mm thick overall, three-layered; outer layer of brownish orange pyramidal, blunt warts up to 0.13 mm tall, underlain by a dull creamy white to off-white layer 0.1–0.15 mm thick, this grading into an innermost, gray to reddish gray layer 0.25–0.3 mm thick. Gleba dark brown (7F8) to dark rusty brown (8F8) when mature, powdery, with numerous, fine, white dissepiments throughout. Odor musky or skunky or of latex when sectioned; taste none. Outer layer of peridium of small pyramidal warts up to 125 μm tall, red-brown at apex and yellow-brown near base; cells short, pigmented, with walls 1–2 μm thick; the pyramids intraspace 50–60 μm deep of hyaline, more or less parallel, inflated cells that are 5–10 μm broad; outer layer grading inward into a disorganized textura epidermoidea of interwoven, hyaline hyphae 10–12 μm wide with walls 2–4 μm thick, grading quickly inward into a darker layer tinted yellow-brown from scattered dark concolorous granules; hyphae immediately adjacent to the gleba occasionally inflated up to 35 μm broad. Mature gleba of ascospores and sinuous, interwoven, hyaline, septate hyphae, these 2–3 μm broad with walls < 0.5 μm thick, intermixed with inflated hyphae up to 6 μm broad and encrusted with brown, amorphous deposits. Asci present in immature gleba, globose, 42–65 μm diam, walls 1–2 μm thick, containing 2–8 ascospores (mostly 8), with a constricted base up to 12 × 7 μm attached to a knot of granulated, pale brown, thick-walled hyphae ± 7 μm wide with walls 2–3 μm thick. Ascospores globose, dark red-brown, (16)20–23(24) μm diam (mean = 21.9 μm) including the irregular, labyrinthine-ridged ornamentation that forms a partial reticulum of dark, crowded ridges 3–4 μm tall; under SEM reticulum walls wavy, fully fused near margins but separating downward into columnar basal elements near ascospore surface; margins with numerous short, irregular, digitate projections.

Holotype. *Henkel 8887* (BRG; ISOTYPES HSU; OSC)

Habit, habitat and distribution. Usually in clusters of 2–5 ascomata in hilly country on red lateritic soils at ~ 710 m in *D. corymbosa* monodominant forest, also in similar terrain in mixed *D. altsonii*, *D. corymbosa* and *A. insignis* forest, infrequently in sandy, alluvial soil partially embedded in humic material on forest floor under *D. corymbosa*, rarely elevated 1–2 m up the bole of a *D. altsonii* in mixed *Dicymbe* forest, or in *D. altsonii*-dominated stands at low elevation on brown sand soils; May–July and December–January rainy seasons; known from the type locality in the Upper Potaro River Basin of Guyana and ~ 100 km east in the Mabura Hill region.

Etymology. Digitatus (Latin adj. A) = digitate, referring to the small, finger-like projections emanating from the outer ascospore ornamentation.

Specimens examined. GUYANA. REGION 8 POTARO-SIPARUNI: Pakaraima Mountains, Upper Potaro River Basin, within a 15 km radius of Potaro base camp at 5°18′04.8″N, 59°54′40.4″W, 710–750 m; old Ayanganna airstrip, 21 May 2000, *S.L. Miller 10041* (BRG; HSU; OSC) and 21 May 2000, *Aime 995* (BRG; LSUM); ~ 200 m southwest of base camp at *D. altsonii* site, 22 May 2000, *Henkel 7448* (BRG; HSU; OSC) and *S.L. Miller 10053* (BRG; HSU; OSC); *Dicymbe* plot 1, 28 May 2000, *Aime 1047* (BRG; OSC; LSUM) and 14 May 2010, *Aime 3914* (BRG; LSUM); vicinity of base camp, 30 May 2000, *Aime 1079* (BRG; OSC; LSUM) and 5 Jul 2004, *Henkel 8744* (BRG; HSU; OSC) and 26 Aug 2007, *Henkel 8887* (HOLOTYPE BRG; ISOTYPE HSU, OSC; ITS GenBank JQ657705); Benny's ridge under *D. corymbosa* on lateritic ironstone

