FUSE

Fungal Systematics and Evolution

VOLUME 1
JUNE 2018
PAGES 1–12

doi:xxxxxxxxxxxx/fuse.2018.xx

Three new black *Elaphomyces* species (*Elaphomycetaceae, Eurotiales, Ascomycota*) from eastern North America with notes on selected European species

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Key words:
ectomycorrhiza
hypogeous fungi
new taxa
sequestrate fungi

Abstract: We describe three new species of *Elaphomyces* from eastern North America. Of the three, *Elaphomyces loebiae* is the rarest, known only from North Carolina and South Carolina, and appears to associate primarily with ectomycorrhizal hardwoods but possibly also with conifers. *Elaphomyces cibulae* is widely distributed but disjunct from Florida, Mississippi, and North Carolina. *Elaphomyces cibulae* seems to primarily associate with *Quercus* species. *Elaphomyces mitchelliae* has the widest distribution of the three species, from Florida, Maryland, North Carolina, Virginia, and West Virginia, and appears to associate with either ectomycorrhizal hardwoods and/or conifers. In the course of comparing our new *Elaphomyces* species to previously described European species we discovered that *E. persoonii* var. *minor* is conspecific in all essential details with and thus a synonym of *E. cyanosporus*.

Published online: 1 December 2018.

INTRODUCTION

The hypogeous, sequestrate genus *Elaphomyces* (*Elaphomycetaceae, Eurotiales, Ascomycota*) forms ectomycorrhizal associations with roots of diverse gymnosperm and angiosperm trees and shrubs around the world (Miller & Miller 1984, Trappe *et al*. 2009, Castellano *et al*. 2016). Species of *Elaphomyces* release aromas that are detected by numerous mammal species that dig them up and utilize them as an important food, thereby dispersing the spores across the landscape (Boudier 1876, Fogel & Trappe 1978, Vogt *et al*. 1981, Genov 1982, Cork & Kenagy 1989, Vernes *et al*. 2004, Vernes & Poirier 2007). The leathery peridium is eaten by the animals and some of the powdery mass of hydrophobic spores within the fruiting bodies are ingested, some released to the air, and some left on the ground, logs, or stumps.

Elaphomyces species have been reported from every continent except Antarctica, and occur in diverse forest habitats ranging from temperate and subarctic conifer forests to lowland tropics (Corner & Hawker 1953, Trappe & Kimbrough 1972, Zhang & Minter 1989, Castellano *et al*. 2011, Reynolds 2011, Castellano *et al*. 2012a, c, 2016, Paz *et al*. 2012, Castellano & Stephens 2017). Recently Paz *et al*. (2017) revised the systematics of the European *Elaphomyces* species. They present all known species from Europe and provide an updated structure to the sections and subsections within *Elaphomyces*.

Our recent work on the genus indicates that eastern North America is likely the epicentre of *Elaphomyces* biodiversity with approximately 30 to 40 species, many of which are still undescribed (Castellano, unpubl. data). Historically in North America, most *Elaphomyces* spp. were assigned European names due to the difficulty in distinguishing species based on published descriptions that were often sparse in details (Trappe & Guzman 1971, Miller & Miller 1984, Luoma & Frenkel 1991, Gomez-Reyes *et al*. 2012). Most North American *Elaphomyces* with a black or dark coloured peridial surface were assigned the name *E. anthracinus*, and others with brown peridial surfaces were named *E. granulatus* or *E. muricatus,* depending on structure of the inner peridium. We now know that these species do not occur in North America. In eastern North America (from Québec south to the Gulf of Mexico), there are currently eight described *Elaphomyces* species, including *E. americanus, E. appalachiensis, E. bartlettii, E. macrosporus, E. oreoides, E. remickii, E. spinoreticulatus, E. verruculosus*, and *E. viridiseptum* (Linder 1939, Trappe & Kimbrough 1972, Zhang & Minter 1989, Castellano *et al*. 2012b, Castellano & Stephens 2017). The aim of the present study was thus to characterise several *Elaphomyces* spp. collected in the USA.

MATERIALS AND METHODS

Species of *Elaphomyces* typically develop below ground, so ascomata were collected by raking away the leaf and upper soil layers in suitable habitats, observing and excavating the area where animals had previously dug, or by looking for *Tolypocladium* species that fruit aboveground while parasitizing the ascomata of *Elaphomyces* species (Castellano *et al*. 2004). Occasionally, specimens had partially emerged from the soil in eroded or disturbed environments like road banks, campgrounds, or trail edges.

Descriptions of macro-morphological characters are based on fresh material. Colours are described in general terms based

on the observations of the authors and collectors. Tissues and spores from dried specimens were rehydrated and examined in 3 % KOH, Melzer's reagent, or cotton blue for study of microscopic characters. Neither tissues nor spores reacted distinctively to Melzer's reagent. Microscopic characters of ascoma tissues and spores were described from 3 % KOH mounts unless otherwise specified. Spore dimensions include ornamentation and are from twenty randomly selected ascospores measured from the holotype collections. Asci of *Elaphomyces* spp. generally disintegrate with maturation, so their features often cannot be recorded. Dried ascospores were mounted on aluminum pegs with double-sided tape and coated with gold for scanning electron microscopy (SEM) with a Quanta 600 FEG scanning electron microscope. Specimens are deposited in the following herbaria as noted for each collection: FLAS, OSC, RMS, ITCV, ISC (Index Herbariorum, referenced May 2017).

TAXONOMY

Elaphomyces cibulae Castellano, Trappe & D. Mitchell, ***sp. nov.*** MycoBank MB821236. Fig. 1.

Etymology: Named "*cibulae*" in honour of the late Dr Bill Cibula of Mississippi, enthusiastic collector of fungi in the Gulf States region of the southern USA.

Type: **USA,** *North Carolina*, Rutherford Co., near Gilkey, 25 Jul. 2012, *T. Elliott* (holotype OSC 149262).

Fresh *ascomata* up to 18 mm tall × 20 mm broad, subglobose to irregularly flattened, completely embedded in white to ivory mycelium and rhizomorphs to form a husk around individual ascomata that incorporates soil, ectomycorrhizae and debris; portions of mycelium staining brown when exposed, when dried off-white to pale brown. *Peridium* covered with mycelium and rhizomorphs to occlude observation of the peridial surface, this peridial surface carbonaceous, black to blue-black, smooth to the naked eye at first but close examination reveals a finely colliculate surface and the ascoma itself somewhat rugose when fresh, wrinkled to pleated when dried from inner peridial collapse. Colliculate surface comprised of flattened, multi-sided, irregularly shaped bumps up to 240 µm broad, ± 40 µm tall, contiguous with each other. Overall the fresh peridium ± 2 mm thick when mature, the carbonaceous outer layer ± 0.05 mm thick, black in section when fresh and dried, inner layer uneven, up to 2 mm thick, pale blue-white to blue-grey to grey, turning grey-green to blue where cut in some specimens, crisp and moist, off-white to grey when dried. *Gleba* when young filled with dense, white, cottony-membranous tissue that bears the asci, eventually forming a powdery, white spore mass containing web-like hyphae, spore mass turning pale grey-blue as spores mature among white capillitial hyphae, becoming dark blue to grey-blue to grey-blue-black or grey-black when fully mature. *Odour* not distinctive to cabbage-like. *Taste* not recorded.

