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Eight new *Elaphomyces* species (*Elaphomycetaceae*, *Eurotiales*, *Ascomycota*) from eastern North America

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Abstract: The hypogeous, sequestrate ascomycete genus *Elaphomyces* is one of the oldest known truffle-like genera. *Elaphomyces* has a long history of consumption by animals in Europe and was formally described by Nees von Esenbeck in 1820 from Europe. Until recently most *Elaphomyces* specimens in North America were assigned names of European taxa due to lack of specialists working on this group and difficulty of using pre-modern species descriptions. It has recently been discovered that North America has a rich diversity of *Elaphomyces* species far beyond the four *Elaphomyces* species described from North America prior to 2012. We describe eight new *Elaphomyces* species (*E. dalemurphyi*, *E. dunlapii*, *E. holtsii*, *E. lougehrigii*, *E. miketroutii*, *E. roodyi*, *E. stevemilleri* and *E. wazhazhensis*) of eastern North America that were collected in habitats from Quebec, Canada south to Florida, USA, west to Texas and Iowa. The ranges of these species vary and with continued sampling may prove to be larger than we have established. Castellano has studied authentic material of all European *Elaphomyces* species published through 2016 and it is interesting to note that many *Elaphomyces* species from eastern North America have morphological similarities but with distinct morphological differences to a number of European *Elaphomyces* species.

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INTRODUCTION

The hypogeous, sequestrate ascomycete genus *Elaphomyces* (*Elaphomycetaceae*, *Eurotiales*, *Ascomycota*) is a morphologically diverse group of species found associated with most ectomycorrhizal forest tree species (Dodge 1929, Corner & Hawker 1953, Zhang & Minter 1989, Trappe *et al.* 2009, Castellano *et al.* 2011, 2012a, c, 2016, 2017, 2018, Reynolds 2011, Paz *et al.* 2017, Molia *et al.* 2020, Shirakawa *et al.* 2020). *Elaphomyces* is unique in the ascomycete, truffle-like group in possessing a gleba of powdery ascospores enclosed by a usually thick, complex peridium. Ascospores of *Elaphomyces* almost always form below the surface of the ground and are most often encased in roots, soil and debris. Ascospores of *Elaphomyces* species are at least partially consumed by numerous animal mycophagists. Animal mycophagy is critical for spore dispersal and the sometimes extremely abundant ascospores of some *Elaphomyces* species are a significant food source (Fogel & Trappe 1978, Vogt *et al.* 1981, Cork & Kenagy 1989, Luoma *et al.* 1991, Vernes *et al.* 2004, 2007, Poirier 2007, Reynolds 2011).

Until recently it was common to erroneously attribute European *Elaphomyces* species names to various North American *Elaphomyces* collections based on overly broad species concepts (Dodge 1929, Hawker 1968, Trappe &

Guzmán 1971, Fogel & Trappe 1976, Vogt *et al.* 1981, States 1983, Miller & Miller 1984, Zhang & Minter 1989, Luoma *et al.* 1991, Cázares *et al.* 1992, Amaranthus *et al.* 1994, North *et al.* 1997, North & Greenberg 1998, Loeb *et al.* 2000, Quandt *et al.* 2015). Prior to 2012 there were only four *Elaphomyces* species described from North America: *E. verrucosus* from California (Dodge 1929); *E. appalachiensis* from Great Smoky Mountains National Park (Linder 1939); *E. viridiseptus* from Florida (Trappe & Kimbrough 1972); and *E. spinoreticulatus* from southeastern Canada (Zhang & Minter 1989). More recently, Castellano *et al.* (2012b) described *E. verruculosus* from northeastern Mexico and southeastern US and reported *E. appalachiensis* from northeastern Mexico. Castellano & Stephens (2017) described *E. americanus*, *E. bartlettii*, *E. macrosporus*, *E. oreoides*, and *E. remickii* from eastern North America. Castellano *et al.* (2018) described *E. cibulae*, *E. loebiae*, and *E. mitchelliae* from southeastern North America. Paz *et al.* (2017) presented a comprehensive molecular phylogeny of European *Elaphomyces* including recognizing 25 (four new) species and nine varieties and designating numerous lectotypes and epitypes. Molia *et al.* (2020) added three new species and selected a lectotype for *Elaphomyces granulatus*. Most importantly they selected epitypes for both *E. granulatus* and *E. muricatus*. Shirakawa & Tanaka (2020) then added two more species in *Elaphomyces*

section *Elaphomyces* from Japan: *E. marmoratus* in subsection *Muricati* and *E. fuscus* in subsection *Elaphomyces*.

It appears that the eastern portion of North America is particularly rich in *Elaphomyces* species. This richness is attributed to the high diversity of *Betulaceae*, *Pinaceae*, and particularly *Fagaceae* species in this region compared to western North America in particular. The eight species we present brings the number of known *Elaphomyces* species in eastern North America to 20, only *E. verrucosus* appears to be restricted to the western USA. One additional planned paper on *Elaphomyces* from eastern North America is forthcoming and will include additional new *Elaphomyces* species from eastern North America and present a comprehensive key to all *Elaphomyces* from North America.

MATERIALS AND METHODS

Elaphomyces typically develop below ground so the ascomata were collected by raking away the upper leaf and soil layers in habitats with ectomycorrhizal trees, observing and excavating the area where animals had previously dug, or by looking for *Tolypocladium* species that fruit above ground while parasitizing the ascomata of *Elaphomyces* species. Occasionally specimens were found partially emergent from the soil in eroded or disturbed environments like road banks, campgrounds, or trail edges.

Descriptions of macromorphological characters are based on fresh material after Castellano *et al.* (2004). Colors are described in general terms based on the observations of the authors and collectors. Preserved specimens were rehydrated and examined in 3 % KOH, Melzer's reagent, or cotton blue. Microscopic descriptions were based on 3 % KOH mounts unless specified. Spore dimensions are from twenty ascospores measured from the holotype collection, dimensions include ornamentation. Dried ascospores were mounted on aluminum pegs with double-sided tape and coated with gold or gold/palladium for scanning electron microscopy (SEM) with a FEI QUANTA 600F environmental scanning electron microscope or a JEOL 5800 LV scanning electron microscope with 10–15 kV. Specimens are deposited in the following herbaria: FLAS, DEWV, ISC, OSC, and RMS (abbreviations after Index Fungorum 2020).

TAXONOMY

Elaphomyces dalemurphyi Castellano & R.A. Healy, *sp. nov.* MycoBank MB835640. Fig. 1.

Etymology: Named for native Oregonian Dale Bryan Murphy (born 12 March 1956), who inspired Michael Castellano with his character, integrity and passion for one's chosen field.

Diagnosis: *Elaphomyces dalemurphyi* resembles *E. papillatus* of Europe in peridial and spore characteristics but distinctly differs by the structure of the peridial surface and the hyphal organization of the peridial layer.

Typus: USA, Iowa, Story Co., Hickory Grove Park, 13 Oct. 1998, R. Healy (**holotype** ISC 435953, **isotype** OSC 150035).

Ascomata irregularly subglobose, up to 10 × 12 × 13 mm. *Peridium* 1.25–2.75 mm thick, outer peridial surface smooth, with pock

marks or indentations, mottled pale yellow-brown background with scattered, orange-brown, irregular areas; outer peridial layer in cross-section, a thin layer of brown to red-brown in patches with a more or less hyaline or off-white coloration in between, inner peridial layer pale gray to pale gray-brown then grading into red-brown near gleba. *Gleba* spore mass powdery, dark brown to nearly black, with numerous pale gray mycelial strands arising from inner peridial wall and traversing gleba. *Odor* of motor oil. *Taste* not recorded. *Peridium in cross-section* two-layered, outer peridial layer 50–80 µm thick, with patches of dark tissue, dark tissue patches up to 70–120 µm broad, composed of red-brown, compact, globose cells overlain by disorganized hyaline cells, also in between dark patches are areas of hyaline, agglutinated, parallel hyphae, ± 5 µm broad, walls 1–2 µm thick; inner peridial layer 1.2–2.7 mm thick, of hyaline, interwoven to somewhat parallel hyphae, 3–5 µm broad, walls 1–2 µm thick, arranged in bundles, 10–15 µm broad, pale red-brown and somewhat larger (up to 30 µm broad) near gleba. *Gleba* of spores and hyaline, septate, smooth, loosely interwoven hyphae, ± 2.5 µm broad, walls < 0.5 µm thick. *Asci* globose, hyaline, 5–7 spores, 18.5–21.5 µm broad, walls ± 2 µm thick, all arising from ascogenous hyphae which form clustered knots of large, hyaline, short-segmented hyphae, walls ± 1 µm thick. *Spores* ornamented, globose to slightly subglobose, variable in size, dark brown (16–)17–19(–20) µm (mean = 17.8 µm) or pale brown spores 20–23 µm (mean = 21.5 µm), pale spores are less ornamented, ornamentation of rods and ridges 1.5–2 µm tall, SEM reveals that the ridges are composed of irregularly fused rods, in KOH singly and in mass dark brown with almost black highlights when mature.

Distribution, habit, habitat and season: Known from Iowa and North Carolina; hypogeous; under *Pinus taeda*, *Quercus alba*, and *Tilia americana*; August and October.

Additional collections examined: USA, North Carolina, Orange Co., east of Chapel Hill, Charles Herman Wilson Park, near picnic area, elevation 552 ft., N35°54.772' W79°2.681', 22 Aug. 2018, M. Castellano (OSC 158592).

Notes: *Elaphomyces dalemurphyi* is placed in *Elaphomyces* section *Elaphomyces* subsection *Papillati* based on the crested spore ornamentation and peridial characters. *Elaphomyces dalemurphyi* resembles the three varieties of *E. papillatus* found in Europe in peridial and spore characteristics but distinctly differs by the structure of the peridial surface, the hyphal organization of the peridial layer, spore size, spore color, or spore ornamentation characteristics.

The first author studied an *Elaphomyces papillatus* collection from Italy (PC 93932) marked “ex ipso, from Vittadini to Tulasne in May 1845”. Castellano data (on file at Forestry Sciences Laboratory, Corvallis, Oregon, USA) from PC 93932 in part is as follows: Peridium rugose to wrinkled, dark brown base with small, very close, nearly black, irregularly-shaped, flat warts, spores globose, in KOH brown singly, dark brown in mass, 16–19 µm, mean = 17.5 µm including ornamentation that appears as rods or spines in clusters or aligned in ridges under light microscopy, SEM reveals the spore ornamentation as rods anastomosed into clusters or irregularly thickened ridges (Fig. 2A, B). Paz *et al.* (2017) separates *E. papillatus* var. *striatosporus* and *E. papillatus* var. *sulphureopallidus* based on DNA and distinct morphological differences. The spores of *Elaphomyces papillatus* var. *striatosporus* are dark sepia to almost black in