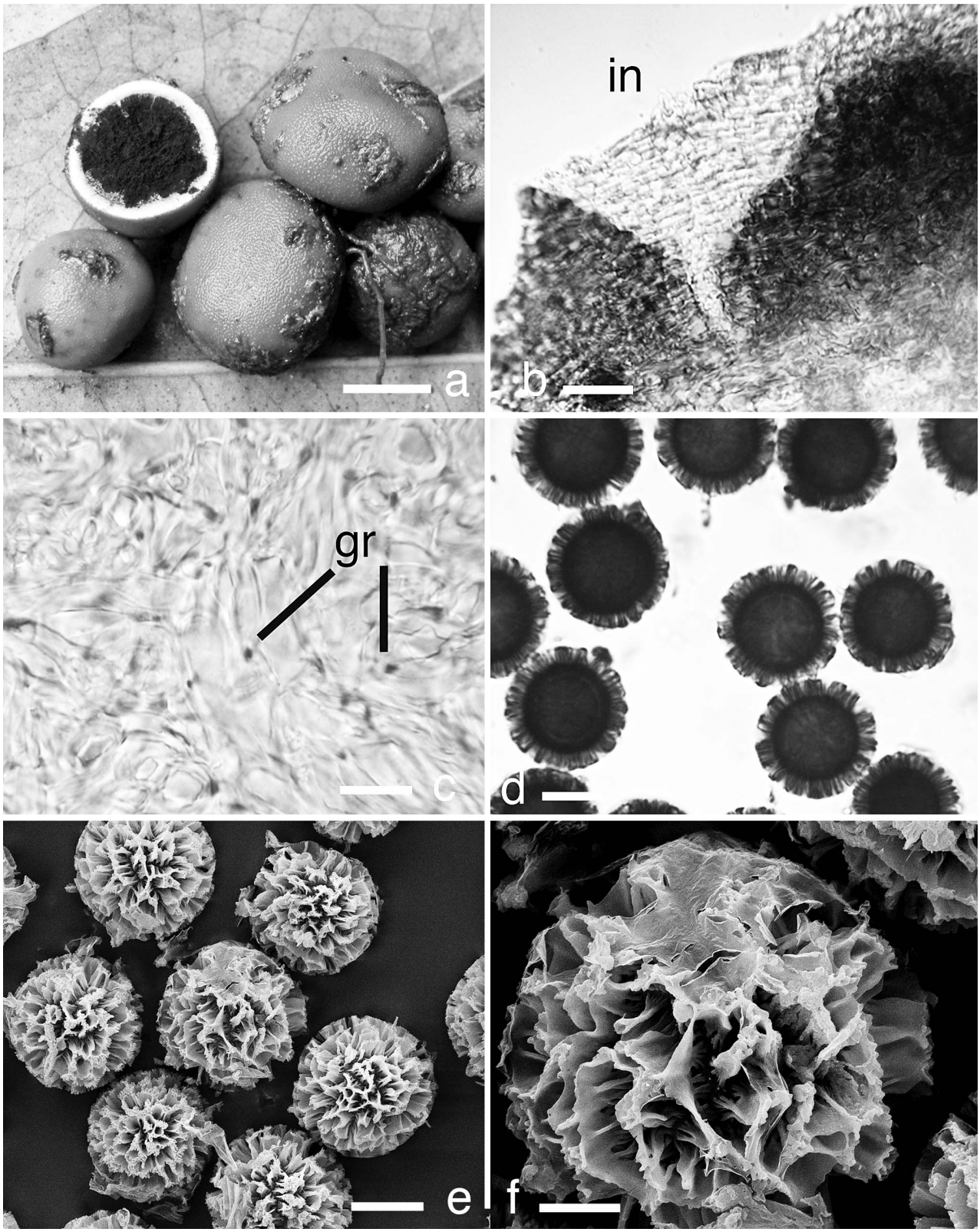

FIG. 2. *Elaphomyces digitatus* (HOLOTYPE; *Henkel 8887*). a. Fresh ascomata including transverse section. b. Outermost peridium layer showing stacked hyaline cells in of space between warts. c. Interior peridium layer with scattered dark granules (gr) interspersed with hyaline hyphae. d. Mature ascospores under bright field microscopy with ornamentation in outline. e. Ascospores under scanning electron microscopy (SEM) with the labyrinthine-ridged ornamentation. f. Individual ascospore under SEM with the digitate protrusions on the wall margins of ornamentation. Bars: a = 10 mm; b = 50 µm; c = 20 µm; d, e =10 µm; f = 5 µm.

soils, 1 Jun 2000, *S.L. Miller 10088* (BRG; HSU; OSC) and 4 Jun 2000, *S.L. Miller 10105* (BRG; HSU; OSC) and 10 Jun 2000, *S.L. Miller 10124* (BRG; HSU; OSC); *Dicymbe* plot 3, 18 May 2001, *Aime 1512* (BRG; OSC; LSUM; LSU GenBank JN713147) and 8 Jun 2002, *Aime 1923* (BRG; OSC; LSUM; LSU GenBank JN713148); near Tadang base camp in mixed *Dicymbe–Aldina* forest on lateritic soils, 27 Dec 2009, *Henkel 9161* (BRG; HSU) and attached directly to roots of *A. insignis*, 29 Dec 2009, *Henkel 9166* (BRG; HSU); REGION 10 UPPER DEMERARA-BERBICE: Mabura Ecological Reserve, field station at 5°09′19.0″N, 58°41′58.9″W, ~ 100 m; vicinity of field station, under *D. altsonii* on brown sand soils, 24 May 2011, *Aime 4284* (BRG; LSUM).

