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## Two new *Elaphomyces* species (Elaphomycetaceae, Eurotiales, Ascomycota) from eastern USA

## アメリカ合衆国東部において採集されたツチダンゴ属（子囊菌門 ユーロチウム目ツチダンゴキン科）の 2 新種

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### Abstract

The hypogeous, sequestrate *Elaphomyces* has a long taxonomic history in Europe and was formally described by Nees von Esenbeck in 1820 from Europe. Until recently, names of European taxa were assigned to most *Elaphomyces* specimens in North America due to lack of specialists working on this group and the difficulty of using pre-modern species descriptions. The genus *Elaphomyces* from North America has received increased attention for the last decade and this has led to numerous new *Elaphomyces* species being described from eastern North America. Here are described two new species of *Elaphomyces* discovered in eastern USA, *E. gouldhoytii* that has similarities to *E. septatus* and *E. maculatus*, and *E. ramulospinus* with similarities to *E. muricatus*.

### 要旨

ツチダンゴ属は地中生のシクエストレート型の子実体を形成する子囊菌類で、1820年にヨーロッパから Nees von Esenbeck によって正式記載され、ヨーロッパにおいて長い分類学史を有する。近年まで北米のツチダンゴ属の標本の多くに対しヨーロッパ産種の種名が適用されてきたが、これは北米に本属の専門家が不在で、かつ、古典的な記載方法に基づいた種の扱いが困難であったことに起因している。北米のツチダンゴ属菌は過去 10 年間にわかに注目を集め、北米東部から多数の新種が記載された。本研究では、*E. septatus* と *E. maculatus* に類似する新種 *E. gouldhoytii*、ならびに *E. muricatus* に類似する新種 *E. ramulospinus* をアメリカ東部から記載する。

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### Introduction

The hypogeous, sequestrate genus *Elaphomyces* Nees (Elaphomycetaceae, Eurotiales, Ascomycota) has received increased attention over the last ten years with numerous new species added and a number of previously described species revised with modern descriptions (Castellano et al., 2011, 2012a, 2012b, 2012c, 2016, 2018, 2021; Castellano & Stephens, 2017; Molia et al., 2020; Paz et al., 2012, 2017; Shirakawa & Tanaka, 2020). *Elaphomyces* ascomata usually have a thick, complex peridium often encased in mycelium, roots, soil and debris, and a powdery gleba.

Prior to 2012, there were five *Elaphomyces* species described

from North America: *E. verrucosus* C.W. Dodge (Dodge, 1929), *E. appalachiensis* Linder (Linder, 1939), *E. viridisepalum* Trappe & Kimbr. (Trappe & Kimbrough, 1972), *E. subviscidus* (Zeller) Trappe & Guzmán (Trappe & Guzmán, 1971), and *E. spinoreticulatus* Zhang & Minter (Zhang & Minter, 1989). A number of other *Elaphomyces* species with European names had been variously reported across North America. Since 2012, 24 new *Elaphomyces* species have been presented from North America by Castellano and colleagues (Castellano et al., 2012b, 2018, 2021; Castellano & Stephens, 2017), primarily from eastern USA.

The rich diversity of *Elaphomyces* species in the eastern portion of

North America, east of the Mississippi River, is attributed to the high diversity of Betulaceae, Pinaceae, and particularly Fagaceae species in this region. In this paper, we extend our knowledge of *Elaphomyces* species from eastern USA and report two new *Elaphomyces* species.

## Materials and methods

Descriptions of macromorphological characters are based on fresh material. 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 (mean) are from 20 ascospores measured from the holotype collection, dimensions include ornamentation. For scanning electron microscopy (SEM), dried ascospores were mounted on aluminum pegs with double-sided tape, coated with gold, and then observed with an AmRay 3300 FE field emission scanning electron microscope (AmRay, Bedford, MA, USA). Specimens are deposited in the following herbaria (Index Herbariorum 2022 [<http://sweetgum.nybg.org/science/ih/>], accessed on 25 Jan. 2022): Cornell University (CUP), University of Florida (FLAS), Instituto Tecnológico de Ciudad Victoria (ITCV), Oregon State University (OSC), and University of Wyoming (RMS).