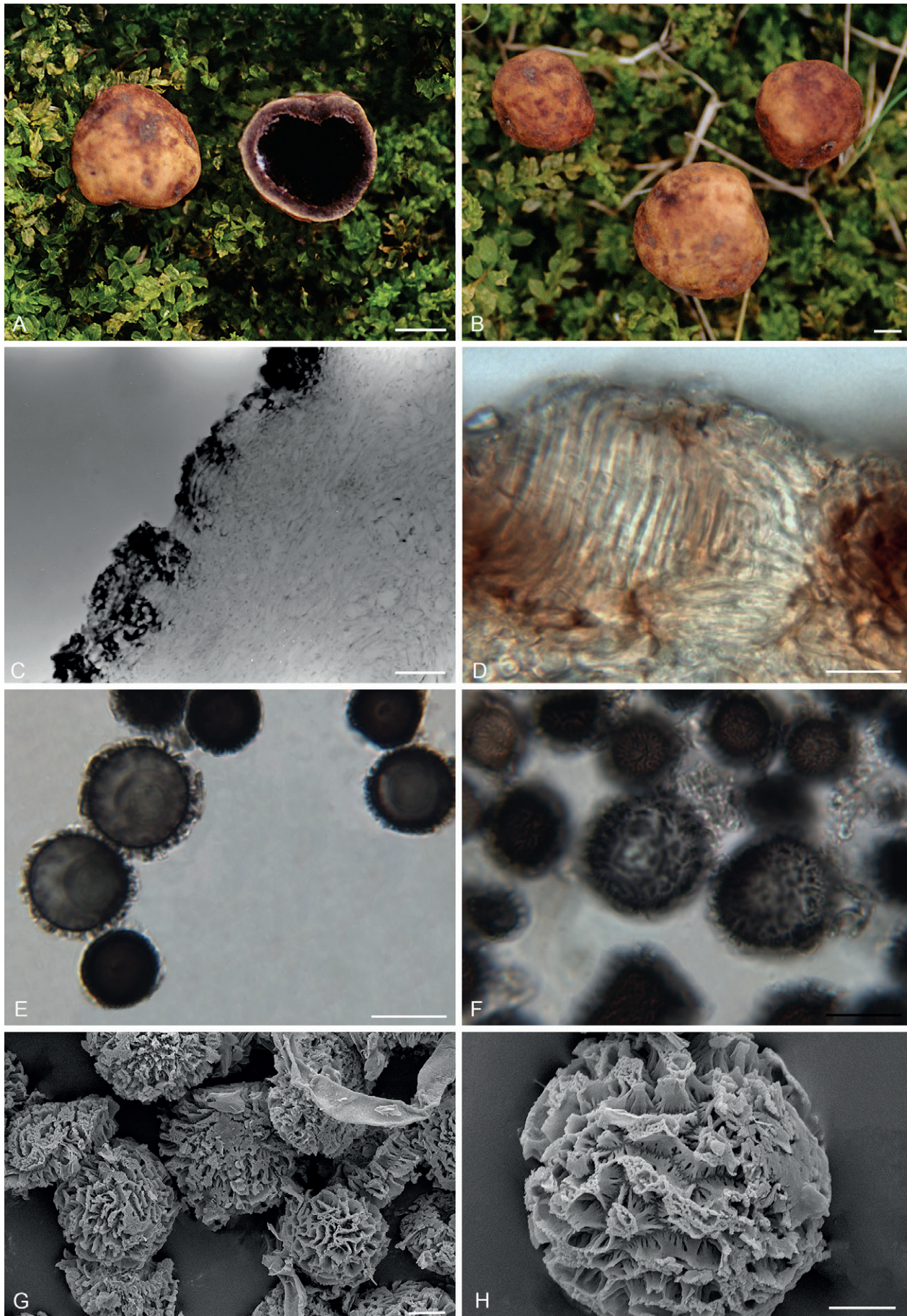


Fig. 1. *Elaphomyces dalemurphyi*. **A.** Ascomata showing peridium in section, gleba, and peridial surface. **B.** Ascoma surface showing smooth peridium. **C.** Cross-sectional view of the peridium showing the small dark patches of tissue. **D.** Magnified dark patches tissue showing the agglutinated, parallel hyphae. **E.** Ascospores in cross-sectional view showing the ornamentation of rods and ridges. **F.** Ascospores in surface view showing the rods and ridges. **G.** SEM micrograph of ascospores showing the rods and ridges. **H.** SEM micrograph of an ascospore showing the fused rods that compose the ridges. A–H (ISC 435953–holotype). Scale bars: A = 20 mm, B = 5 mm, C = 30 μ m, D–G = 10 μ m, H = 2.5 μ m.

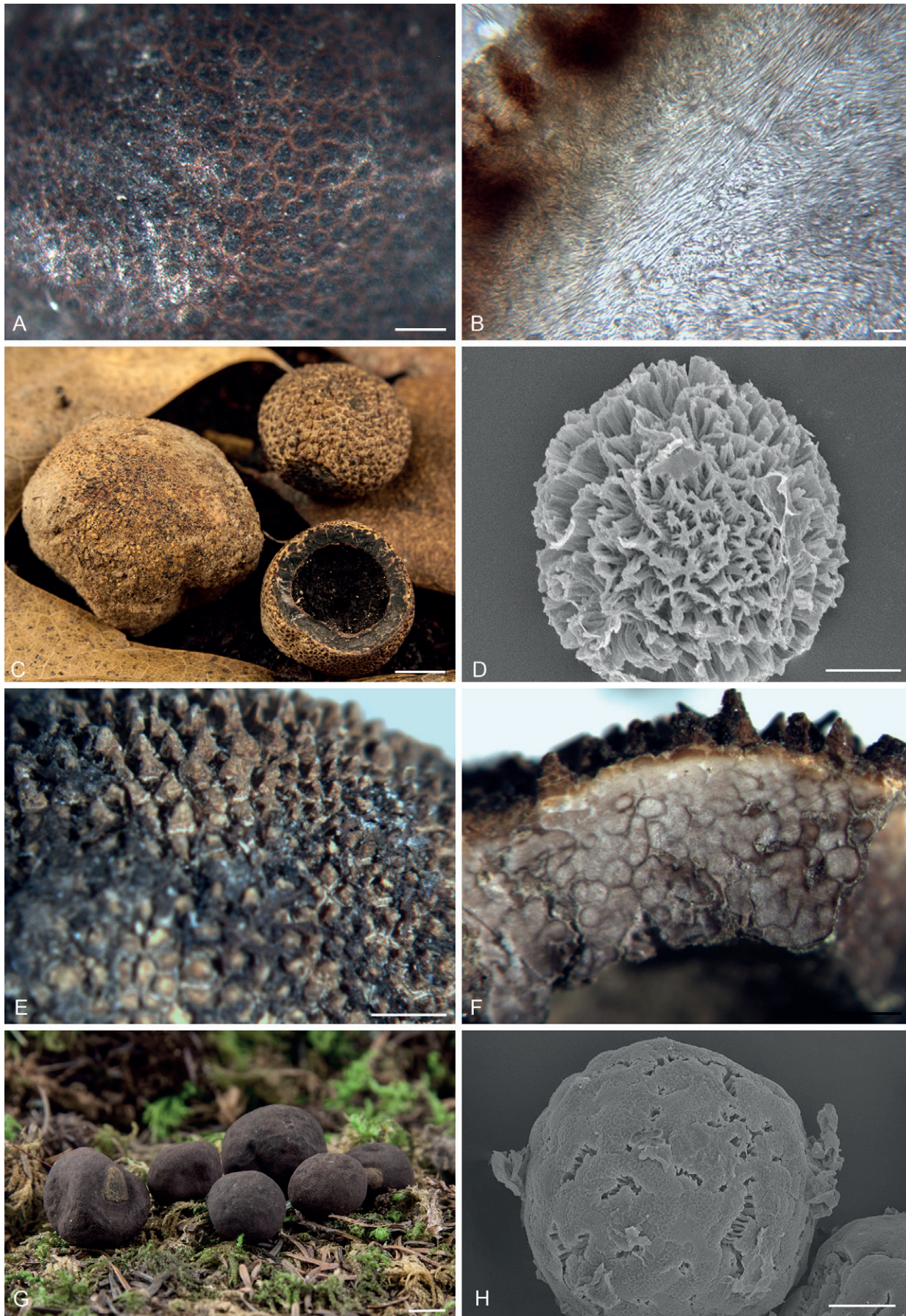


Fig. 2. *Elaphomyces* species from Europe. **A.** Ascogonia showing peridial surface. **B.** Ascogonium in cross-section showing the marbled inner peridium. **C.** Structure of the outer peridium. **D.** Ascogonia in cross-sectional view showing the ornamentation of rods. **E.** Ascogonia in surface view showing the coarse rods and tufts. **F.** SEM micrograph of ascogonia showing the short ridges. **G.** SEM micrograph of an ascogonium showing the irregular arrangement of the rods to form ridges. **H.** SEM micrograph showing the anastomosed rods that form irregular ridges. **A–B** (*E. papillatus* – PC 93932), **C–D** (*E. verrucosus* – Gardner 268), **E–F** (*E. decipiens* – PC 93894), **G–H** (*E. maculatus* – FH “Portion autotici C. Vittadini”). Scale bars: **A** = 20 mm, **B** = 5 mm, **C** = 30 μ m, **D–G** = 10 μ m, **H** = 2.5 μ m.

mass, (10–) 14–16(–18) μm , with ornamentation that is variously shaped, somewhat minutely rugose to often striate (Kers 1980). The spores of *Elaphomyces papillatus* var. *sulphureopallidus* are hyaline but otherwise similar in size and ornamentation to *Elaphomyces papillatus* var. *striatosporus*.

We agree with Molia *et al.* (2020) that *E. striatosporus* is the correct rank.

Elaphomyces dunlapii Castellano, D. Mitchell & Crabtree, *sp. nov.* MycoBank MB835641. Fig. 3.

Etymology: “*dunlapii*” – in honor of Mr. Randall Dunlap of Greenville, South Carolina, discoverer of the type collection.

Diagnosis: Distinguished from all other *Elaphomyces* species in North America by its nearly smooth, brittle, carbonaceous peridium, spore size and ornamentation characteristics. Distinguished from the similar *E. anthracinus* of Europe by its much thinner outer and inner peridial layers, its widely spaced, low rounded papillae on the peridial surface (compared to the compact contiguous papillae found on the peridial surface of *E. anthracinus*), and its spores which are slightly smaller in size.

Typus: USA, South Carolina, McCormick Co., Sumter National Forest, northwest of McCormick, east of Little River, south side of Hwy 28, approximately 0.5 miles from Long Cane Bridge heading north, May 2003, *R. Dunlap* (**holotype** OSC 149098).

Ascomata globose to subglobose or irregular with a rather smooth raised basal area which is distinctly different from the rest of the surface, up to 30 mm broad, dimpled with a central mound, smooth and cracked, not papillate, completely enveloped in dark brown mycelium, ectomycorrhizal roots and soil; mycelium not staining. **Peridium** \pm 800 μm thick, peridial surface \pm 200 μm thick, black, brittle, appearing smooth to the naked eye but closer examination reveals widely spaced, small, low, rounded papillae, \pm 60 μm broad; immediately underlain by a narrow, gray band \pm 100 μm in thickness then fairly abruptly a uniform, off-white to very pale gray inner peridial layer, \pm 500 μm thick. **Gleba** spore mass powdery, dark brown to black. **Odor** not recorded. **Taste** not recorded. **Peridium in cross-section** two-layered, outer peridial layer, \pm 200 μm thick, of dark brown to mostly black, compact hyphae (so much so as to obscure most of the structure), walls \pm 1 μm thick, inner peridial layer \pm 600 μm thick, of hyaline (near gleba) to pale gray to gray (near epicutis), septate, interwoven hyphae, up to 5 μm broad, walls \pm 2 μm thick; outer gray hyphal layer has numerous dark crystalline structures scattered throughout, inner pale zone is devoid of crystalline structures. **Gleba** of spores and hyaline, septate, sinuous, slightly encrusted hyphae, \pm 2 μm broad, walls $<$ 0.5 μm thick. **Asci** not observed. **Spores** ornamented, globose but usually somewhat flattened on multiple sides, (19–)20–21(–22) μm (mean = 20.4 μm), walls \pm 1 μm thick, very dark brown to black in KOH singly and in mass when mature, ornamentation appearing almost smooth on the surface but with some scattered knobs, SEM reveals a fine honeycomb beneath a plate-like covering that is not homogeneous.

Distribution, habit, habitat and season: Known only from Missouri, North Carolina and South Carolina; hypogeous; under *Cornus florida*, *Quercus alba*, *Q. stellata*, *Q. velutina*, and other *Quercus* spp.; May, July and August.

Additional collections examined: USA, Missouri, Camden Co., Ha Ha Tonka State Park, Spring trail, N37° 58' 21.6" W92° 46' 23.8", 24 Aug. 2016, *C. Crabtree*, CDC 0591 (OSC 159156); North Carolina, Pisgah National Forest, 22 Jul. 1995, *D. Mitchell* (OSC 150034, DEWW).