Peridium two-layered with an additional ephemeral mycelial covering: outer layer 35–50 µm thick, carbonaceous, of dark brown, compact, septate, parallel hyphae, 2.5–3.5 µm broad, the outer ±20 µm portion of very dark brown hyphae, walls ± 1 µm broad; inner layer up to 2 mm thick when fresh, 400–700 µm thick when dried, of compact, uniform, hyaline to pale grey, fascicled hyphae (bundles of 5–10 hyphae), individual hyphae mostly 3.5–8(–9) µm broad, fascicles in a cross-hatched pattern, walls 1.5–3.5 µm thick, occasional disjunct amorphous areas of pale brown pigment diffused through the hyphae; outer mycelial covering composed of interwoven, much-branched, hyaline to pale green, slightly encrusted hyphae, 2–3 µm broad, walls <0.5 µm thick. *Gleba* of spores and hyaline, septate, extensively branched, compact, interwoven hyphae up to 4 µm broad, walls <0.5 µm thick. *Asci* more or less globose, distorted somewhat from maturing spores, hyaline, 29–33 µm broad at maturity, walls ± 1 µm thick, 8-spored. *Spores* globose, sometimes with a flattened side, (13–)14–15 µm broad (mean = 14.6 µm broad), walls ± 1 µm thick, in KOH yellow-brown to red-brown singly and in mass when mature, ornamented with rods, short ridges or a labyrinthine pattern, 1–2 µm tall.

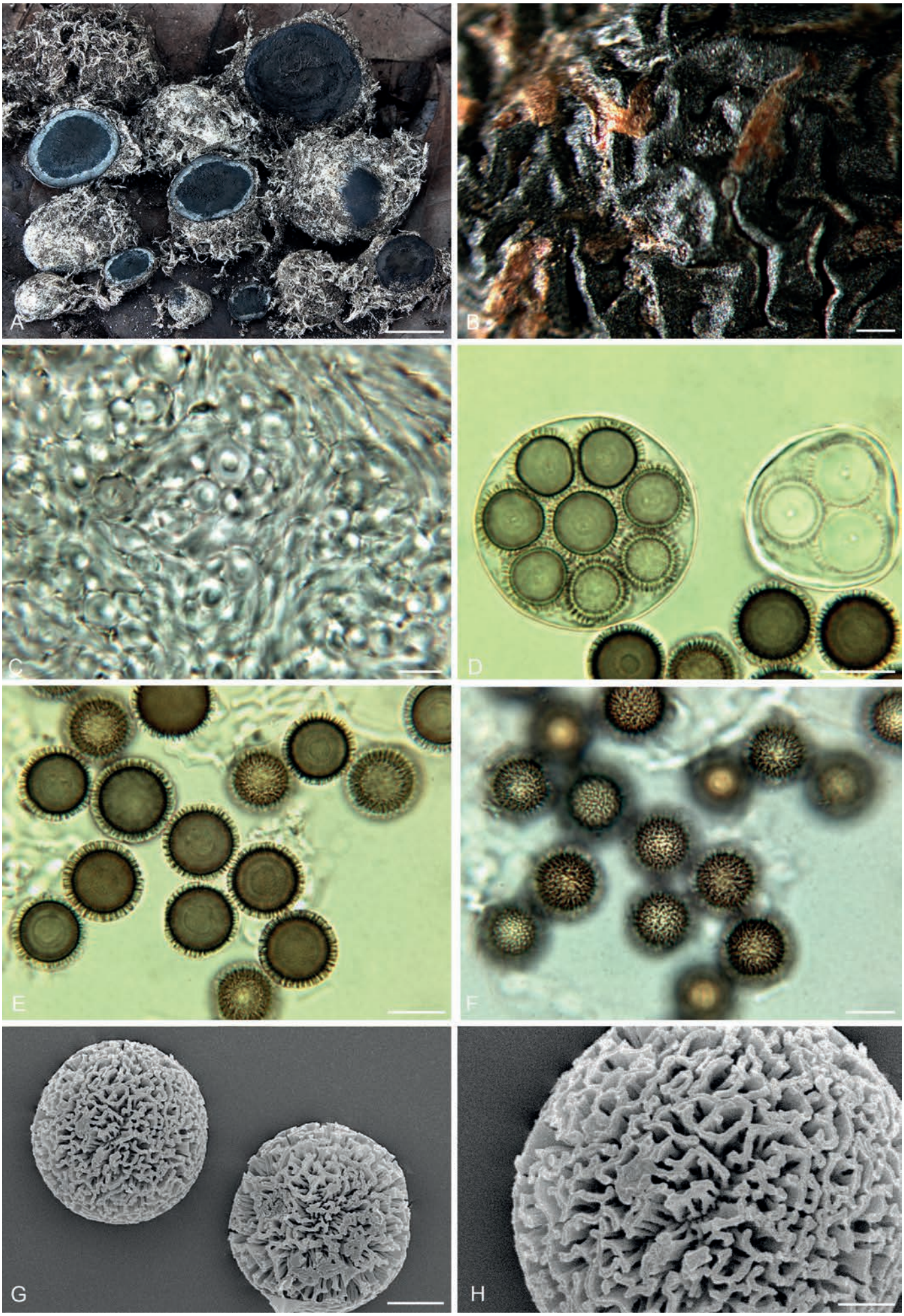
Distribution, habit, habitat, and season: Known from Florida, Mississippi, and North Carolina; hypogeous; under *Quercus virginiana*, *Quercus* sp. and possibly *Pinus* sp.; February, June through August.