## Results

During the course of examining thousands of *Elaphomyces* collections during the last 15 years, I have come across numerous undescribed *Elaphomyces* species based on significant quantitative morphological differences. In addition, I have attempted to receive on loan and study as many different previously described *Elaphomyces* species as possible. This background work has enabled me to compile a detailed record of macro- and micro-characters of nearly all described *Elaphomyces* species from either type, authentic or type locality material. The two new *Elaphomyces* species presented below are a recent product of this work.

## Taxonomy

### *Elaphomyces gouldhoytii* Castellano, sp. nov.

[Mycobank ID: MB 843351]

Fig. 1.

**Diagnosis:** *Elaphomyces gouldhoytii* is distinguished from all other species in North America by its black carbonaceous peridium composed of tall warts and the unusually large spores.

**Etymology:** Named in honor of the late Professor Gould Jennings Hoyt Jr. (1922–2012) of Paul Smith's College, New York, forester and outdoorsman extraordinaire that single-handedly instilled in me a

love for forestry and the natural world.

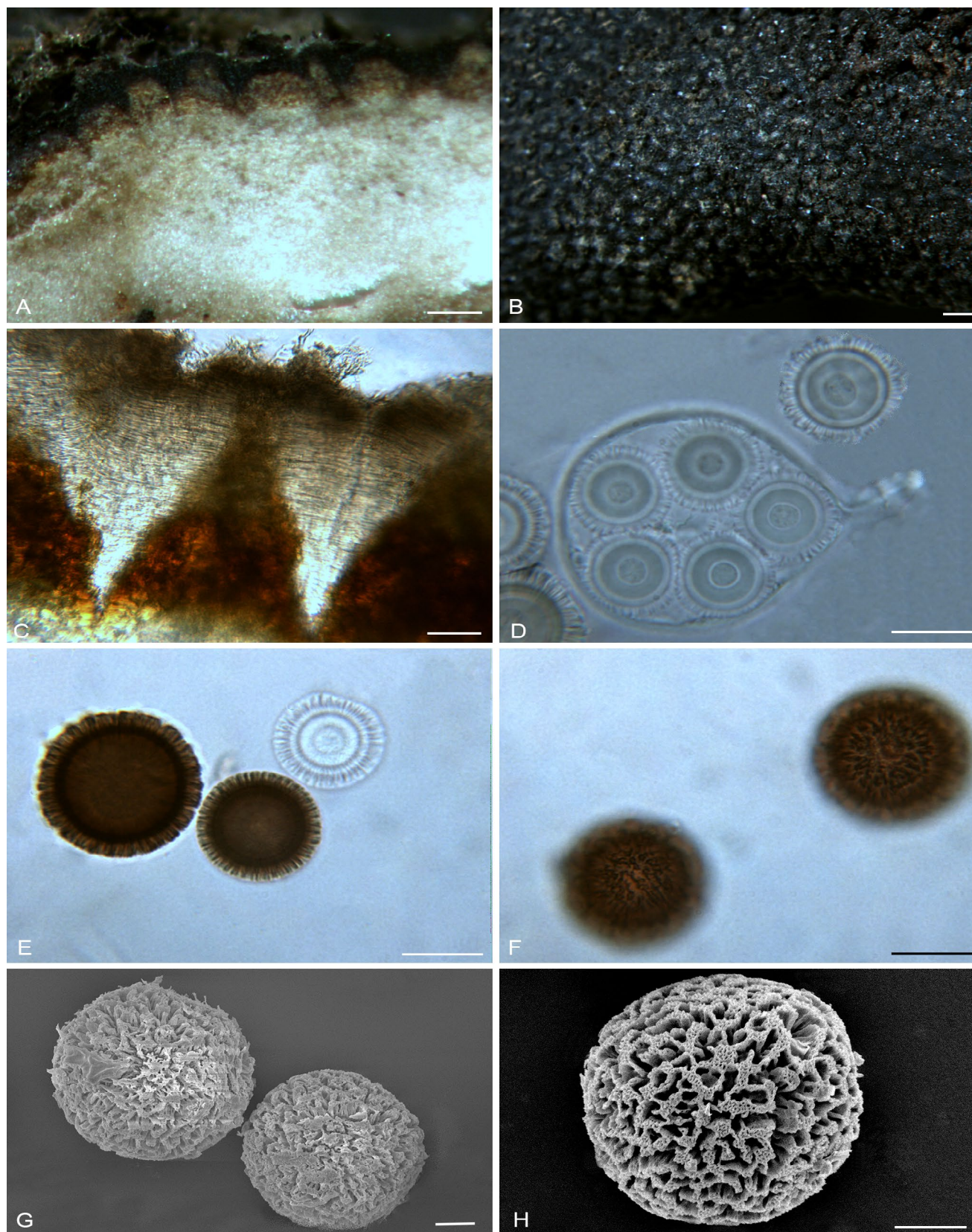
**Type:** USA, New York, Thompsons Co., Danby, Michigan Hollow, 1 Oct. 1980, J.M. Trappe & R. Korf (holotype OSC 40649; isotypes CUP, ITCV).