Notes: *Elaphomyces dunlapii* is placed in *Elaphomyces* section *Ceratogaster* subsection *Sclerodermei* based on the lack of mycelial patches on the peridial surface. *Elaphomyces dunlapii* resembles *E. anthracinus* of Europe in some peridial and spore characters. The spores of *E. anthracinus* are somewhat larger (20–24 μm , mean = 21.9 μm) than *E. dunlapii* (20–21 μm , mean = 20.4 μm) but of similar ornamentation. The peridium of *E. anthracinus* is much thicker in both outer and inner layers (750–820 μm outer carbonaceous layer, \pm 1 000 μm thick inner layer) than *E. dunlapii* (\pm 200 μm thick outer carbonaceous layer, \pm 500 μm thick inner layer). In addition, the peridial surface of *E. dunlapii* has widely spaced, low rounded papillae compared to the contiguous low papillae found on the peridial surface for *E. anthracinus*.

The first author studied an *Elaphomyces anthracinus* collection in TO (also OSC 149163) marked “Esemplare originale di Vittadini da “Museo di Paris”. Castellano (on file at Forestry Sciences Laboratory, Corvallis, Oregon, USA) recorded data from this collection in part as follows: Peridial surface appearing smooth to the naked eye, close examination reveals a distinct pattern of contiguous, black, low circular, rounded papillae, not warty or angular, ascoma base with a peduncle, peridial surface black, spores globose, in KOH brown to dark brown singly, slightly darker in mass, 20–24 μm , mean = 21.9 μm , including ornamentation that appears punctate or dimpled under light microscope, in cross-section appearing with some flattened sides in outline to the spore, SEM reveals the spore ornamentation as a fine complete reticulum partially or nearly completely overlain with an over-lapping, plate-like material (see plate 2 in Castellano *et al.* 2018). We agree with Dodge (1929) that *E. pyriformis* (the first author studied authentic Vittadini material in FH marked “ex ipso auctore”) and *E. plumbeus* (the first author studied authentic material in Marburg marked “Laurasen, Germany, April 1890”) are conspecific. Paz *et al.* (2017) present *E. anthracinus f. talosporus* as differing from *E. anthracinus f. anthracinus* by its globose-polyhedral spores with six faces (flattened sides) and asci with 2–5 spores and an inner peridium that darkens towards the gleba.

Elaphomyces holstii Castellano & R.A. Healy, *sp. nov.* MycoBank MB835642. Fig. 4.

Etymology: “*holstii*” named for Bernhart Paul Holst, accomplished educator in the late 1800’s from Boone County, Iowa and donator of the land for the Holst State Forest Preserve.

Diagnosis: *Elaphomyces holstii* resembles *E. americanus*, *E. barrioi*, *E. decipiens*, *E. muricatus*, and *E. verrucosus* in overall ascomata and spore characteristics. *Elaphomyces americanus* has smaller spores and smaller asci with a different structure to the inner peridium. *Elaphomyces barrioi* has smaller spores. *Elaphomyces decipiens* has larger spores and low plate-like warts. *Elaphomyces muricatus* has a much thicker peridium and spores without a partial reticulum. *Elaphomyces verrucosus* also has larger spores and a very thick overall peridium.

Typus: USA, Iowa, Boone Co., near Ames, Holst State Forest Preserve, N42.11748° W93.9794°, 31 Aug. 1997, *R. Healy RH70* (**holotype** ISC-F-0075954, **isotype** OSC 150022).

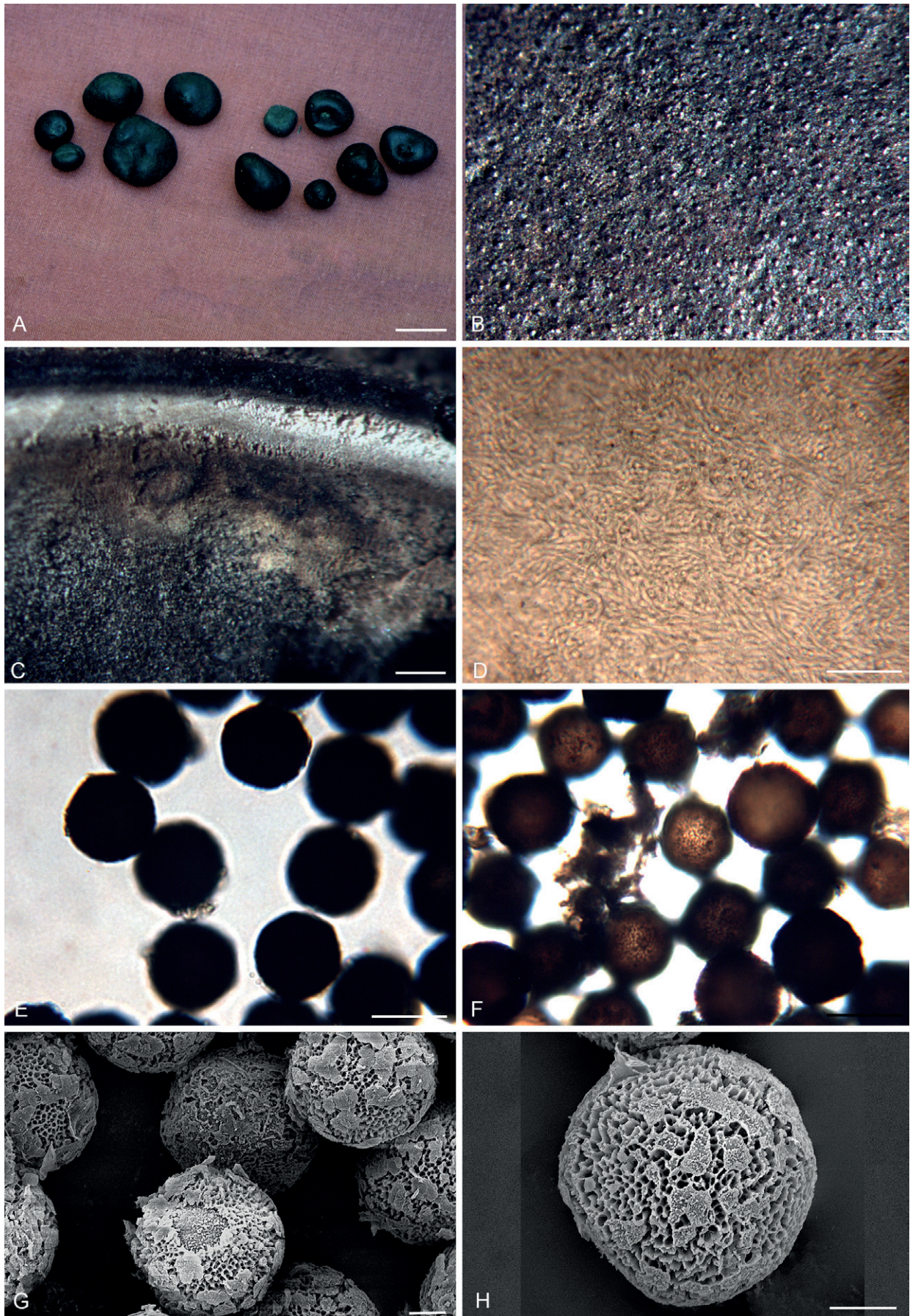


Fig. 3. *Elaphomyces dunlapii*. **A.** Ascomata showing the various shapes. **B.** Ascoma surface showing the small, low, rounded papillae. **C.** Peridial structure showing the rather thick, black carbonaceous layer underlain by a narrow, gray middle layer then fairly abruptly a uniform, off-white to very pale gray inner layer. **D.** Inner peridial layer of hyaline to pale gray to gray interwoven hyphae. **E.** Ascospores in cross-sectional view showing the flattened sides. **F.** Ascospores in surface view showing fine texture of the ornamentation. **G.** SEM micrograph of ascospores showing the ornamentation of a fine honeycomb beneath a plate-like covering. **H.** SEM micrograph of an ascospore showing the irregularly shaped plates that partially covers the spore surface. A–H (OSC 149098 – holotype). Scale bars: A = 10 mm, B = 500 μ m, C–F = 10 μ m, G = 5 μ m, H = 2 μ m.

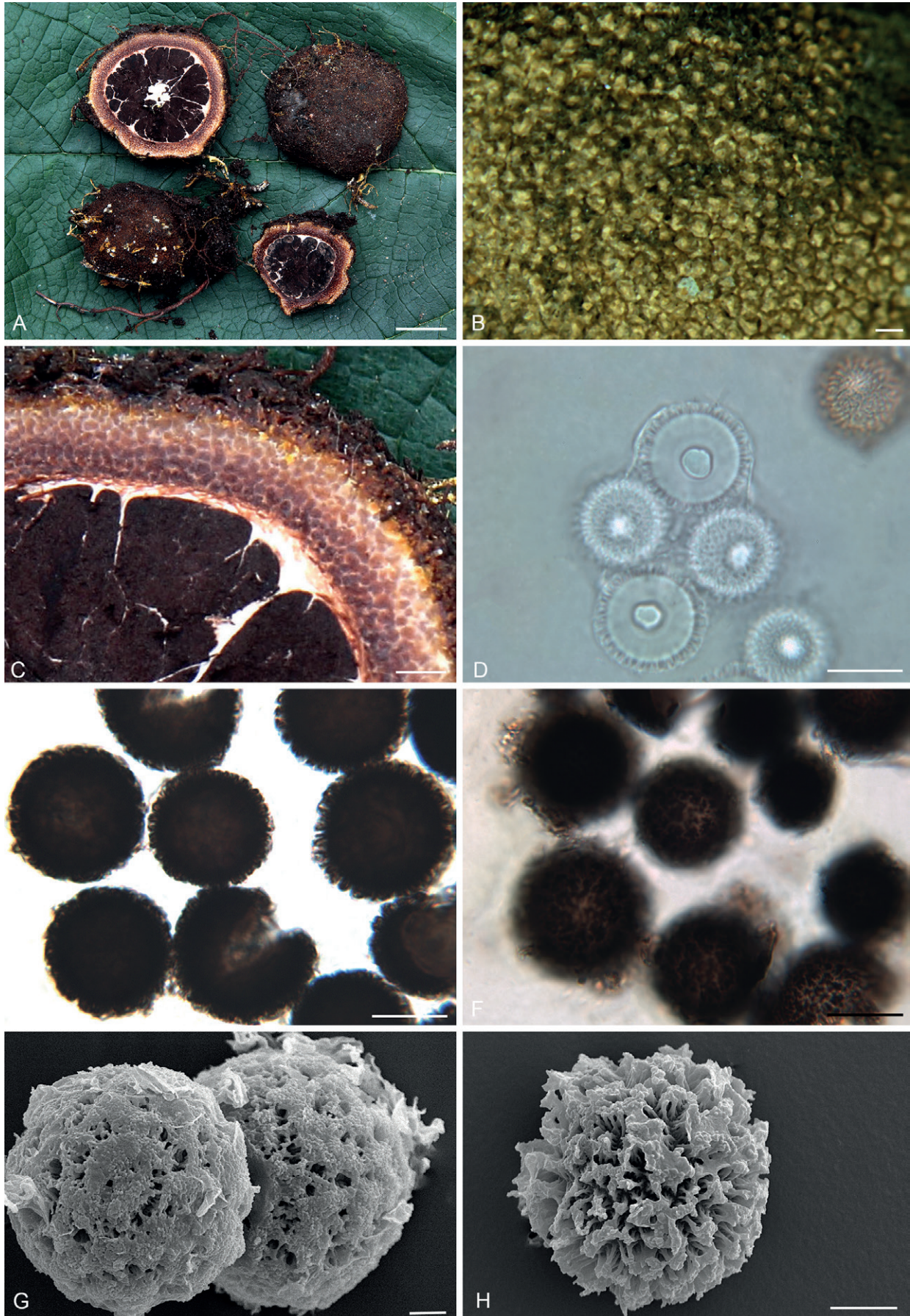


Fig. 4. *Elaphomyces holstii*. **A.** Ascomata showing peridium in section, gleba, and peridial surface with some roots, soil and debris attached. **B.** Ascoma surface showing peridial warts. **C.** Cross-sectional view of the peridium and gleba showing the marbled inner peridium. **D.** Ascus showing four immature ascospores, two in surface view and two in cross-sectional view. **E.** Ascospores in cross-sectional view showing the ornamentation of rods and spines. **F.** Ascospores in surface view showing the ornamentation pattern. **G.** SEM micrograph of ascospores showing the ornamentation pattern covered with amorphous material. **H.** SEM micrograph of an ascospore showing the fused rods. A–H (ISC-F-0075954 – holotype). Scale bars: A = 20 mm, B = 5 mm, C = 30 μ m, D–G = 10 μ m, H = 2.5 μ m.