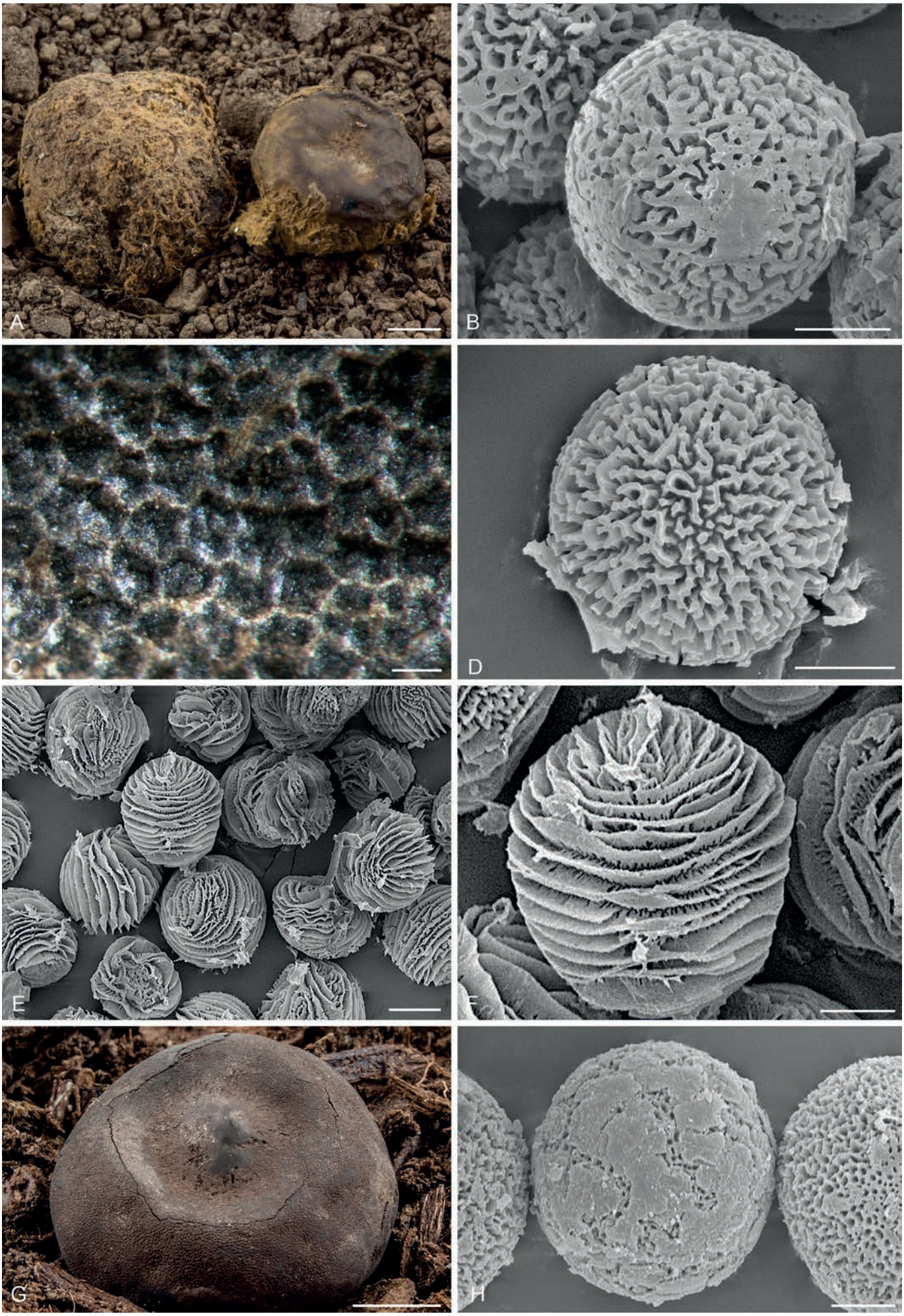
Additional collections examined: **USA**: *Florida*, Polk Co., along county road 54, just west of Champions Gate Blvd., north side of road next to small pond, 25 Feb. 2012, *M. Castellano,* OSC 148261; *Mississippi*, Hancock Co., Steenis Space Center, "old" Gainesville, near corner of Ambrose St. and Union St., under a very large, old-growth *Quercus virginiana* named "Jeremiah," 4 Jun. 1976, *J. Trappe 4601,* OSC 36196; same locality, 6 Aug. 2007, *M. Castellano,* OSC 150018.

Notes: We assign *Elaphomyces cibulae* to section *Malacodermei* on the basis of the thin, somewhat smooth, soft peridium and the small spores.

Castellano studied *Elaphomyces citrinus* collections in Torino (in OSC 149172) marked "Vittadini collection" and Kew (161174) marked "ex herb Berkeley from Vittadini." Castellano (unpubl. data) recorded data from OSC 149172 in part as follows: Dried specimens with peridial surface black, appearing wrinkled or wavy, covered by dark orange-yellow to orange-brown hyphae; spores globose, in KOH brown singly, slightly darker in mass, 15–16(–18) µm broad, mean = 15.8 µm including ornamentation that appears labyrinthine under light microscopy, in section appearing spiny to coarsely spiny; SEM reveals the spore ornamentation as anastomosed rods forming an irregular labyrinth with flattened apices (Fig. 2A–B). Dodge (1929) does not cite a type but reports it as common around Milan. Vittadini, a professor of medicine at the University of Milan, shared many of his truffle collections with contemporary mycologists in several European herbaria. How collections in his herbarium were dealt with after his death is uncertain but the

Fig. 1. *Elaphomyces cibulae*. **A.** Ascomata showing peridial surface, gleba, and peridium in section. **B.** Ascoma surface showing wrinkled nature of the peridium after drying and the finely colliculate surface. **C.** Peridial structure of the inner peridium showing bundled/clustered hyphae. **D.** Asci showing immature and mature spore development. **E.** Ascospore in cross-section showing height and pattern of the ornamentation. **F.** Ascospores in surface view. **G.** SEM micrograph of ascospores showing rod-like ornamentation. **H.** SEM micrograph of an ascospore showing the complex structure to the rod structure. A–F (OSC 149262 – holotype), G & H (OSC 36196). Bars: A = 10 mm, B = 500 µm, C–F = 10 µm, G = 5 µm, H = 2 µm.





Milan herbaria have no Vittadini collections. Mattirolo (1907) reconstituted many Vittadini collections at the Herbarium of the University of Turin (TO) with generous cooperation from other herbaria; the specimens are marked accordingly but with little or no information on their origin, associated habitat or specific collector(s). *Elaphomyces cibulae* as dried resembles *E. citrinus* in the black, carbonaceous outer peridium and in spore characters. The enveloping mycelium surrounding the ascomata of *E. citrinus* is orange-yellow to orange-brown coloured when dried, whereas *E. cibulae* has white to ivory mycelium when dried. Most strikingly are the blue tones of the inner peridium and gleba in fresh ascomata. These blue tones fade with time. In addition, the peridial layers of *E. cibulae* are much thicker than *E. citrinus*, and the spores of *E. cibulae* are slightly smaller than *E. citrinus*.

Castellano studied *Elaphomyces mutabilis* collections from TO marked "Originale de Vittadini – don. Dal Museo di Paris," and in Paris (PC 93923), marked "ex ipso, Mai 1845." Castellano (unpubl. data) recorded data from both collections above in part as follows: Dried peridial surface black, apparently smooth to the naked eye but under magnification revealing a fine pattern of multisided, contiguous, flattened bumps, 250–350 µm broad, completely and persistently covered by floccose, off-white mycelium, spores globose, in KOH brown singly, darker in mass, 14–15 µm broad, mean = 14.5 µm broad including ornamentation that appears as rods, spines, and short ridges under light microscopy, SEM reveals the spore ornamentation as spines or rods anastomosed into short, irregularly-shaped ridges that form an irregular labyrinth, apices flattened (Fig. 2C–D). Dodge (1929), Ceruti (1960), Montecchi & Sarasini (2000), and Rubio *et al*. (2006) all report that *E. mutabilis* when fresh has distinct blue tones to the inner peridium that fade to off-white or very pale blue over time and when dried. Dodge (1929) reports the type from "oak forests near Milan, July to November" but does not designate a collection as type. Castellano also studied PC 93924 marked as *E. mutabilis* var. *flocciger* Tul. & C. Tul., France, Nantes, leg. Menier (the likely type for this variety) and found it to be conspecific with *E. mutabilis*. Dried *E. cibulae* resembles *E. mutabilis* in the blue tones of the inner peridium and gleba when fresh and in the colour of the enveloping mycelium surrounding the ascoma when dried, the black, carbonaceous outer peridium and in spore characters. The inner peridium of *E. mutabilis* has larger sized hyphae and also scattered dark red-brown hyphal segments beneath the outer carbonaceous layer. These elements are lacking in *E. cibulae*. The carbonaceous, outer peridial layer in *E. mutabilis* is constructed of puzzle-like hyphal segments not the parallel hyphae found in *E. cibulae*.