**Description:** Ascomata subglobose to irregular, up to 15 mm tall, 32 mm broad, completely enveloped in a pale yellow-brown to dark brown mycelium; mycelium not staining. Peridium 2–3 mm thick, outer surface 200–250 µm thick, dark brown, minutely verrucose to naked eye but actually of tall conical warts (200–250 µm tall, 150–275 µm broad) embedded in the mycelium, subcutis 2–2.8 mm thick, uniform, white, leathery, overlain by a thin covering of brown-black to black hyphae to give the appearance of a black peridium and obscuring the details of the warts. Gleba spore mass powdery, dark brown, with white web-like hyphae. Odor fungoid. Taste not recorded. Peridium two-layered: epicutis of septate, disorganized, compact, red-brown to yellow-brown hyphae, 3–4 µm broad, walls ± 1 µm thick, these hyphae grade into the subcutis by the loss of pigment; subcutis of hyaline, septate, compact, elongate, interwoven, cells, up to 7 µm broad, walls 1–2 µm thick; outer hyphal layer is periclinal and of parallel, short-segmented, septate, more or less uniform pale yellow-brown hyphae, 2–4 µm broad. Gleba of spores and hyaline, septate, sinuous to curly smooth hyphae, 2–3 µm broad, walls <0.5 µm thick. Asci globose with a tapered attachment point, 35–45 µm broad, hyaline, 6-spored, walls ± 3 µm thick when young, thinning as spores mature, arising from knots of short, irregularly curved or contorted clustered hyphae, up to 4 µm broad. Spores ornamented, globose, 33–37 µm (mean = 34.9 µm), immature spores abundant and 25–26 µm broad, also numerous dark pigmented small spores 20–25 µm broad; walls ± 1 µm thick, in KOH singly and in mass hyaline at first then dark red-brown when mature, ornamentation a coarse labyrinth of anastomosed rods with holes at the tops of the ridges (only apparent with SEM), 2–3 µm tall.

**Distribution, habit, habitat and season:** Known from Massachusetts, New York, North Carolina, and West Virginia; hypogeous; under *Picea rubra*, *Pinus strobus*, and *Tsuga canadensis*; August to November.

**Other specimens examined:** USA, Massachusetts, Middlesex Co., 3 miles east of Concord, 16 Nov. 1984, J. Trappe (OSC 149097). New York, Essex Co., Newcomb, College of Environmental Science Forest, Huntington Wildlife Forest, Sept. 1989, K.F. LoBuglio 102 (OSC 149095, OSC 149096). North Carolina, Mitchells Co., Pisgah National Forest, Craver's Gap, 30 Aug. 1995, S. Loeb & F. Tainter (OSC 150046); Transylvania Co., Pisgah National Forest, Devil's Courthouse, 15 Aug. 1996, S. Loeb & F. Tainter 130 (OSC 150045). West Virginia, Tucker Co., Blackwater State Park, 12 Oct. 2007, C.





**Fig. 1.** *Elaphomyces gouldhoytii*. A: Ascoma showing peridium in section. B: Ascoma surface showing conical warts embedded in dark brown mycelium. C: Cross-sectional view of the peridium showing the patches of mycelium filling the spaces between warts. D: Immature ascospores with in an ascus. E: Ascospores in cross-sectional view showing the ornamentation of rods and ridges. F: Ascospores in surface view showing the density of 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: OSC 40649 (holotype). Scale bars: A = 2.5 mm, B = 3 mm, C = 90  $\mu$ m, D = 10  $\mu$ m, E, F = 18  $\mu$ m, G = 6  $\mu$ m, H = 9  $\mu$ m.