Ascomata subglobose to irregularly shaped, up to 15 × 18 × 22 mm, completely embedded in a white to off-white mycelial mat which forms a husk around individual ascoma and incorporating soil, ectomycorrhizal roots, and debris; mycelium not staining. *Peridium* overall ± 1.1 mm thick when mature, outer peridial layer ± 350 µm thick, of distinct warts, contiguous at base, sometimes with apparent canals between warts, blunt to pointy at apex, 4–5-sided at base, pale brown to brown or yellow-brown, warts up to 500 µm broad, up to 350 µm tall, inner peridial layer ± 800 µm thick, marbled with brown to rose-brown matrix and off-white veins, matrix darker as it nears gleba, almost black at gleba, matrix elongated, chambers extending towards surface. *Gleba* spore mass powdery, dark brown to black with pale brown to brown or rose-brown hyphae emanating from inner peridium, when young pale tan from tan asci and white cottony mycelium. *Odor* strong, somewhat acrid. *Taste* not recorded. *Peridium in cross-section* two-layered, outer peridial layer of yellow-brown warts, 260–350 µm tall and up to 500 µm broad at base, warts constructed of yellowish hyphae in a *textura intricata* near base grading into red-brown hyphae towards wart surface, hyphae ± 2 µm broad, in between warts are distinctly parallel, stacked, hyaline hyphae, 4–5 µm broad, inner peridial layer ± 800 µm thick, of hyaline, compact, interwoven hyphae, 4–5 µm broad, finer towards the epicutis, with randomly spaced sutures characterized by pale red-brown, tiny, adherent amorphous particles. *Gleba* of spores and hyaline, elongate, smooth, crooked, septate, loosely interwoven hyphae, 1.5–2.5 µm broad, walls < 0.5 µm thick. *Asci* globose to subglobose, hyaline, 31–40 µm broad, walls 2–3 µm thick, 4(–5)-spored, arising from ascogenous hyphae, when young pedicellate, up to 7 µm broad by 10 µm long, tapered. *Spores* globose, 25–30(–31) µm (mean = 27.6 µm); walls ± 1 µm thick, in KOH brown when mature singly and in mass, when immature hyaline and distinctly ornamented with tall spines or rods, when mature ornamentation of rods and short ridges composed of fused rods that are darker than spore wall, partially reticulate, ± 2 µm tall, often the ornamentation is covered with amorphous material, also with some smaller, dark, aborted spores, ± 15 µm broad, mostly smooth or with very low ornamentation.

Distribution, habit, habitat and season: Known from Iowa, North Carolina, and Québec Province, Canada; hypogeous; under *Carya ovata*, *C. tomentosa*, *Quercus alba*, *Q. rubra*, or *Q. virginiana*; May through September.

Additional collections examined: **Canada**, Québec Province, Montreal, 6 Jun. 1956, *F. Rolland-Germain* (OSC 28712). **USA**, Iowa, Boone Co., Iowa State University, 4-H camp, 23 Jul. 1978, *C. Walker* (OSC 39145, E 00204004); Emmet Co., Fort Defiance State Park, 8 Aug. 1998, *S. Brown RH208* (ISC 435962, OSC 150028); Lee Co., near Farmington, Shimek State Forest, 7 Jul. 2000, *R. Healy RH688* (FLAS-F-66148, OSC 150027); Dubuque Co., Mines of Spain State Park, 13 Jun. 1999, *R. Healy RH369* (ISC, OSC 150026); Lucas Co., Stephens State Forest, 25 May 1999, *R. Healy RH335* (ISC, OSC 150025); North Carolina, Yancey Co., Pisgah National Forest, Valley of the Black Mountains, Woody Ridge trail, 17 Aug. 2012, *T.F. Elliott* (OSC 158255).

Notes: *Elaphomyces holstii* is assigned to *Elaphomyces* section *Elaphomyces* subsection *Muricati* based on the brown peridial surface and the marbled inner peridium. *Elaphomyces holstii* resembles the North American *E. americanus* and *E. verrucosus*

and the European *E. barrioi*, *E. decipiens* and *E. muricatus* in overall ascomata and spore characteristics.

Elaphomyces americanus differs from *E. holstii* by the former's larger spores (mean = 30.9 µm), larger asci (42–46 µm) containing 4–8 spores, and by the makeup of the inner marbled peridial layer (Castellano & Stephens 2017). The marbling of the inner peridial layer in *E. americanus* is comprised of white to off-white veins against a tan to brown matrix, the opposite of the makeup of the inner peridial layer for *E. holstii*.

Elaphomyces verrucosus is characterized by very low warts (80–90 µm tall by 210–250 µm broad at base), a very thick overall peridium (4–5 mm), an inner peridium with dark veins against a brown to purplish-brown matrix, and large spores (32–35 µm broad). Dodge (1929) reports *E. verrucosus* spores as 22–25 µm, with a mean = 22.5 µm. The first author studied the holotype (Gardner 268) of *E. verrucosus* and it is obvious that the small spores are aborted spores common to some species in *Elaphomyces* section *Elaphomyces*. The mature spores in the *E. verrucosus* holotype measured 32–35 µm. In contrast *E. holstii* has a thinner overall peridium (± 1.1 mm), smaller spores (25–30 µm, mean = 27.6 µm), and the inner peridial layer has white veins against a dark matrix (Fig. 2C, D). Paz *et al.* (2017) synonymize *E. verrucosus* under *E. decipiens* while at the same time recognizing that their clade of *E. decipiens* (fig. 2 in their paper) has cryptic species within, those being the three collections from western North America. We distinguish *E. verrucosus* from the European *E. decipiens* based on the slightly smaller spores of *E. verrucosus* (32–35 µm, mean = 33.5 µm), and the very thick (4–5 mm) overall peridium with an inner matrix that is brown to purplish brown matrix with very dark veins. *Elaphomyces decipiens* has slightly larger spores (32–38(–42) µm, mean = 35.4 µm) and a thinner peridium with an inner peridium that has a dark, nearly black matrix and nearly white veins. Castellano has nearly 500 collections of *Elaphomyces* specimens from across western North America that will be presented in a forthcoming paper focused on western North American. *Elaphomyces barrioi* has smaller spores, 19–24 µm wide (Paz *et al.* 2017). *Elaphomyces decipiens* has larger spores (32–38(–42) µm, mean = 35.4 µm) and low plate-like warts. *Elaphomyces muricatus* has a much thicker peridium that is constructed in opposite fashion to *E. holstii* and spores without a partial reticulum.

The first author studied an *Elaphomyces decipiens* collection from Italy (TO, also OSC 149180) marked "Italy, Lombardia, leg Vittadini, sent to Andisonne" that has duplicates in the Paris Cryptogamie (PC 93894, also studied by Paz *et al.* 2017), Patouillard (F 25817) and Dodge (F 258146) herbaria. Castellano (on file at Forestry Sciences Laboratory, Corvallis, Oregon, USA) data from PC 93894 and OSC 149180 in part is as follows: Peridium somewhat smooth to the naked eye or with low flat warts, often warts nearly absent to reveal an alligator-like texture of mottled patches of brown to yellow-brown warts that are plate-like, spores globose, in KOH brown to dark red-brown singly, darker in mass, 32–38(–42) µm, mean = 35.4 µm including ornamentation that appears as rods and short ridges or clustered rods under light microscope, in cross-section the spore outline appearing spiny, SEM reveals the spore ornamentation as rods anastomosed into clusters or short ridges (Fig. 2C, D).

The first author studied *Elaphomyces muricatus* collections from Sweden marked "Småland, Femsjö, leg & det. Elias Fries" (UPS 06/58 1) and marked "Upland, Kungsparken, 18-XI-1856, leg E.P. Fries" (UPS 06/58 14). Castellano data (on file at Forestry Sciences Laboratory, Corvallis, Oregon, USA) from UPS 06/58 1

and UPS 06/58 14 in part is as follows: Peridium with distinct brown, tall (500–750 μm) pyramidal warts with quad- to quint-angular bases, peridium 1.2–2.6 mm thick, inner peridium an off-white to pale gray matrix with brown to dark brown veins, spores globose, in KOH pale brown to dark brown singly, darker in mass, spores 24–30 μm broad, mean = 26.8 μm broad (often with smaller, very dark aborted spores intermixed) including ornamentation that appears as warts or clustered rods under light microscopy, SEM reveals the spore ornamentation as rods anastomosed into clusters or very short ridges (Fig. 2E, F). Paz *et al.* (2017) report three varieties for *Elaphomyces muricatus*: var. *muricatus*, var. *reticulatus*, and var. *variegatus*. We accept var. *variegatus sensu* Paz *et al.* (2017) as the true *E. muricatus sensu* Fries based on the cryptic morphological data they present and the microphotographs of the spores which clearly show the coarse clusters of rods on the spore surface for *Elaphomyces muricatus* var. *variegatus*. Interestingly it is also the only variety that has a collection from near where Fries collected; Småland, Femsjö. Paz *et al.* (2017) does not list a collection for Sweden under var. *muricatus*. Certainly, this species complex needs further study. Castellano has studied authentic collections of *E. muricatus* (as listed above) as well as for *E. reticulatus* and *E. variegatus*. Some of the confusion surrounding these species concepts may arise from the fact that Vittadini did not study any authentic material from Fries. Molia *et al.* (2020) selected an epitype from a recent collection from Småland, Femsjö and state that ascomata of *E. muricatus* “show acute, pyramidal warts on the cortex surface and the peridium is clearly marbled in cross-section.” They also designate Willdenow, C.L. (1787), *Florae Berolinensis Prodrromus*, plate VII, fig. 19 as lectotype.

Elaphomyces lougehrigii Castellano, R.A. Healy & Marzitelli, *sp. nov.* MycoBank MB835643. Fig. 5.

Etymology: Named in honor of the dauntless late Heinrich Ludwig (Lou) Gehrig (born 19 June 1903, died 2 June 1941), who inspired Michael Castellano with his grit and determination in the face of adversity and his graciousness, and excellence in his chosen field.

Diagnosis: Distinguished from all other *Elaphomyces* species in North America by the pale yellow-green to yellow-green mycelium forming a well-developed husk enveloping the black carbonaceous peridium. Distinguished from the similar *E. maculatus* of Europe by the much larger spores that have plate-like structures covering rods.

Typus: USA, Iowa, Story Co., Ames, Iowa State University, Reactor Woods, 7 Oct. 1998, R. Healy 294 (**holotype** ISC 435952, **isotype** OSC 150033).