Castellano studied an *Elaphomyces virgatosporus* Hollós collection from TO (also in OSC 149131) marked "Hungary, Nógrád Comite, Litke, 8 November 1904, Under *Fagus*." Castellano (unpubl. data) recorded data from this collection in part as follows: Peridial surface of black, variably sized and shaped, flattened warts, completely covered by dark brown mycelium, spores globose, in KOH brown to dark brown singly, darker in mass, (18–)19–22 µm broad, mean = 20.2 µm broad, including ornamentation that appears as long, continuous ridges in a pseudo-concentric pattern under light microscopy, SEM reveals the spore ornamentation as spines or rods anastomosed into long ridges in a pseudo-concentric pattern, apices flattened (Fig. 2E–F). We do not designate a type collection, as the type should reside in the Hungarian Natural History Museum at Budapest (BP), but collections are unavailable to study except on location. Dried *Elaphomyces cibulae* resembles *E. virgatosporus* in its black, carbonaceous outer peridium and spore size. The dried enveloping mycelium of *E. cibulae* is white to ivory colour, whereas *E. virgatosporus* has dark brown mycelium. The long-ridged spore ornamentation that forms a concentric pattern in *E. virgatosporus* is distinctly different from the irregularly shaped labyrinth ornamentation pattern of *E. cibulae* spores.

Elaphomyces loebiae Castellano & T.F. Elliott ***sp. nov.*** MycoBank MB821237. Fig. 3.

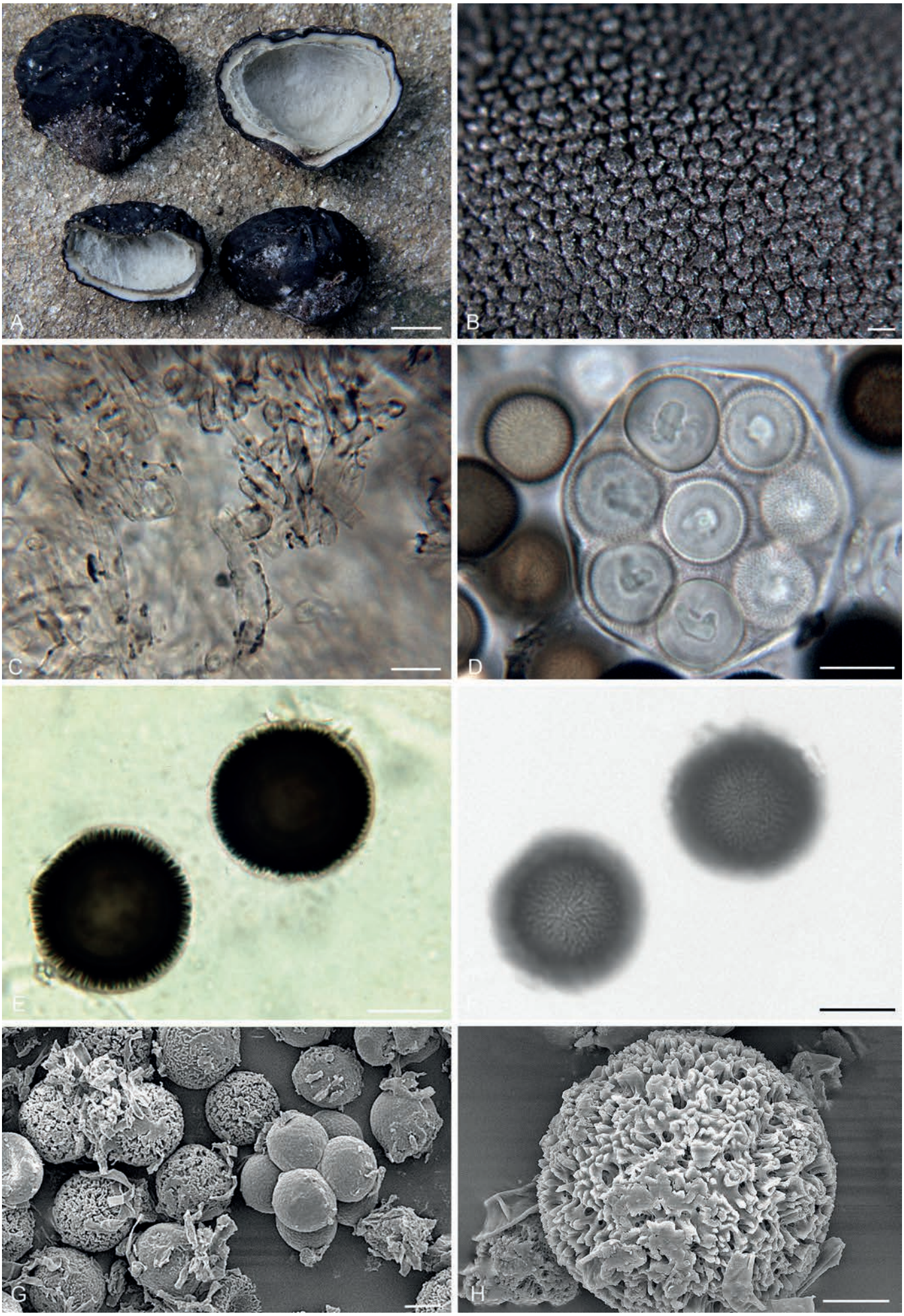
Etymology: Named "*loebiae*" in honour of Dr Susan C. Loeb, research ecologist with the US Forest Service and collector of the type specimens.

Type: **USA**, *North Carolina,* Haywood Co., Nantahala National Forest, Reinhart Gap, 15 Aug. 1996, *S. Loeb & F. Tainter 130* (holotype - OSC 149108).

Ascomata as dried subglobose or irregularly-shaped, up to 23 × 35 mm. *Fresh peridium* ± 1.1 mm thick, outer layer ± 300 µm thick, peridial surface black, brittle, uniformly papillate, papillae flattened or sometimes rounded, irregular in outline, contiguous, 140–260 µm broad at base; inner layer ± 800 µm thick, somewhat pale grey adjacent to epicutis then soon off-white and uniform in texture, specimens supplied for study had been thoroughly cleaned beforehand, so information on material covering specimens is lacking. *Gleba* composed of a dark brown to black, powdery spore mass. *Odour* not recorded. *Taste* not recorded.