Stihler (OSC 149093).

***Elaphomyces septatus* collection studied:** FRANCE, Larrau, St. Joseph Forest, 18 May 1985, M. G  rard & F. Lescourrent (OSC 149128).

***Elaphomyces maculatus* collection studied:** FH in the Patouillard herbarium (ex. herbarium O. Mattirollo) marked “Portion autotici C. Vittadini”.

**Note:** *Elaphomyces gouldhoytii* is placed in *Elaphomyces* section *Ceratogaster*, subsection *Sclerodermei* because of the lack of yellow, greenish or purplish mycelial patches on the peridial surface (Paz et al., 2017).

The black carbonaceous peridium composed of tall warts and the unusually large spores of *E. gouldhoytii* clearly separate it from all other North American *Elaphomyces* species. The European *E. maculatus* Vittad. (spores 37–41  $\mu\text{m}$ , mean = 39.1  $\mu\text{m}$ ) and *E. septatus* Vittad. (spores 30–35  $\mu\text{m}$ , mean = 33.0  $\mu\text{m}$ ) have similarly-sized large spores but both of the European species have a smooth, black carbonaceous peridium versus the black tall warts of *E. gouldhoytii*.

Castellano studied *E. septatus* collections from France and Hungary, no authentic or type material of this species could be located. Castellano data (on file at Forestry Sciences Laboratory, Corvallis, Oregon, USA) from OSC 149128 is as follows: Peridial surface black, appearing smooth to the naked eye, covered by dark brown hyphae, spores globose, 30–35  $\mu\text{m}$ , mean = 33.0  $\mu\text{m}$  including ornamentation that appears finely granulate in patches under light microscopy, in section appearing as short spines imbedded in an uneven, lumpy matrix conveying a dark outline to the spore, SEM reveals the spore ornamentation as spines or rods overlain with amorphous, rounded, irregular plates, plate surface roughened.

Castellano studied an *E. maculatus* collection from Italy (FH in the Patouillard herbarium (ex. herbarium O. Mattirollo) marked “Portion autotici C. Vittadini”). Castellano data (on file at Forestry Sciences Laboratory, Corvallis, Oregon, USA) on the FH collection in part is as follows: Peridial surface black, nearly smooth, with tiny, widely spaced, low papillae with floccose, green mycelium, spores globose, (35–)37–41(–42)  $\mu\text{m}$ , mean = 39.1  $\mu\text{m}$  including ornamentation that appears in surface view as a finely wrinkled bumpy surface, in section appearing bumpy under light microscopy, SEM reveals the spore ornamentation as coarse spines, up to 2  $\mu\text{m}$  tall (see Fig. 2g, h in Castellano et al., 2021).

***Elaphomyces ramulospinus* Castellano, sp. nov.**

[Mycobank ID: MB 843352]

**Diagnosis:** *Elaphomyces ramulospinus* is distinguished from all other species in North America by its tall orange-brown peridial warts and relatively small spores (20–25  $\mu\text{m}$ , mean = 25.0  $\mu\text{m}$ ) that are clearly smaller than other *Elaphomyces* in subsection *Muricati* that occur in North America (*E. americanus* Castellano, *E. holstii* Castellano & R.A. Healy, *E. verrucosus* C.W. Dodge, and *E. wazhazhensis* Crabtree & Castellano). The spore ornamentation of tall, forked spines anastomosed to form a fine punctuation to the spore surface is unique in this subsection and clearly differentiate it from other *Elaphomyces* species in Europe.