Ascomata globose, subglobose to irregularly depressed, with variously sized and shaped, raised areas scattered across surface, up to 12 \times 25 mm broad, completely embedded in a pale yellow-green to yellow-green mycelium which forms a well-developed husk around individual ascoma and incorporating soil, ectomycorrhizal roots, and debris; mycelium not staining. **Peridium** overall 2–3 mm thick when mature, outer peridial layer \pm 500 μm thick, appearing smooth to the naked eye but in reality, with tiny, carbonaceous, compact, black papillae, \pm 100 μm broad, inner peridial layer 1.5–2 mm thick, leathery, pale gray-brown to gray-brown, slightly paler around the perimeter next to the outer

peridium. **Gleba** spore mass powdery, black. **Odor** not recorded. **Taste** not recorded. **Peridium in cross-section** two-layered, outer layer carbonaceous, \pm 500 μm thick, of very dark brown to black, compact, inflated cells, arranged in stacked parallel bundles, cells 3.5–5 μm broad, walls 1–2 μm thick, hyphae just inside outer peridium are pale red-brown as in inner peridial layer, no crystalline structures; inner peridial layer 1.5–2 mm thick, of compact, hyaline, interwoven, hyphae, 4–7.5 μm broad, walls 1–2 mm thick; crust hyphae in KOH yellow to brown, slightly swollen up to 7.5 μm , with many adherent particles, in Melzer’s reagent these crust hyphae are smooth, hyaline to pink-brown or gray-brown. **Gleba** of spores and hyaline, septate, sparsely branched, loosely interwoven hyphae, 2.5–3 μm broad, walls $<$ 0.5 μm thick. **Asci** globose, subglobose to somewhat angular in shape, 65–75 μm broad, hyaline, 8-spored, walls \pm 2 μm thick, arising from typical knots of large, contorted interwoven hyphae, \pm 5 μm broad. **Spores** ornamented, globose (29–)30–32(–33) μm (mean = 30.9 μm); with small, rounded warts, rough in appearance, in cross-section knobby appearing disorganized, up to 2 μm tall, apparently double-walled; in KOH, dark red-brown when mature singly and in mass, surface with dark spots against dark red background. SEM reveals overlapping small, warty protuberances in a disorganized pattern completely covering spore surface.

Distribution, habit, habitat and season: Known from Florida, Iowa, and Québec Province, Canada; hypogeous; under *Ostrya virginiana* and *Quercus alba*; September and October.

Additional collections examined: **Canada**, Québec Province, Montreal, 1 Oct. 1992, F. Marzitelli (OSC 149106). **USA**, Florida, Alachua Co., Sugarfoot Hammock, 1 mile west of Gainesville, south of SW 20th Ave., west of Interstate 75, 300 yards east of entrance to limestone caves, 16 Sep. 1983, J. Gibson (FLAS 58695);

Notes: *Elaphomyces lougehrigii* is placed in *Elaphomyces* section *Ceratogaster* subsection *Maculati* based on the spore size, thick peridium and mycelial patches on the peridial surface. *Elaphomyces lougehrigii* resembles three *Elaphomyces* species from Europe in some ascoma or spore characteristics: *E. citrinus*, *E. leveillei*, and *E. maculatus*. The yellow to green colored hyphae covering the black peridial surface of *E. lougehrigii* somewhat resembles the surface hyphae of all three European species. *Elaphomyces citrinus* has orange-yellow to brown-yellow hyphae on a thin, pliable, carbonaceous peridial surface, and small, reticulate spores (15–16 μm , mean = 15.8 μm). *Elaphomyces leveillei* has pale tan to yellow to yellow-brown peridial surface hyphae and smaller spores (26–28 μm , mean = 26.6 μm) covered by smooth plate-like structures over spines or rods. *Elaphomyces maculatus* usually has greenish peridial surface hyphae with a persistent disc-like patch of distinct greenish hyphae at the base and very large spores (37–41 μm , mean = 39.1 μm) with coarse spines 3 μm tall. *Elaphomyces lougehrigii* has pale yellow-green to yellow-green surface hyphae but without a persistent patch of hyphae at the base and large spores (30–32 μm , mean = 30.9 μm) covered by small, warty protuberances in a disorganized pattern completely covering the spore surface.

The first author studied an *Elaphomyces citrinus* collection (TO, also in OSC 149172) marked “Vittadini collection” and Kew (161174) marked “ex herb Berkeley from Vittadini.” Castellano (on file at Forestry Sciences Laboratory, Corvallis, Oregon, USA) recorded data from OSC 149172 in part as follows: Dried specimens with peridial surface black, appearing wrinkled

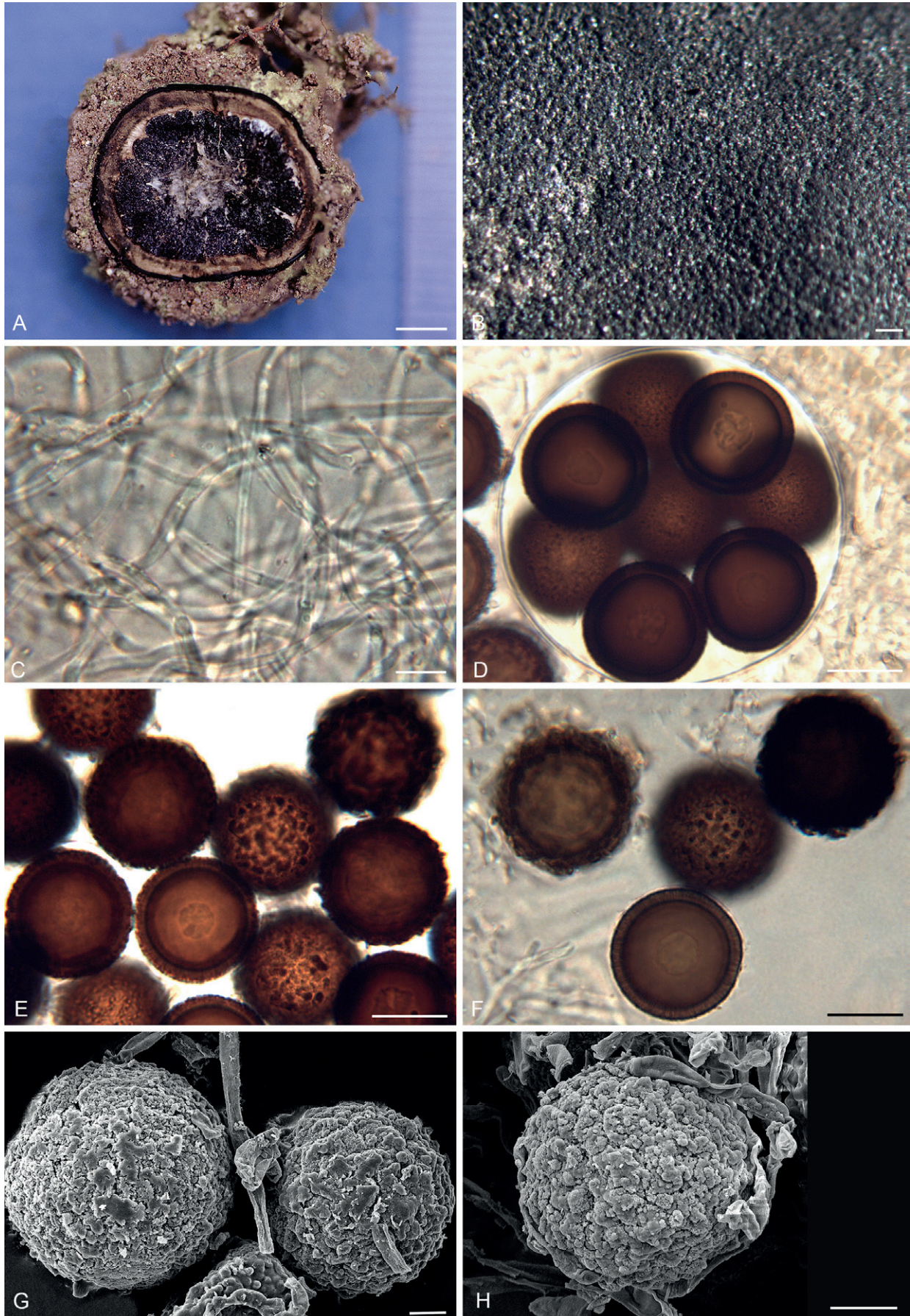


Fig. 5. *Elaphomyces lougehrigii*. **A.** Ascomata cut in half showing peridium in section, gleba, and surrounding hyphae, roots and soil. **B.** Ascoma showing compact, small papillae on peridial surface. **C.** Hyaline, septate, sparsely branched, loosely interwoven hyphae of the gleba. **D.** Ascus showing eight immature ascospores. **E.** Ascospores in surface view showing the pattern of the ornamentation. **F.** Ascospores in cross-sectional view showing the ornamentation pattern. **G.** SEM micrograph of ascospores showing the ornamentation of overlapping, small, warty protuberances in a disorganized pattern. **H.** SEM micrograph of an ascospore showing the various dimensions of the knobby ornamentation. A–H (ISC 435952 – holotype). Scale bars: A = 10 mm, B = 500 µm, C = 10 µm, D = 15 µm, E–G = 10 µm, H = 5 µm.

or wavy, peridial surface 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 a flattened apices (see plate 2 in Castellano *et al.* 2018). Dodge (1929) does not cite a type but reports it as common around Milan.

The first author studied an *Elaphomyces leveillei* collection in Kew, K162150. Tulasne (1841) lists specimens from Meudon, Clamart, and Chaville communes in suburbs surrounding Paris. Castellano could not locate any Tulasne material of this species in Paris (PC) but it appears this collection in Kew may be the same as cited by Paz *et al.* (2017) as PC 0167755 (lectotype) with the same collection details. Castellano (on file at Forestry Sciences Laboratory, Corvallis, Oregon, USA) recorded data from K162150 in part as follows: Peridium ornamented with pustulate to tuberculate bumps, partially covered by pale tan, pale yellow-brown to yellow hyphae, peridial surface black, base indented, spores globose, in KOH dark brown singly, slightly darker in mass, 26–28 μm , mean = 26.6 μm including ornamentation that appears pustulate (bumpy) under light microscope, in section appearing with a flattening of the spore outline at least on a portion of many 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 (see plate 4 in Castellano *et al.* 2018).

The first author studied an *Elaphomyces maculatus* collection from Italy (FH in the Patouillard herbarium (ex. herbarium O. Mattiolo) marked “Portion autotici C. Vittadini”). Castellano (on file at Forestry Sciences Laboratory, Corvallis, Oregon, USA) data on the FH collection in part is as follows: Peridium black, nearly smooth, with tiny widely spaced, low papillae with floccose, green mycelium, spores globose, in KOH brown to dark brown singly, darker in mass, (35–)37–41(–42) μm , mean = 39.1 μm including ornamentation that appears in surface view as a finely wrinkled bumpy surface, in section appearing bumpy under light microscope, SEM reveals the spore ornamentation as coarse spines, up to 2 μm tall (Fig. 2G, H).

Elaphomyces miketroutii Castellano, *sp. nov.* MycoBank MB835644. Fig. 6.

Etymology: Named in honor of Michael Nelson (Mike) Trout (born 7 August 1991, Vineland, New Jersey), who continually inspires Michael Castellano with his old-school professionalism and determination to continually improve his skills in his chosen field.

Diagnosis: Distinguished from all other similar *Elaphomyces* species in North America by the brown to dark brown mycelium forming a well-developed husk enveloping the black carbonaceous peridium. Distinguished from the similar *E. nopporensis* (Imai 1929) from Japan in the color of the enveloping mycelium surrounding the sporocarp. *Elaphomyces miketroutii* has much larger spores than *E. nopporensis*.

Typus: USA, Florida, Alachua Co., just south of Windsor, Owens-Illinois County Park, east side of Newnans Lake at end of SE 16th Ave., 3 May 2006, M. Castellano (**holotype** OSC 149081, **isotype** FLAS-F-65662).

Ascomata subglobose to irregular, up to 22 × 25 mm, completely embedded in a brown to dark brown mycelial mat which forms a husk around individual ascoma and incorporating much sand, ectomycorrhizal roots, and debris; mycelium not staining. *Peridium* 1–1.9 mm thick, epicutis \pm 400 μm thick, carbonaceous, black, of rounded to subacute, low warts, 300–350 μm wide, embedded in brown to dark brown hyphae that occasionally obscures the warts, subcutis 1–1.5 mm thick, uniform, pale gray-tan, subcutis shrinks when dried or as it matures. *Gleba* stuffed with bright white mycelium when young, when mature spore mass powdery, black, with black, web-like hyphae. *Odor* not recorded. *Taste* not recorded. *Peridium* two-layered, epicutis, carbonaceous, \pm 400 μm thick, of brown to dark brown to nearly black, septate, short-segmented, compact, subparallel to parallel hyphae, 3–4 μm broad, walls \pm 1 μm thick, arranged periclinally; subcutis 1–1.5 mm thick, of hyaline to pale tan, septate, compact, interwoven cells, up to 8 μm broad, walls 1–2 μm thick, abundant lipid droplets obscuring detail. *Gleba* of spores and thin-walled, hyaline, septate, somewhat branched, sinuous hyphae, 3–4 μm broad. *Asci* globose, 60–75 μm broad, hyaline at first then darkening to brown with spore maturity, walls \pm 1 μm thick, 4- or 8-spored, arising from knots of short, irregularly curved or contorted clustered hyphae, up to 14 μm broad. *Spores* ornamented, globose, 32–34 μm broad (mean = 33.2 μm); walls \pm 1 μm thick, in KOH dark brown singly and in mass when mature, ornamentation a fine labyrinth, \pm 2 μm tall, with much debris in mature mounts from sloughing of ornamentation.