Commentary. The orange peridium of *E. digitatus* is distinctive in the genus, and this along with the subepigeous presentation of ascomata in clusters makes them relatively easy to find in the field. The European species *E. aculeatus* Vittad., *E. anthracinus* Vittad., *E. echinatus* Vittad., *E. leveillei* Tul. & C. Tul. and *E. leucosporus* Vittad. have ascospores of similar size as *E. digitatus*, but each has a black carbonaceous peridium and a different pattern of ascospore ornamentation. *Elaphomyces cyanosporus* and *E. persoonii* have similarly reticulate ascospore ornamentations with short, finger-like projections from the wall margin, but both have significantly larger ascospores than *E. digitatus* (28.0 µm and 31.3 µm vs. 21.9 µm mean diam respectively). *Elaphomyces austrogranulatus* Castellano, Trappe & Vernes from Australia has a brown peridium and relatively small ascospores like *E. digitatus* but its ascospore ornamentation is irregularly verrucose (Castellano et al. 2011). Other Australian *Elaphomyces* species with ascospores of similar size have a black peridium.

Elaphomyces digitatus was found fairly frequently in long-term *D. corymbosa* macrofungal study plots in the Upper Potaro Basin, with ascomata recorded in 16.3% of 630, 100 m^2 sampling quadrats over 7 y (Henkel et al. 2011). The congener *E. compleximurus* (described above) was never found in the study plots but recovered sporadically in ''off-plot'' collecting forays. In addition to *Dicymbe* spp., *E. digitatus* appears to form mycorrhizae with the papilionoid leguminous tree *A. insignis*. In one collection of *E. digitatus* (*Henkel 9166*) the ascomata were found directly attached to ECM roots of this confirmed ECM host (Smith et al. 2011).