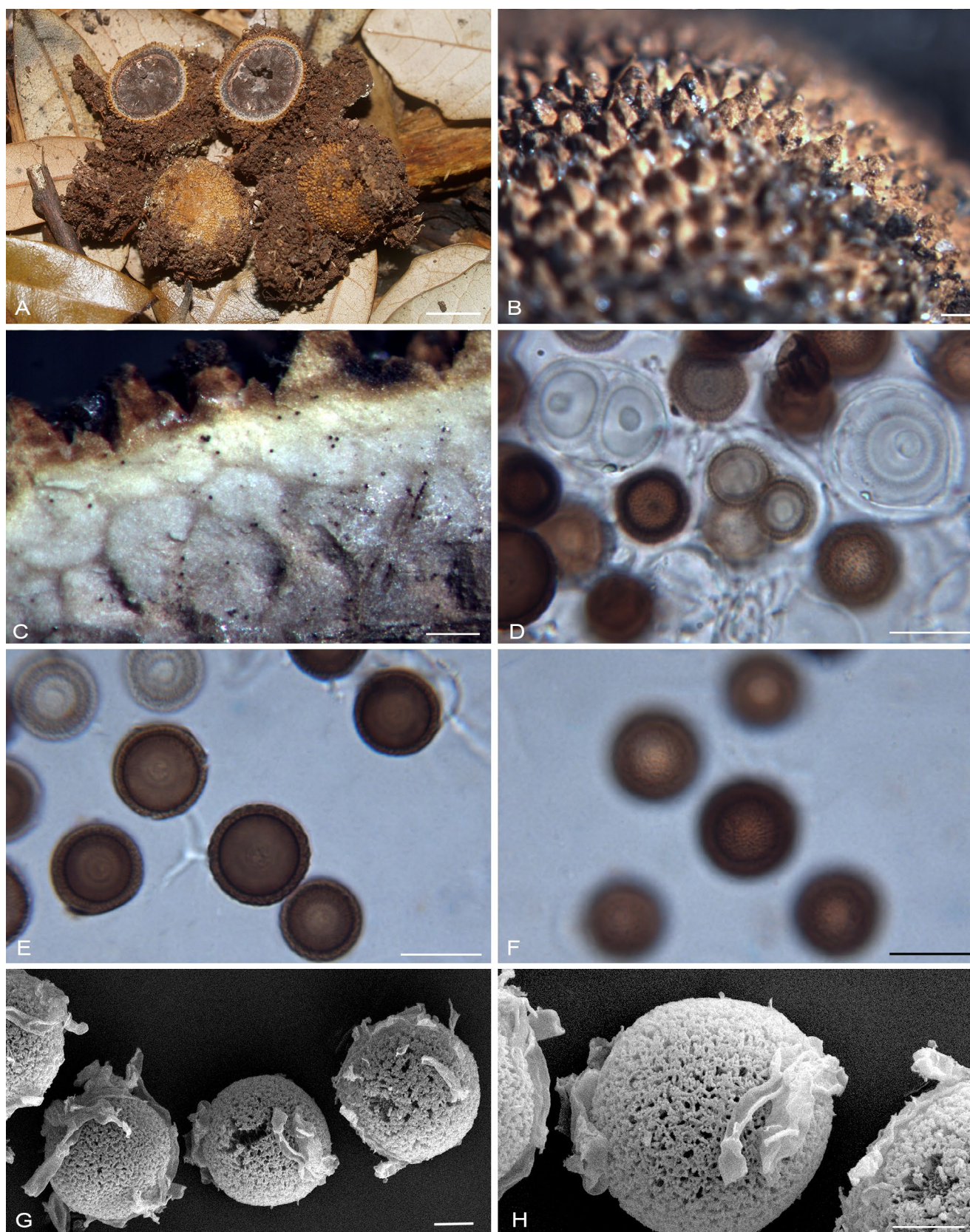
**Etymology:** “ramulospinus” ramul – forked, spinus – spines, in reference to the forked spines of the spores.

**Type:** USA, Florida, Hillsborough Co., Tampa, University of Southern Florida campus, 31 Aug. 1977, J.M. Trappe (holotype OSC 38881).