Distribution, habit, habitat and season: Known from Florida, Louisiana and Texas; hypogeous; under *Quercus nigra* and *Q. virginiana*; January, May and November.

Additional collections examined: USA, Florida, Alachua Co., just south of Windsor, Owens-Illinois County Park, east side of Newnans Lake at end of SE 16th Ave., 25 Feb. 2012, M. Castellano (OSC 149367 is also FLAS-F-65663, OSC 149084 is also FLAS-F-65664, OSC 149085 is also FLAS-F-65665); Louisiana, St. Tammany Parish, Delta Regional Primate Center, 31 Jan. 1969, A.L. Welden (OSC 149083, NO 7237). Texas, Nacogdoches Co., 1 Nov. 1984, K. O'Halloran (OSC 149082).

Notes: *Elaphomyces miketroutii* is placed in *Elaphomyces* section *Malacodermei* based on the dense mycelial coating on the peridial surface. *Elaphomyces miketroutii* resembles *E. nopporensis* (Imai 1929) from Japan in the color of the enveloping mycelium surrounding the sporocarp. *Elaphomyces miketroutii* has much larger spores than *E. nopporensis* (15–17.5 μm , mean = 15 μm). OSC 149083 and OSC 149085 are parasitized by *Typocladium* sp.

Elaphomyces roodyi Castellano & D. Mitchell, *sp. nov.* MycoBank MB835645. Fig. 7.

Etymology: Named for William C. Roody of West Virginia, accomplished collector and photographer of fungi and provider of the field photo for this species.

Diagnosis: *Elaphomyces roodyi* resembles *E. appalachiensis*, *E. atropurpureus*, and *E. roseolus* in the thin, smooth, pliable peridial surface and in the color of the enveloping mycelium surrounding the ascoma but has distinctly larger spores than any of these species.

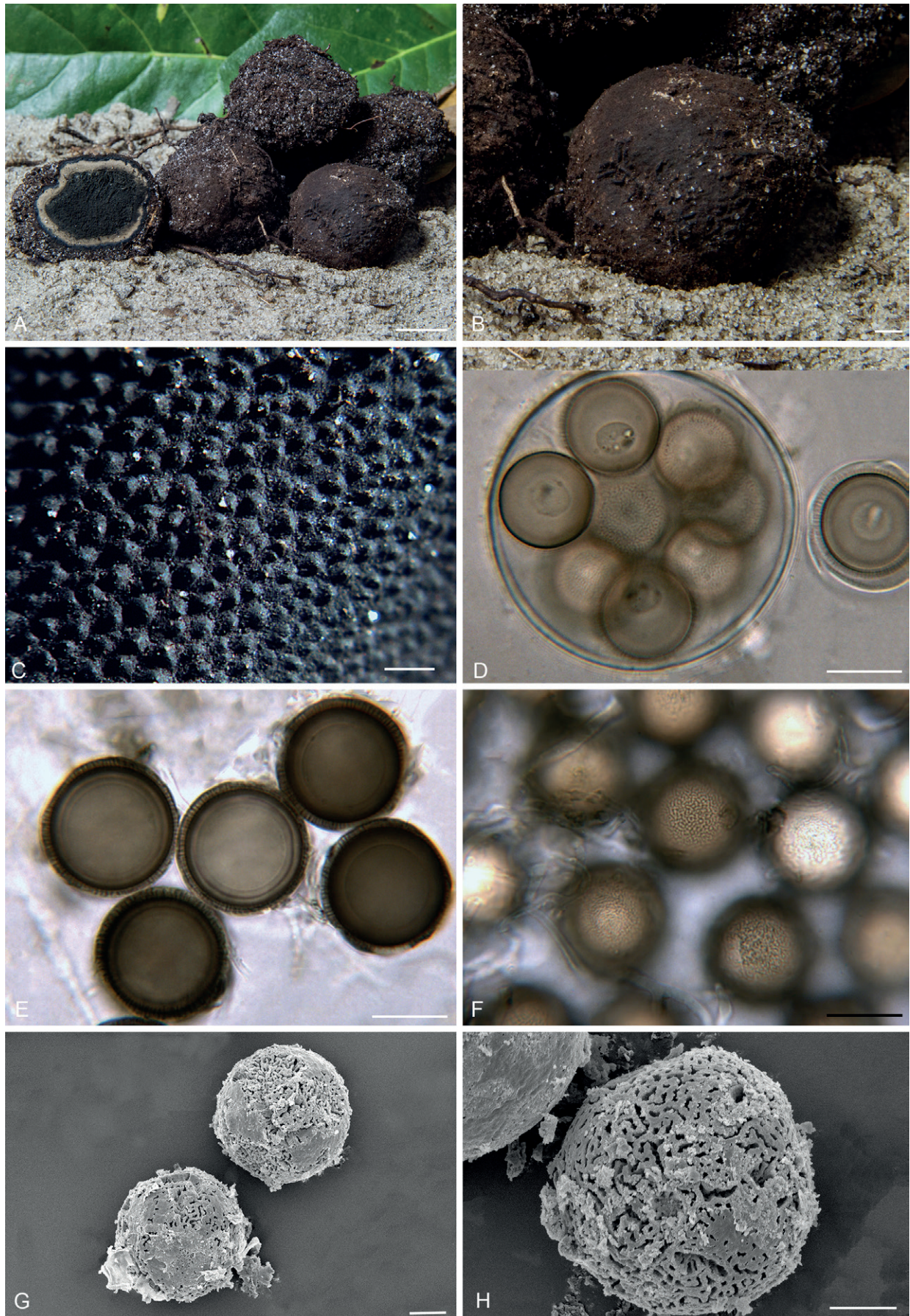


Fig. 6. *Elaphomyces miketrouitii*. **A.** Ascomata showing peridium in section and gleba with adherent dark brown mycelium and sand particles. **B.** Ascomata closeup showing envelope of ectomycorrhizal roots, dark brown hyphae and sand particles. **C.** Closeup of the ascoma surface showing the small, black warts. **D.** Ascus with eight immature ascospores within. **E.** Ascospores in cross-sectional view showing the ornamentation of rods which compose the labyrinth. **F.** Ascospores in surface view showing the fine labyrinth ornamentation. **G.** SEM micrograph of ascospores showing the partially angular sides with fine ornamentation. **H.** SEM micrograph showing closeup of the fine labyrinth. A–B (OSC 149367), C–H (OSC 149081 – holotype). Scale bars: A = 10 mm, B = 0.5 mm, C = 700 μ m, D = 20 μ m, E–F = 15 μ m, G = 4 μ m, H = 8 μ m.

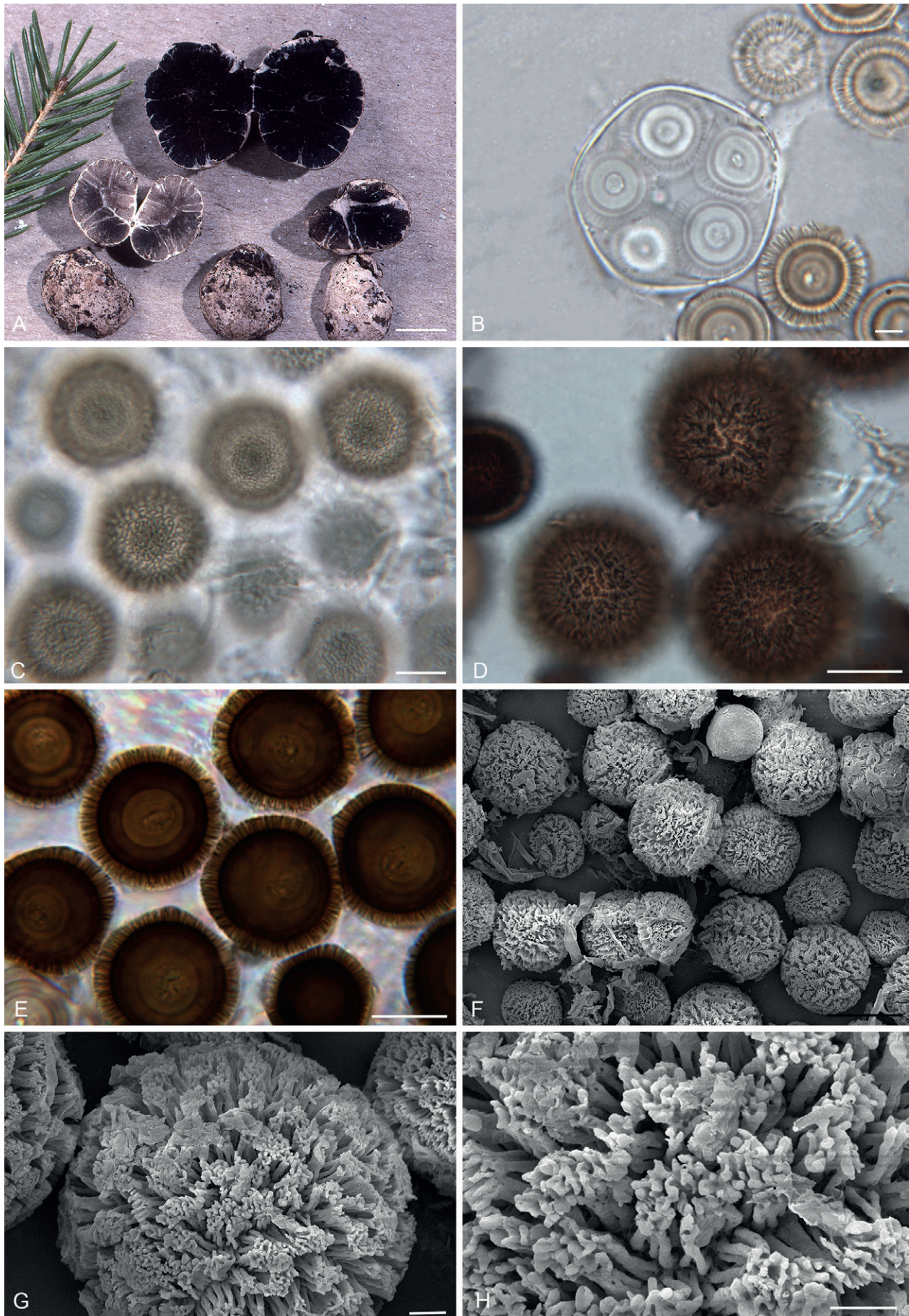


Fig. 7. *Elaphomyces roodyi*. **A.** Ascomata showing peridium in section, gleba, and peridial surface with adherent white mycelium. **B.** Ascus showing immature ascospores. **C.** Immature ascospores in surface view showing ornamentation. **D.** Ascospores in surface view showing the ornamentation pattern. **E.** Ascospores in cross-sectional view showing the ornamentation of rods. **F.** SEM micrograph of ascospores showing the ornamentation pattern. **G.** SEM micrograph of ascospore showing the clustering of rods. **H.** SEM micrograph showing the anastomosed rods that form clusters. A–H (OSC 149220 – holotype). Scale bars: A = 20 mm, B = 5 mm, C = 30 μ m, D–G = 10 μ m, H = 2.5 μ m.

Typus: USA, West Virginia, Pocahontas Co., Monongahela National Forest, Odey Run Bog, along Forest Service road 267, N38° 28' 7" W79° 54' 55", 22 Apr. 1997, C. Stihler & D. Mitchell (**holotype** OSC 149220, isotype DEWV).