Peridium as dried two-layered; outer layer ± 300 µm thick, of compact cells with dark brown to black walls ± 1 µm thick, in between papillae are parallel hyphae with dark brown to black walls < 0.5 µm thick; inner layer ± 800 µm thick, its outer pale grey portion ± 200 µm thick, of short, segmented, much branched, loosely interwoven hyphae 4–7 µm broad, walls 1–2 µm thick, encrusted with small dark granules, the inner off-white portion of hyaline, occasionally sinuous, to somewhat periclinal to interwoven or even interlaced hyphae, mostly ± 4(–8) µm broad, rarely up to 12 µm broad, walls 1–2 µm thick. *Gleba* of spores and hyaline, septate, sinuous hyphae, 1–2 µm broad, walls <0.5 µm thick. *Asci* globose, 45–55 µm broad, hyaline, walls ± 1 µm thick, 8-spored, arising from hyaline,

Fig. 2. Various *Elaphomyces* species from Europe. **A.** *Elaphomyces citrinus* ascomata showing peridial surface and adherent yellow toned mycelium (LE 162869). **B.** *Elaphomyces citrinus* SEM micrograph of ascospores showing the fine detail of the ornamentation (OSC 149172). **C.** *Elaphomyces mutabilis* ascomata showing wart structure and adherent off-white mycelium (OSC 149119). **D.** *Elaphomyces mutabilis* SEM micrograph of ascospores showing the fine detail of the ornamentation (OSC 149121). **E.** *Elaphomyces virgatosporus* SEM micrograph of ascospores showing spiral pattern of the ornamentation (OSC 149131). **F.** *Elaphomyces virgatosporus* SEM micrograph of ascospores showing the fine detail of the ornamentation (OSC 149131). **G.** *Elaphomyces anthracinus* ascoma showing peridial surface (W2008-1095). **H.** *Elaphomyces anthracinus* SEM micrograph of ascospores showing the ornamentation of irregular plates overlaying a fine reticulum (OSC 149163). Bars: A, G = 5 mm, B, D, F, H = 5 µm, C = 250 µm, E = 10 µm.



clustered, contorted, short segmented hyphae ± 6 µm broad. *Spores* globose, 21–22(–23) µm broad (mean = 21.7 µm), walls ± 1 µm thick, in KOH singly and in mass very dark red-brown to nearly black when mature, ornamentation opaque at maturity, consisting of rods or spines anastomosed at their apices to form a fine, irregular labyrinthine-like surface, 1–2 µm tall.

Distribution, habit, habitat, and season: Known only from North Carolina and South Carolina; hypogeous; under *Betula alleghaniensis, Fagus grandifolia*, *Picea rubens*, *Quercus rubra,* and *Tsuga canadensis*; March, May, July, and August.

Additional collections examined: **USA**, *North Carolina*, Haywood Co., Nantahala National Forest, Haywood Gap, 10 Jul. 1996, *S. Loeb & F. Tainter 106* (OSC 149107); Rutherford Co., Union Mills, along Painters Gap Rd., 22 Mar. 2007, *T. Elliott* (OSC 149237); *South Carolina*, Horry Co., Huntington State Park, 25 May 2012, *T. Elliott* (OSC 149109).

Notes: We assign *Elaphomyces loebiae* to section *Ceratogaster*, subsection *Sclerodermei* based on the absence of mycelial patches on the peridial surface.

Castellano studied an *Elaphomyces aculeatus* collection in TO (in FH 258126) marked "Originale di Vittadini dono al Museo di Paris." Castellano (unpubl. data) recorded data from this collection in part is as follows: Peridial surface appearing off-white to brown because of overlaying hyphae. Actual peridial surface is black, carbonaceous and warty, and the thick layer of overlying hyphae can be rubbed off to expose the apices of the warts and give the surface a black-spotted appearance against a brown background. Spores globose, in KOH brown to dark brown singly, slightly darker in mass, 21–24 µm broad, mean = 22.5 µm broad, including ornamentation that appears as irregular, short ridges under light microscopy, in section appearing warty in outline, SEM reveals the spore ornamentation as a fine, complete reticulum without overlying material (Fig. 4A–B). The somewhat smooth peridial surface and spore ornamentation of *E. loebiae* differs significantly from *E. aculeatus*. Castellano also studied a collection marked "*Elaphomyces rubescens*, 2 Aug. 1890, Eisenkaute, Herb Hesse" (OSC 158064) and found it to be conspecific with *E. aculeatus*.

Castellano studied an *Elaphomyces anthracinus* collection in TO (in OSC 149163) marked "Esemplare originale di Vittadini da Museo di Paris." Castellano (unpubl. data) recorded data from this collection in part as follows: Peridial surface black, appearing smooth to the naked eye, close examination reveals a distinct pattern of low, circular, rounded papillae, not warty or angular, ascoma base with a peduncle. Spores globose, in KOH brown to dark brown singly, slightly darker in mass, 20–24 µm broad, mean = 21.9 µm broad, including ornamentation that appears punctate or dimpled under light microscopy, in section appearing with some flattened sides in outline to the spore, SEM reveals the spore ornamentation as a fine, complete reticulum overlain with over-lapping, plate-like material partially or nearly completely (Fig. 2G–H). We agree with Dodge (1929) that *E. pyriformis* Vittad. (Castellano studied authentic Vittadini material in FH marked "ex ipso auctore") and *E. plumbeus* Hesse (Castellano studied authentic material in Marburg marked "Laurasen, Germany, April 1890") are conspecific. Dried *Elaphomyces loebiae* resembles dried *E. anthracinus* in its black, carbonaceous peridial surface covered with minute to small papillae and in having spores that are similarly sized, both approx. 21.5–22 µm broad with ornamentation. The spore ornamentation of *E. loebiae* is characterised by rods or spines anastomosed at their apices to form a fine, irregular labyrinth, whereas the spore ornamentation of *E. anthracinus* appears as a fine, complete reticulum overlain with plate-like material that is partially or nearly completely overlapping.

Elaphomyces loebiae resembles *E. cantabricus* in spore size and ornamentation and its black, carbonaceous peridial surface, but *E. cantabricus* has distinct sharp warts on the peridial surface in contrast to the papillae on the peridial surface of *E. loebiae*.

Elaphomyces loebiae resembles *E. spirosporus* in spore size, but the spore ornamentation of *E. spirosporus* appears as clearly spiraled ridges compared to the irregularly, labyrinthine-like spore ornamentation of *E. loebiae*.