ACKNOWLEDGMENTS

This research was made possible by grants to TWH from the National Science Foundation grant DEB-0918591, National Geographic Society's Committee for Research and Exploration, Smithsonian Institution's Biological Diversity of the Guiana Shield Program, Linnaean Society of London, Humboldt State University Foundation and to MCA from the Explorer's Club, Washington Group and NSF DEB-0732968. Additional support was provided by a grant to SLM from NSF DEB-1050292. Dillon Husbands functioned as Guyanese field counterpart for several of the expeditions and assisted with collection and description of specimens. Additional field assistance in Guyana was provided by Mimi Chin, Christopher Andrew, Leonard Williams, Valentino Joseph, Francino Edmond and Luciano Edmond. Research permits were granted by the Guyana Environmental Protection Agency. This paper is No. 182 in the Smithsonian Institution's Biological Diversity of the Guiana Shield Program publication series.

LITERATURE CITED

Aime MC, Vilgalys R, Miller OK Jr. 2005. The Crepidotaceae (Basidiomycota, Agaricales): phylogeny and taxonomy of the genera and revision of the family based on molecular evidence. Am J Bot 92:74–82, doi:10.3732/ajb.92.1.74

Castellano MA, Beever RE, Trappe JM. 2012. Sequestrate fungi of New Zealand: *Elaphomyces* and *Rupticutis* gen. nov. (Ascomycota, Eurotiales, Elaphomycetaceae). NZl J Bot. In press.

———, Trappe JM, Maser Z, Maser C. 1989. Keys to ascospores of the genera of hypogeous fungi of north temperate forests with special reference to animal mycophagy. Eureka, California: Mad River Press. 186 p.

———, ———, Vernes K. 2011. Australian species of *Elaphomyces* (Elaphomycetaceae, Eurotiales, Ascomycota). Aust Syst Bot 24:32–57, doi:10.1071/SB10012

Cooke MC. 1892. Handbook of Australian fungi. London: Williams & Norgate. 457 p.

Corner EJH, Hawker LE. 1953. Hypogeous fungi from Malaya. J Br Mycol Soc 36:125–137, doi:10.1016/S0007-1536(53)80057-4

Henkel TW. 2003. Monodominance in the ectomycorrhizal *Dicymbe* corymbosa (Caesalpiniaceae) from Guyana. J Trop Ecol 19:417–437, doi:10.1017/S0266467403003468

———, Aime MC, Chin M, Miller SL, Vilgalys R, Smith ME. 2011. Ectomycorrhizal fungal sporocarp diversity and discovery of new taxa in *Dicymbe* monodominant forests of the Guiana Shield. Biodivers Conserv, doi:10.1007/s10531-011-0166-1

———, James TY, Miller SL, Aime MC, Miller OK Jr. 2006. The mycorrhizal status of *Pseudotulostoma volvata* (Elaphomycetaceae, Eurotiales, Ascomycota). Mycorrhiza 16:241–244, doi:10.1007/s00572-006-0040-2

Holmgren PK, Holmgren NH, Barnett LC. 1990. Index herbariorum I. The herbaria of the world. New York: New York Botanical Garden. 693 p.

Kornerup A, Wanscher JH. 1978. Methuen handbook of colour. 3rd ed. London: Eyre Methuen. 252 p.

Miller OK Jr, Henkel TW, James TY, Miller SL. 2001. *Pseudotulostoma*, a new genus in the Elaphomycetaceae from Guyana. Mycol Res 105:1268–1272, doi:10.1017/S095375620100466X

Rodway L. 1918. Botanical notes. Papers and proceedings of the Royal Society of Tasmania 1917. p 105–110.

Smith ME, Henkel TW, Aime MC, Fremier AK, Vilgalys R. 2011. Ectomycorrhizal fungal diversity and community structure on three co-occurring leguminous canopy tree species in a Neotropical rainforest. New Phytol 192:699–712, doi:10.1111/j.1469-8137.2011.03844.x