Ascomata subglobose to irregularly shaped, up to 6 × 10 mm broad, covered with white mycelium that slowly stains rose. *Peridium* thin, ± 200 µm thick, surface felty, a single layer, pliable, extending into the gleba at regular intervals. *Gleba* hyphae white, spore mass powdery, chocolate brown or darker. *Odor* mild or indistinct. *Taste* not recorded. *Peridium in cross-section* a single layer, ± 400 µm thick, of pale yellow-tan, septate, compact to loosely interwoven hyphae 4–5 µm broad, walls ± 1 µm thick, appearing somewhat coarse from debris. *Gleba* with hyaline, slightly curly to sinuous at times, loosely interwoven hyphae 2–5 µm broad, walls ± 1 µm thick. *Asci* globose to somewhat angular, hyaline, 45–55 µm broad, 5- or 6-spored, walls ± 1 µm thick, arising from knots of short irregular hyphae. *Spores* ornamented, globose, 36–41 µm (mean = 38.8 µm); walls 1–2 µm thick, in KOH red-brown singly and in mass, ornamentation of rods and clumps that form a uniform labyrinthine ridge system, ± 4 µm tall, immature spores 22–26 µm broad, hyaline to pale yellow, walls ± 2 µm thick, with tall (± 4 µm) rods and labyrinthine ridges; intermixed with mature spores are numerous dark brown-red spores, 24–26 µm broad, with the same ornamentation but the ornamentation only 3 µm tall.

Distribution, habit, habitat and season: Known only from a single collection from West Virginia; hypogeous; under *Picea rubens* at approximately 1 283 m elevation; April.

Notes: *Elaphomyces roodyi* is within *Elaphomyces* section *Malacodermei* based on the thin, smooth, pliable peridial surface. *Elaphomyces roodyi* resembles *E. appalachiensis*, *E. atropurpureus*, and *E. roseolus* in the color of the enveloping mycelium surrounding the ascoma but has distinctly larger spores than any of the other species assigned in section *Malacodermei*. *Elaphomyces appalachiensis* has small spores (10–13 µm, mean = 10.95 µm) and purple mycelium enveloping the ascoma (Linder 1939, Castellano *et al.* 2012). *Elaphomyces atropurpureus* also has small spores (11–12(–13) µm, mean = 11.7 µm) and purple enveloping mycelium. Paz *et al.* (2017) describes *E. roseolus* with pink-brown hyphae covering the scrobiculate peridial surface, and spores 9–11 µm with thick rods forming an irregular labyrinth.

The first author studied an *Elaphomyces atropurpureus* collection from Italy (PC 93889) marked “ex ipso”. Castellano data (on file at Forestry Sciences Laboratory, Corvallis, Oregon, USA) from PC 93932 in part is as follows: Peridial surface dark purple covered with purple, tomentose mycelium and scattered ectomycorrhizas, spores globose, in KOH brown singly, dark brown in mass, 11–12(–13) µm (mean = 11.7 µm), including ornamentation that appears as clustered rods or spines usually aligned in short lines under light microscope, SEM reveals the spore ornamentation as rods anastomosed into clusters or irregularly thickened ridges.

Elaphomyces stevemilleri Castellano, *sp. nov.* MycoBank MB835646. Fig. 8.

Etymology: Named in honor of esteemed mycologist Dr. Steve L. Miller, University of Wyoming, accomplished truffle and co-collector of the holotype.

Diagnosis: *Elaphomyces stevemilleri* resembles *E. mitchelliae* from eastern North America, *E. cyanosporus*, and *E. persoonii* from Europe. It differs from these three species by its smaller spores that are not reticulate.

Typus: USA, Florida, Alachua Co., Owens-Illinois Park, Newnans Lake, N29° 38.172' W82° 12.005', 11 Aug. 1985, M. Castellano & S. Miller (**holotype** OSC 149099, **isotypes** RMS and FLAS-F-65666).

Ascomata subglobose to irregularly flattened, up to 17 mm × 20 mm, often with a distinct basal protuberance, completely enveloped in a pale brown to brown to dark brown mycelium with ectomycorrhizal roots and sand; mycelium not staining. *Peridium* 100–300 µm thick, carbonaceous, dark brown to black, peridial surface appearing smooth to the naked eye but with a hand lens somewhat evanescent, low, irregularly-shaped papillae, basal protuberance covered with warts that are pyramidal-shaped, warts up to 250 µm tall, up to 350 µm broad, inner peridial layer 1–2 mm thick, leathery, uniform in thickness, white to dark gray, in addition a paper-thin layer next to gleba which is dark gray; peridium thinning with age until only the dark carbonaceous layer remains. *Gleba* spore mass powdery, dark gray, dark gray-brown to black, with dark web-like hyphae. *Odor* not recorded. *Taste* not recorded. *Peridium in cross-section* two-layered, outer peridial layer, 100–300 µm thick, of compact, dark brown, interwoven hyphae, ± 4 µm broad, walls ± 1 µm thick; inner peridial layer 1–2 mm thick, of hyaline to pale tan (near epicutis), septate, compact, interwoven hyphae, ± 5 µm broad, walls 1–2 µm thick. *Gleba* of spores and hyaline, septate, elongate, sinuous hyphae, 2–3 µm broad, walls < 0.5 µm thick. *Asci* globose, hyaline, 52–55 µm broad, walls ± 2 µm thick when young, thinner when full of nearly mature spores, 8-spored, arising from knots of short, irregularly curved or contorted clustered hyphae up to 4 µm broad. *Spores* ornamented, globose, 20–23 µm (mean = 21.4 µm); walls ± 1 µm thick, in KOH singly and in mass hyaline at first then dark brown when mature, ornamentation of spines embedded or enclosed in an enveloping covering which appears as a fine labyrinth under the light microscope, ± 2 µm tall.

Distribution, habit, habitat and season: Known only from Florida and Iowa; hypogeous; under *Quercus nigra*, *Q. virginiana* and *Quercus* sp.; August.

Additional collections examined: USA, Florida, Alachua Co., Owens-Illinois County Park, Newnans Lake, 11 Aug. 2007, M. Castellano (FLAS-F-65667, OSC 149239); Calhoun Co., Chipola River County Park, along Hwy 20, east of Clarksville, N30° 25.907' W85° 10.302', 9 Aug. 2007, M. Castellano (FLAS-F-65668, OSC 149238); Lake Co., Lake Griffin State Park, in picnic area, N28° 51.427' W81° 54.044', 14 Aug. 2007, M. Castellano (FLAS-F-65669, OSC 149240); Iowa, Story Co., Hickory Grove State Park, 17 Aug. 1996, R. Healy (ISC-F-0075947, OSC 150021).

Notes: We place *E. stevemilleri* in *Elaphomyces* section *Ascoscleroderma* based on the distinct protuberance at the base. *Elaphomyces stevemilleri* resembles *E. mitchelliae* from North America (Castellano *et al.* 2018) and *E. cyanosporus*, *E. foetidus* (*sensu* Paz *et al.* 2017), and *E. persoonii* from Europe. Based on study of a few European collections of *Elaphomyces echinatus*, *E. foetidus* and *E. morettii* (Castellano data on file at Forestry Sciences Laboratory, Corvallis, Oregon, USA) we believe that *E. foetidus* is a synonym of *E. morettii*, while *E. echinatus* is

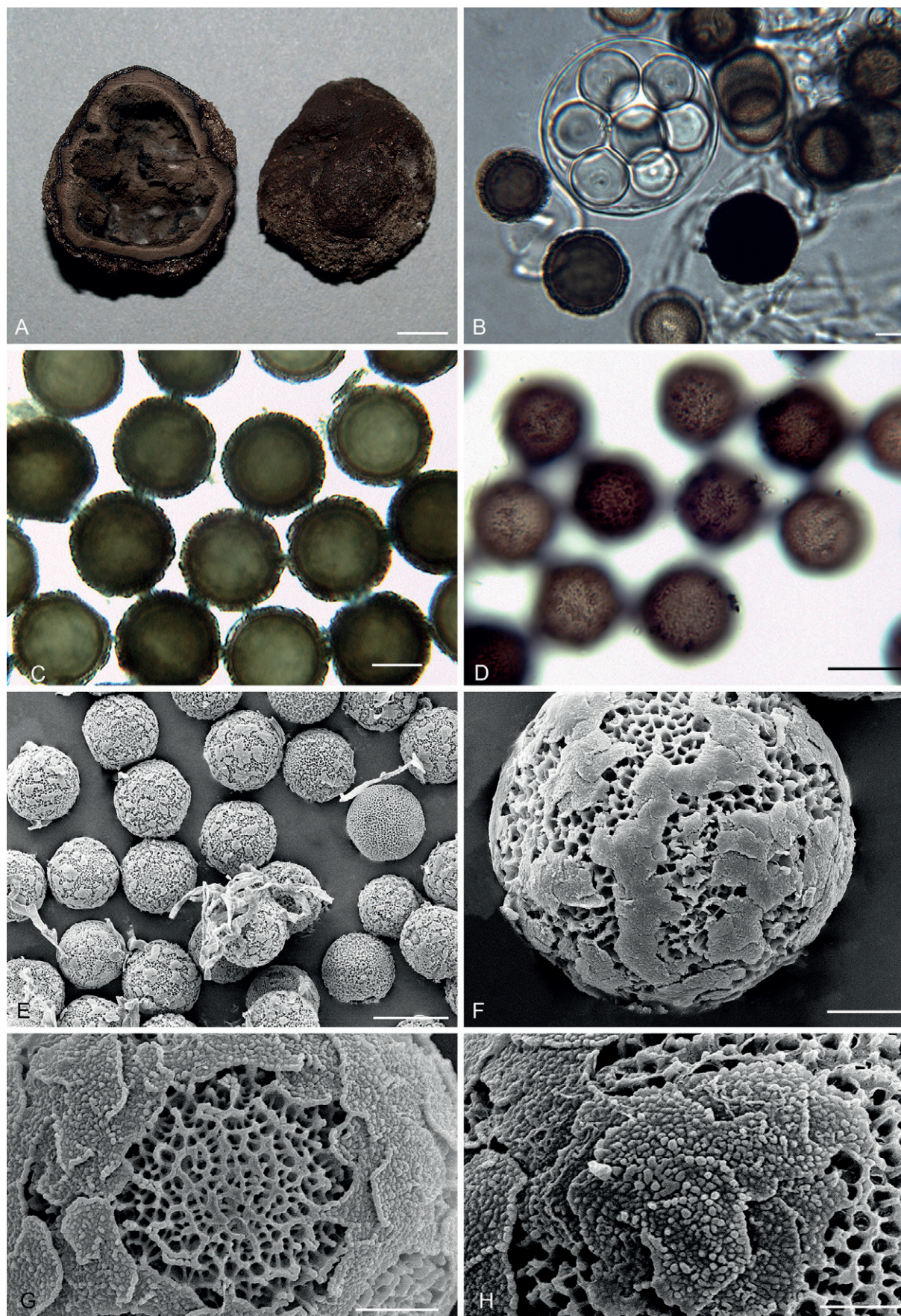


Fig. 8. *Elaphomyces stevemilleri*. **A.** Ascomata showing peridium in section, gleba, and peridial surface. **B.** Ascus showing eight immature ascospores, some in surface view, some in cross-sectional view. **C.** Ascospores in cross-sectional view showing the ornamentation as spiny to knobby in outline. **D.** Ascospores in cross-sectional view showing the ornamentation of a fine honeycomb overlain with plate-like structures. **E.** SEM micrograph of ascospores showing the of a fine honeycomb overlain with plate-like structures. **F.** SEM micrograph showing the fine detail of the fine honeycomb overlain with plate-like structures. **G.** SEM micrograph showing the fine detail of the fine honeycomb beneath the plate-like structures. **H.** SEM micrograph showing the fine detail of the plate-like structures. A–H (OSC 149099 – holotype). Scale bars: A = 20 mm, B = 5 μ m, C = 10 μ m, D–E = 20 μ m, F = 5 μ m, G–H = 2 μ m.

distinct. Paz *et al.* (2017) present three varieties for *E. morettii* but do not list an authentic collection of *E. morettii* that they studied even though they recognized that it exists in UPS. We believe this complex needs further molecular and morphological study to truly establish *E. morettii* and sort out potential cryptic species.