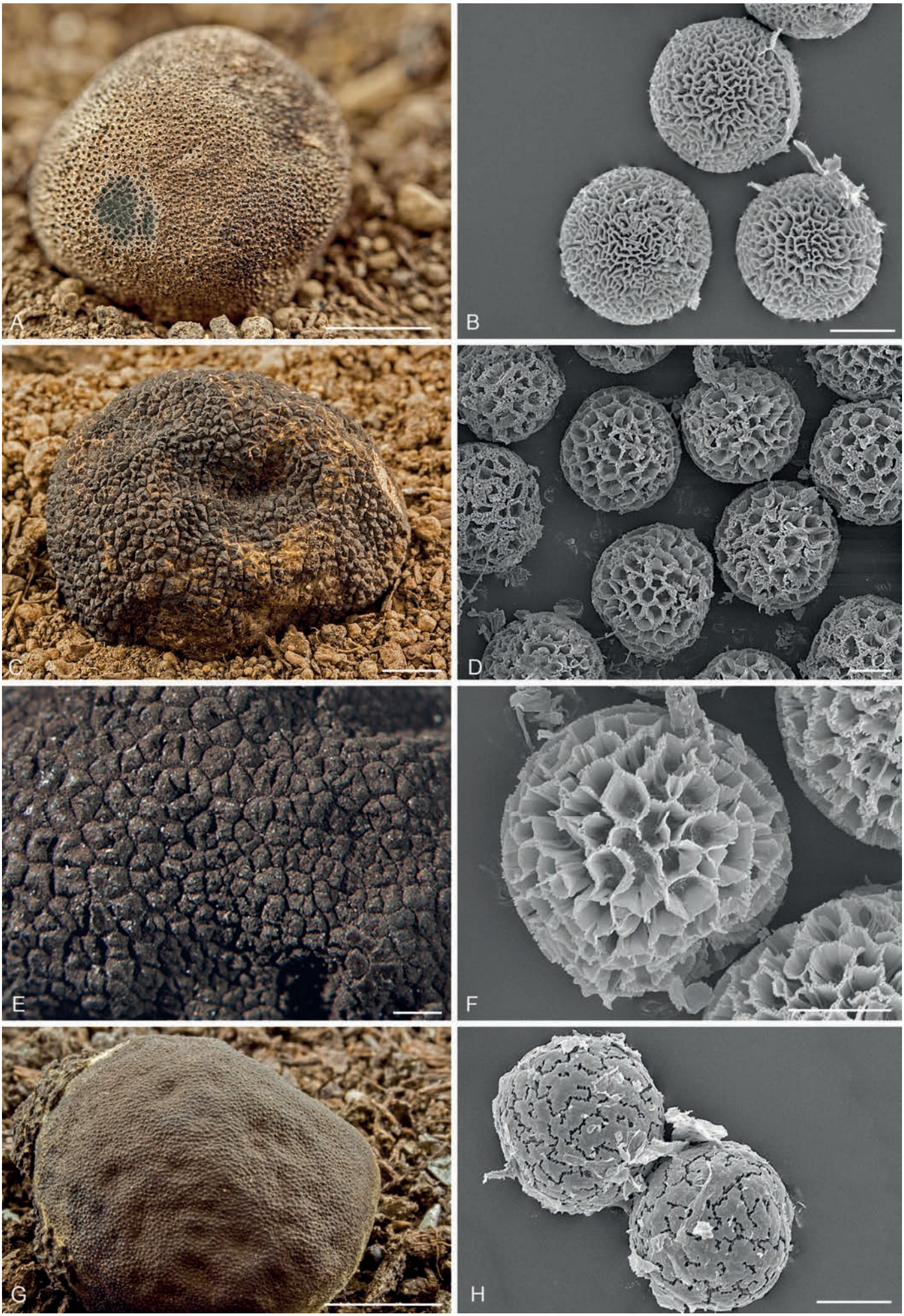
Elaphomyces mitchelliae Castellano & T.F. Elliott, ***sp. nov.*** MycoBank MB821238. Fig. 5.

Etymology: Named "*mitchelliae*" (*iae* – after) in honour and recognition of Donna Mitchell of West Virginia, accomplished collector of sequestrate taxa.

Type: **USA**, *Florida*, Alachua Co., along State Route 325, ± 1/2 mile north of junction with SR346, 10 Aug. 1985, *M. Castellano & S. Miller* (holotype - OSC 149206; isotype - RMS).

Dried ascomata subglobose to somewhat turbinate, 11–18 × 14–40 mm, completely embedded in a yellow to green-yellow mycelial mat which forms a husk around individual ascomata and incorporates much sand, ectomycorrhizae and debris, the mycelium is sparse on the upper part of the ascomata (found particularly in between the warts), more dense near base but above the stipitate basal projection; the hyphae with heavy deposits of amorphous yellow material which, along with the hyphae, turns orange to magenta with KOH and releases a red pigment, under magnification the mycelial covering immediately adjacent to the surface is actually brown and gives rise to the yellowish hyphae; KOH on ascoma surface instantly red, soon brown-black, ETOH instantly black (from show-through of peridium). *Peridium* 3–4 mm thick when fresh, 2.5–3 mm when dried, outer layer 500–900 µm thick when fresh, carbonaceous, black, brittle, with a surface of rounded to angled, irregularly shaped warts, individual warts constructed in a compound, stellate pattern, warts 250–300 µm tall; inner layer a composite of several layers, 2.5–3 mm thick when fresh, leathery, uniform to banded, pale brown to pale yellow-brown to dark grey-brown (near gleba), KOH on darkening but not distinctive; at the base of the ascoma the carbonaceous layer is thickened as a dense, black hyphal mass incorporating roots and debris, often forming

Fig. 3. *Elaphomyces loebiae*. **A.** Ascomata showing peridial surface, immature gleba, and peridium in section (OSC 149109). **B.** Ascoma showing small rounded warts on peridial surface. **C.** Inner layer (2nd) of the peridium with encrusted hyphae. **D.** Asci showing 8 immature spores. **E.** Ascospore in cross-section showing height and pattern of the ornamentation. **F.** Ascospores in surface view showing the ornamentation pattern. **G.** SEM micrograph of ascospores showing the labyrinthine pattern to the clumpy ornamentation. **H.** SEM micrograph showing the fine detail of the ornamentation. B–H (OSC 149108 – holotype). Bars: A = 10 mm, B = 500 µm, C = 10 µm, D = 15 µm, E–G = 10 µm, H = 5 µm.



a projection up to 11 mm long × 8 mm broad (usually 3 × 3 mm or less). *Gleba* stuffed with bright white mycelium when young, when mature developing a powdery spore mass that is dark green-blue, dark grey-blue to dark grey-brown, finally grey-black, with concolourous web-like hyphae. *Odour* not distinctive. *Taste* not recorded.

Peridium 7-layered, 1st layer carbonaceous, ± 200 µm thick, of golden yellow to dark brown to black, multi-sided plates or warts with slightly raised edges, many hyphae between plates and also on the centre of the plate where it has a depression, in profile the plates wart-like with depression between the warts; 2nd layer 200–230 µm thick, of dark brown, irregularly inflated cells up to 10 × 15 µm, walls 0.5 µm thick; 3rd layer 120–140 µm thick, of hyaline to pale tan, compact, interwoven hyphae, 4–5 µm broad, walls 0.5 µm thick; 4th layer 1–1.2 mm thick, of ectomycorrhizas that are enveloped with dark brown-black hyphae, this layer irregularly structured with more ectomycorrhizas found near the ascomata base and none found at top of ascomata; 5th layer 200–250 µm thick, hyaline to pale tan, loosely interwoven, parallel to somewhat bundled hyphae, 4–5 µm broad; 6th layer 800–900 µm thick, of hyaline to pale tan, distinctly bundled, cord-like, compactly parallel hyphae, 5–7 µm broad, bundles slightly wavy or sinuous; 7th layer 200–275 µm thick, of hyaline to pale tan, short-segmented, contorted, irregularly shaped, compact, interwoven hyphae or cells, 4–5(–9) µm broad. *Gleba* composed of spores and hyphae that are hyaline, septate, somewhat branched, sinuous, loosely interwoven, 3–5 µm broad, walls <0.5 µm thick. *Asci* globose, 65–70 µm broad, hyaline, walls 2–3 µm thick, 4- or 8-spored, arising from knots of short, irregularly curved or contorted clustered hyphae, up to 14 µm broad; occasionally forming asci up to 140 µm broad filled with 11 or 12 spores. *Spores* globose, (23–)24–27(–28) µm broad (mean = 25.5 µm broad); walls 2–3 µm thick, in KOH dark grey-brown to olive singly and in mass when mature, ornamentation a partial to complete reticulum, alveoli 4–5 sided, (3–)5 µm broad, ± 3 µm tall.

Distribution, habit, habitat, and season: Known from Florida, Maryland, North Carolina, Virginia, and West Virginia; hypogeous; under *Fagus grandifolia*, *Pinus serotina, P. taeda*, *Quercus alba, Q. coccinea, Q. laurifolia, Q. montana, Q. nigra, Q. rubra,* or *Q. virginiana*; February, April through May, September, and November.

Additional collections examined: **USA,** *Florida,* Alachua Co., Cross Creek, 17 Sep. 1980, *J. Trappe* 5951 (OSC 149202) and 5955 (OSC 40154; PERTH); Newman Lake, Owens-Illinois County Park, 3 May 2006, *M. Castellano* (OSC 149210; same locality, 11 Aug. 1985, *M. Castellano & S. Miller* (OSC 149209, RMS); same locality, 6 May 1987, *M. Castellano, D. Luoma, & T. O'Dell* (OSC 149103); same locality, 14 Jun. 2012, *M. Smith 602* (OSC 149216, FLAS); same locality, 25 Feb. 2012, *M. Castellano & M. Smith* (OSC 149215 & OSC 149213); same locality, 11 Aug. 2007, *M. Castellano* (OSC 149212); same locality, 23 Nov. 1981, *Col. E. Dickstein* (FLAS 55379); ±13 miles southeast of Gainesville, 5.3 miles north of Cross Creek, 24 Aug. 1979, *G. Benny, J. Kimbrough, L. Jacobs, & J. Gibson* (FLAS 52090, OSC 39528); Polk Co., Lake Kissimmee State Park, picnic area, 17 Aug. 2007, *M. Castellano* (OSC 149211); *Maryland,* Anne Arundel Co., Patuxent Research Refuge, Laurie-Bowie Rd., 20 Apr. 1966, *F.A. Uecker, O.K. Miller, & L. Stevens* (OSC 149887); *North Carolina*, Brunswick Co., Wilmington, across from Belk-Beery, 8 Sept. 1984, *S. Miller 806* (RMS, OSC 150029); Rutherford Co., Painters Gap Rd., 3 miles east of Cove Rd., 13 Jul. 2011, *T. Elliott* (OSC 149214); *Virginia*, Fairfax Co., no locality, 8 May 1926, *E.G. Artzberger* (OSC 149888); Accotink, 30 Jun. 1968, unknown collector (OSC 149101); West Virginia, Barbour Co. northwest portion of county, 18 Apr. 1992, *D. Mitchell* (OSC 149203).

Notes: We assign *Elaphomyces mitchelliae* to section *Asco-scleroderma* within *Elaphomyces* based on its distinct base.

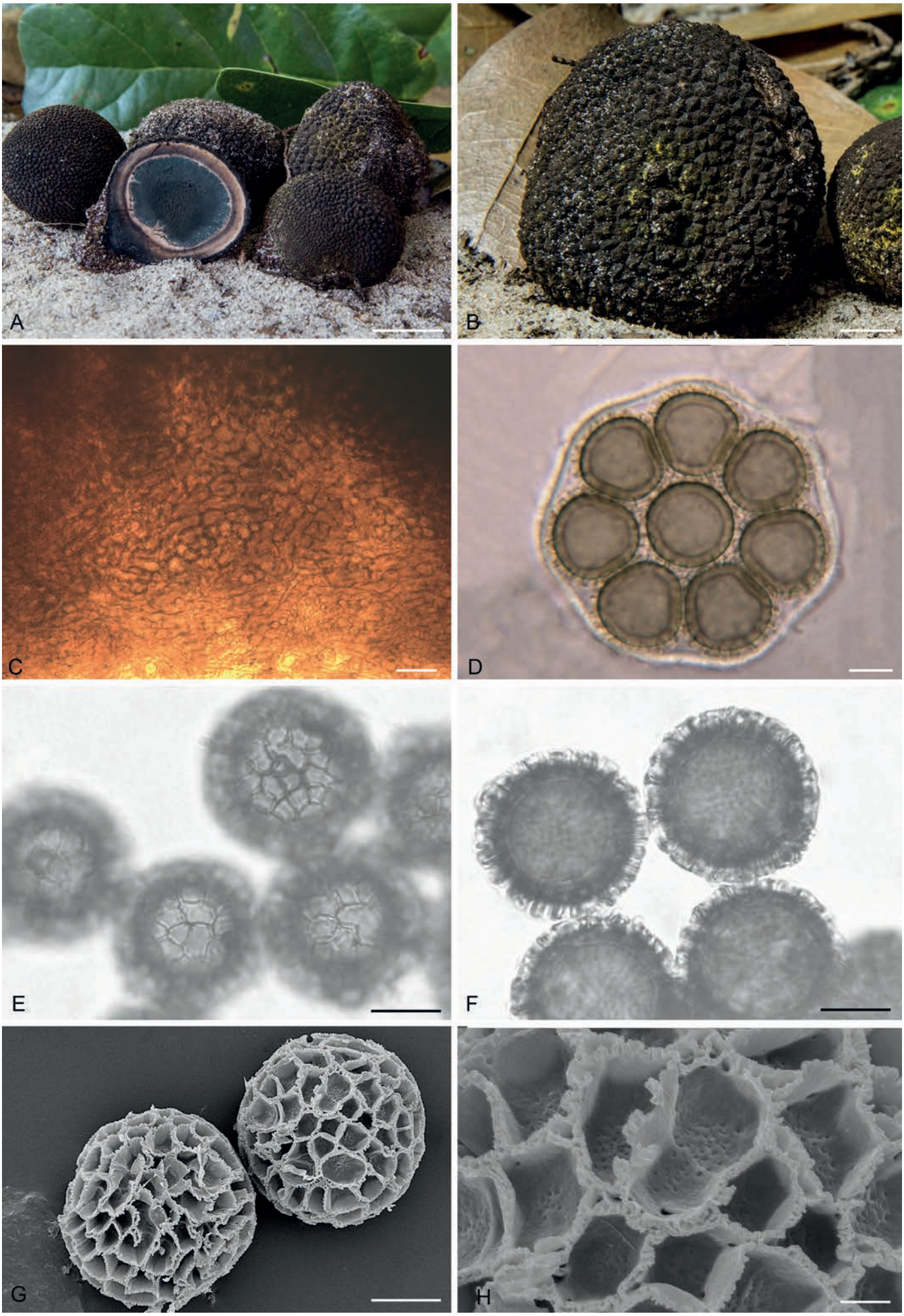
Samuelson *et al*. (1987) provide an ultrastructural study of spore ornamentation of the holotype collection of this species (as *E. persoonia*).