Elaphomyces mitchelliae has a very complex 7-layered peridium and larger spores (24–27 µm broad, mean = 25.5 µm broad) ornamented with a distinct reticulum. The spores of *E. cyanosporus* and *E. personii* are larger than *E. stevemilleri* and the spore ornamentation is distinctly reticulate.

The first author studied an *Elaphomyces cyanosporus* 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 (on file at Forestry Sciences Laboratory, Corvallis, Oregon, USA) 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 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 (see plate 4 in Castellano *et al.* 2018).

The first author studied an *Elaphomyces personii* var. *minor* collection in Kew (K161168 marked Meudon, close to Paris, summer 1841, col. Tulasne). The Tulasne brothers (1841) list specimens from Meudon, southwest of Paris but do not designate a type collection. Castellano could not locate any Tulasne material of this species in Paris (PC or FH, although Paz *et al.* (2017) cite material from PC). *Elaphomyces personii* var. *minor* is conspecific in all essential morphological details with *E. cyanosporus* and aligns well with the molecular analysis by Paz *et al.* (2017).

The first author studied *Elaphomyces personii* collections from TO - OSC 149124, W2008-1079 and K162166 marked as from Vittadini. Castellano recorded data (on file at Forestry Sciences Laboratory, Corvallis, Oregon, USA) from OSC 149124 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 (see plate 4 in Castellano *et al.* 2018).

Elaphomyces wazhazhensis Crabtree & Castellano, *sp. nov.* MycoBank MB835647. Fig. 9.

Etymology: “wazhazhensis” wazhazhe from Wa Zha Zhe – the tribal name used by the Osage Nation peoples of the Great Plains of North America, meaning “children (people) of the middle waters”; *ensis* – pertaining to or originating in. The majority of collections, including the type collection, come from the ancestral lands of the Osage Nation and from the Osage River region of Missouri.

Diagnosis: The nearly smooth or very low alligator-like plate structure of the peridial surface of *Elaphomyces wazhazhensis* easily separates it from *E. holstii* from North America and *E.*

muricatus from Europe. The nearly smooth to very low plate-like peridial surface characters of *E. wazhazhensis* are similar to *E. decipiens* from Europe and *E. verrucosus* of western North America. The spores of both *E. decipiens* (32–38 µm, mean = 35.4 µm) and *E. verrucosus* (32–37 µm) are much larger than those of *E. wazhazhensis* (27–31 µm, mean = 29 µm).

Typus: USA, Missouri, Camden Co., Ha Ha Tonka State Park, Turkey Pen Hollow trail, in access road, N37.97897° W92.74873°, 2 Aug. 2007, *C. Crabtree* CDC0579 (**holotype** OSC 150032).

Ascomata globose to irregularly subglobose, up to 16 × 21 mm, completely embedded in fine sand, ectomycorrhizal roots, debris, and brown mycelium. **Peridium** ± 3 mm thick when mature, outer peridial layer 300–400 µm thick, somewhat smooth or with small to large flat warts, often warts nearly absent to reveal an alligator-like texture of mottled patches of brown to yellow-brown warts that are plate-like, flat, irregularly-sided, 240–320 µm broad, with narrow, pale cracks between plates often filled with fine sand, when cleaned of fine sand the cracks are darker than the plates, one specimen dark brown nearly black on surface, apparently from exposure; in cross-section inner peridial layer 2.5+ mm thick, marbled with white veins and pale gray-brown matrix near surface then rapidly rose-brown to dark brown or nearly black near gleba, matrix up to 750 µm broad between veins. **Gleba** spore mass powdery, black, with numerous white to pale tan web-like hyphae attached to inner peridial layer and invaginating into gleba, when in gleba usually more rose-tan. **Odor** when present slight to pungent, pine-like to burnt oil. **Taste** not recorded. **Peridium in cross-section** two-layered, thick, ± 400 µm thick, with hyaline and golden brown patches, of bundles of hyphae ± 100 µm broad, hyphae short segmented, branched, pale yellow brown, compact, up to 5 µm broad, walls ± 1 µm thick, in disorganized bunches, somewhat periclinal directly underneath plates then quickly becoming hyaline and more disorganized with interwoven hyphae and overall slightly larger diameter, intergrading into a layer of finer hyphae, ± 3 µm broad, loosely interwoven in a hyaline matrix, lastly the layer next to gleba is darker of brown, loosely interwoven hyphae, walls < 0.5 µm thick. **Gleba** of spores and scattered hyphae; web-like hyphae in bundles, pale brown, somewhat branched hyphae, 1–3 µm mm broad, with much amorphous debris scattered amongst hyphae, walls 1 µm thick. **Asci** not observed. **Spores** ornamented, globose, 27–31(–33) µm (mean = 29 µm); walls ± 1 µm thick, mostly brown to occasionally dark brown when mature singly and in mass, ornamentation of coarse rods and tufts of rods forming short ridges, 2–3(–4) µm tall.

Distribution, habit, habitat and season: Known from Louisiana and Missouri; hypogeous; under *Carya tomentosa*, *Quercus alba*, *Q. marilandica*, *Q. palustris*, *Q. phellos*, *Q. stellata*, *Q. velutina* or *Q. virginiana*; August and September.

Additional collections examined: USA, Missouri, Barton Co., Prairie State Park, day use area, 24 Aug. 2017, *C. Crabtree* CDC0599 (OSC 159155); same data except 29 Aug. 2017, *C. Crabtree* CDC0601 (OSC 159154); same data except 21 Sep. 2017, *C. Crabtree* CDC0603 (OSC 159153); Camden Co., Ha Ha Tonka State Park, Turkey Pen Hollow trail, in access road, N37.97897° W92.74873°, 2 Aug. 2007, *C. Crabtree* CDC0580 (OSC 150031); same data but 24 Aug. 2016, *C. Crabtree* CDC0593 (OSC 158016); same data but River Cave Flatwoods, along access road to unburnt unit, N37° 58' 42.1" W92° 45' 5.2", 27 Jun. 2007, *C. Crabtree* 0578 (OSC 150030); same data as

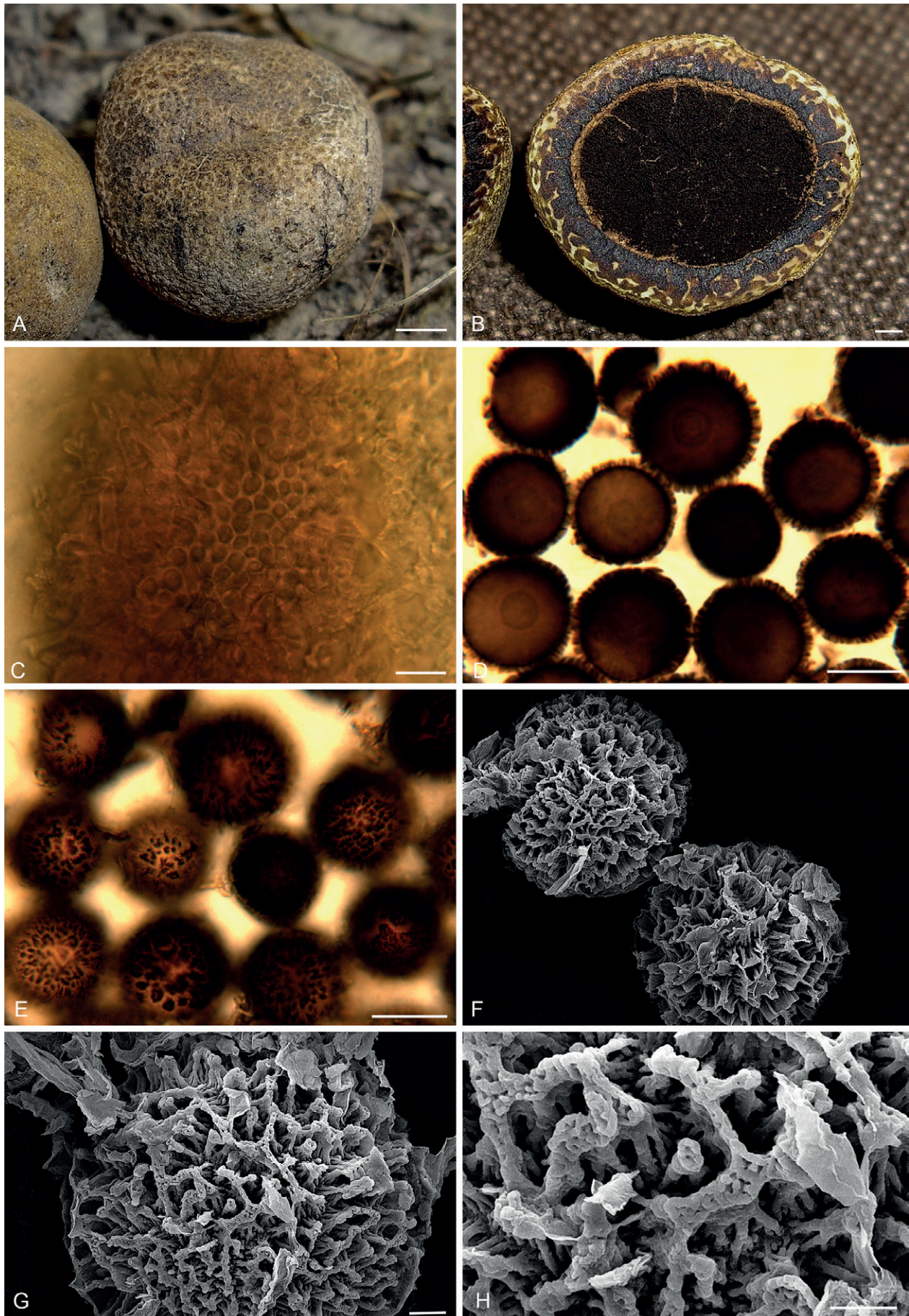


Fig. 9. *Elaphomyces wazhazhensis*. **A.** Ascomata showing peridial surface. **B.** Ascoma in cross-section showing the marbled inner peridium. **C.** Structure of the outer peridium. **D.** Ascospores in cross-sectional view showing the ornamentation of rods. **E.** Ascospores in surface view showing the coarse rods and tufts. **F.** SEM micrograph of ascospores showing the short ridges. **G.** SEM micrograph of an ascospore showing the irregular arrangement of the rods to form ridges. **H.** SEM micrograph showing the anastomosed rods that form irregular ridges. A–H (OSC 150032 – holotype). Scale bars: A = 20 mm, B = 5 mm, C = 30 μ m, D–G = 10 μ m, H = 2.5 μ m.

OSC 150030 but 20 Jul. 2016, *C. Crabtree 0585* (OSC 159157); Greene Co., Republic, 4827 South Claremont Dr., north of residence, 10 Sep. 2018, *C. Crabtree CDC 0609* (OSC 158593),

Notes: *Elaphomyces wazhazhensis* is assigned to *Elaphomyces* section *Elaphomyces* subsection *Muricati* based on the marbled inner peridium. *Elaphomyces wazhazhensis* resembles *E. holstii* from North America and three *Elaphomyces* species from Europe, *E. barrioi*, *decipiens* and *E. muricatus*, in ascoma or spore characteristics (see previous species for data on *E. barrioi*, *E. decipiens* and *E. muricatus*). The peridial characters of *E. barrioi* and *E. decipiens* are very close to *E. wazhazhensis* but the spores of *E. decipiens* are much larger (32–38 µm, mean = 35.4 µm) and the spores of *E. barrioi* are much smaller (19–24 µm). The spores of *E. muricatus* are similar in ornamentation pattern to *E. wazhazhensis* but the distinctly tall, pointy, peridial warts of *E. muricatus* easily separates it from *E. wazhazhensis* that has flat, plate-like warts. See the discussion under *Elaphomyces holstii* for remarks concerning molecularly unique western North American collections that resemble *E. decipiens* and *E. muricatus* from Europe.

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Conflict of interest: The authors declare that there is no conflict of interest.

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