Castellano studied an *Elaphomyces cyanosporus* Tul. & C. Tul. collection in Kew (K161175). Tulasne (1841) lists specimens from Meudon, Clamart, and Chaville in the area surrounding Paris. Castellano could not locate any Tulasne material of this species in Paris (PC or FH). Castellano (unpubl. data) recorded data from K161175 in part as follows: Dried peridium ornamented with flat, coarse, irregularly shaped black warts, peridial surface black, base subturbinate, spores globose, in KOH dark brown singly, slightly darker in mass, 27–30 µm broad, mean = 28.0 µm broad, including ornamentation that is a complete reticulum with alveoli 3–4 µm broad × 2–3 µm tall under light microscopy, SEM reveals the spore ornamentation as a complete reticulum with coarse ridges (Fig. 4E–F). The spores of *E. cyanosporus* are slightly larger than *E. mitchelliae,* and the alveoli are smaller. In addition, the peridial surface of *E. cyanosporus* consists of flat, irregularly shaped warts, whereas the peridial surface of *E. mitchelliae* has distinct, rounded to angled warts in a compound stellate pattern.

Castellano studied an *Elaphomyces persoonii* var. *minor* Tul. & C. Tul. collection in Kew (K161168). The Tulasne brothers (1841) list specimens from Meudon, Clamart, and Chaville in the area surrounding Paris but do not designate a type. Castellano could not locate any Tulasne material of this species in Paris (PC or FH). Castellano (unpubl. data) recorded data from K161175 in part as follows: Peridial surface of black, flat, coarse, irregularly shaped warts, ascoma base subturbinate, spores globose, in KOH dark brown singly, slightly darker in mass, 27–30 µm broad, mean = 28.0 µm broad including ornamentation that is a complete reticulum, alveoli 3–4 µm broad × 2–3 µm tall under light microscopy, SEM reveals the spore ornamentation as a complete reticulum with coarse-looking ridges. *Elaphomyces persoonii* var. *minor* is conspecific in all essential details with *E. cyanosporus*.

Castellano studied an *Elaphomyces leveillei* Tul. & C. Tul. collection in Kew herbarium (K162150). Tulasne (1841) lists specimens from Meudon, Clamart, and Chaville in the area

Fig. 4. Additional *Elaphomyces* species from Europe. **A.** *Elaphomyces aculeatus* ascomata showing peridial surface and the black warts overlain by yellow-brown hyphae (LE 162850). **B.** *Elaphomyces aculeatus* SEM micrograph of ascospores showing the labyrinthine-like ornamentation (OSC 149159). **C.** *Elaphomyces persoonii* ascomata showing wart structure and adherent yellow toned mycelium (LE 162885). **D.** *Elaphomyces persoonii* SEM micrograph of ascospores showing reticulate ornamentation (OSC 149365). **E.** *Elaphomyces cyanosporus* ascomata showing wart structure. **F.** *Elaphomyces cyanosporus* SEM micrograph of ascospores showing the reticulate ornamentation (OSC 149176). **G.** *Elaphomyces leveillei* ascomata showing papillate peridial surface and some adherent yellow toned mycelium (W2000-805). **H.** *Elaphomyces leveillei* SEM micrograph of ascospores showing the ornamentation of irregular plates (OSC 149116). Bars: A, G = 1 cm, B, D, F, H = 10 µm, C = 5 mm, E = 500 µm.



surrounding Paris but does not designate a type. Castellano could not locate any Tulasne material of this species in Paris (PC). Castellano (unpubl. data) recorded data from K162150 in part as follows: Peridial surface of black, pusticulate (bumpy) to tuberculate, partially covered by pale tan, pale yellow-brown to yellow hyphae, ascoma base indented, spores globose, in KOH dark brown singly, slightly darker in mass, 26–28 µm broad, mean = 26.6 µm broad including ornamentation that appears pusticulate under light microscopy, in section appearing with flattening of spore outline at least on a portion of numerous spores, SEM reveals the spore ornamentation as spines or rods overlain with amorphous, small, irregular plates to form a discontinuous surface, plate surface slightly roughened (Fig. 4G–H). The spores of *E. leveillei* are similar in size (26–28 µm broad, mean = 26.6 µm broad) but have an ornamentation of irregularly shaped, discontinuous plates compared to the distinct reticulum of *E. mitchelliae*. In addition, the peridial surface of *E. leveillei* consists of bump-like features compared to the distinct rounded to angled warts in a compound stellate pattern of *E. mitchelliae*.

Castellano studied *Elaphomyces persoonii* Vittad. collections from TO - OSC 149124, Wien - W2008-1079 and Kew - K162166 marked as Vittadini. Castellano (unpubl. data) recorded data from Trappe 1470 in part as follows: Ascoma subturbinate to turbinate, peridial surface of large black warts, with dark brown hyphae seen between warts, spores globose, in KOH brown to dark brown singly, slightly darker in mass, 29–33(–35) µm broad, mean = 31.3 µm broad including ornamentation that is a complete reticulum, up to 5 µm tall, alveoli irregular, up to 5–7 µm across under light microscopy, SEM reveals the spore ornamentation as a complete reticulum with the digitate edges along the alveoli (Fig. 4C–D). The spores of *E. persoonii* are larger (29–33 µm broad, mean = 31.3 µm broad) and have taller alveoli walls (up to 5 µm tall) compared to the spores of *E. mitchelliae*. In addition, the peridial surface of *E. persoonii* has flat warts compared to the distinct rounded to angled warts in a compound stellate pattern of *E. mitchelliae.*

ACKNOWLEDGEMENTS

We thank the following colleagues for sharing *Elaphomyces* collections included in this study: S. Loeb, S. Miller, D. Mitchell, F. Tainter. We also appreciate the opportunity to study specimens from the following herbaria: FH, FLAS, K, MB, PC, TO, and W.

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Fig. 5. *Elaphomyces mitchelliae.* **A.** Ascomata showing peridial surface, gleba, and peridium in section (OSC 149215). **B.** Ascoma showing peridial warts with some adherent yellow mycelium (OSC 149213). **C.** Outer peridium showing the pale, stacked hyphae between the darker wart tissues. **D.** Asci showing 8 immature spores. **E.** Ascospores in surface view showing the ornamentation pattern. **F.** Ascospores in cross-sectional view showing the height and pattern of the ornamentation. **G.** SEM micrograph of ascospores showing the distinct reticulate pattern. **H.** SEM micrograph of ascospores showing the structure of the alveoli. C, E–F (OSC 40154); D (OSC 40154); G–H (OSC 149103). Bars: A = 20 mm, B = 5 mm, C = 30 µm, D–G = 10 µm, H = 2.5 µm.

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