**Description:** Ascomata irregularly subglobose, up to 9 mm tall  $\times$  30 mm broad. Peridium  $\pm$  2 mm thick, outer surface of orange-brown, pyramidal (4–6 sided) warts, up to 400  $\mu\text{m}$  tall, up to 600  $\mu\text{m}$  wide at base, darker near the tips than at base, contiguous with each other at base, warts not uniform over entire surface, warts are smaller on sides and below, in cross-section the warts are mottled yellow and yellow-brown, more evenly yellow near base, grading from pale white to pale gray where inner marbled area begins; subcutis 1.4–1.6 mm thick, outer portion of subcutis irregularly marbled, matrix somewhat globose-shaped, 330–400  $\mu\text{m}$  broad, paler than inter-venation, veins 30–60  $\mu\text{m}$  broad, slightly but distinctly darker than matrix, inner portion of subcutis distinctly darker overall but of similar structure, darker inner portion 200–225  $\mu\text{m}$  in thickness. Gleba spore mass powdery, dark brown to nearly black, with numerous off-white to pale brown mycelial strands arising from an inner peridial wall, and traversing the powdery spore mass. Odor not recorded. Taste not recorded. Peridium two-layered: epicutis, 400–600  $\mu\text{m}$  thick, of golden brown-yellow compact, septate, disorganized, interwoven hyphae, 4–5  $\mu\text{m}$  broad, walls  $\pm$  2  $\mu\text{m}$  thick, distinctly and abruptly different from interwoven hyphae, interwoven hyphae stacked perpendicular, hyaline, straight, septate, 3–4  $\mu\text{m}$  broad; subcutis 1.4–1.6 mm thick, of hyaline (somewhat brown near gleba), septate, compact, interwoven hyphae, 3–5  $\mu\text{m}$  broad, walls  $\pm$  2  $\mu\text{m}$  thick. Gleba of spores and hyaline, septate, smooth, curly and loosely interwoven hyphae, 1–3  $\mu\text{m}$  broad, walls  $\pm$  1  $\mu\text{m}$  thick. Asci hyaline, walls 2  $\mu\text{m}$  thick, variably sized and shaped depending on spore number within, 1-spored asci globose, 27–33  $\mu\text{m}$  broad, 2-spored asci ovate, 28–31  $\mu\text{m}$  broad, 3-spored asci irregular, 29–35  $\mu\text{m}$  broad, 4-spored asci subglobose, 32–37  $\mu\text{m}$  broad, all arising from clustered knots of large, hyaline, short-segmented hyphae, walls  $\pm$  1

Fig. 2.





**Fig. 2.** *Elaphomyces ramulospinus*. A: Ascomata showing peridial surface of warts and peridium in section showing the powdery spore mass of the gleba. B: Ascoma surface showing the tall pyramidal warts. C: Cross-sectional view of the peridium showing the marbled tissue of the inner peridium. D: Asci showing single, double and four-spored asci. E: Ascospores in cross-sectional view showing the ornamentation of tall forked spines forming a continuous surface. F: Ascospores in surface view showing the fine punctation of the spore surface. G: SEM micrograph of ascospores showing the fine spines that anastomosed to form a fine punctate spore surface. H: SEM micrograph of an ascospore showing composition of the fused spines to form the punctate surface. A: T32037; B–H: OSC 38881 (holotype). Scale bars: A = 15 mm, B, C = 500 µm, D–F = 20 µm, G = 6 µm, H = 7.5 µm.



µm thick. Spores ornamented, globose, highly variable in size (20–25 (–27) µm, mean = 25.0 µm) or (16–18 µm, these spores appear to be immature or aborted), color brown to dark brown in KOH singly and in mass, ornamentation 2–3 µm tall, ornamentation of tall, forked interconnected spines, spore surface appears finely punctate under light microscopy with a distinct dark inner wall.

**Distribution, habit, habitat and season:** Known from Florida and Virginia; hypogeous; under *Betula alleghaniensis*, *B. lenta*, *Quercus hemisphaerica*, *Q. laurifolia*, *Q. prinus*, *Q. rubra*, and *Q. virginiana*; January, February, April, June through September, and November.

**Other specimens examined:** USA, Florida, Alachua Co., Newnans Lake, just south of Windsor, Owens-Illinois County Park, 17 Sept. 1980, J. Trappe (OSC 46707); near Gainesville, 27 Aug. 1977, J. Trappe (OSC 38946); southwest of Gainesville, Sugarfoot Hammock, 13 Jan. 1982, C. Larez (FLAS 54503); Marion Co., Ocala National Forest, Fore Lake, 21 Aug. 1977, J. Trappe (OSC 38882); Polk Co., Ronald Reagan Parkway, just west of Champions Gate Blvd., north side of road next to small pond, 25 Feb. 2012, M. Castellano (OSC 150036); Sumter Co., 4 miles west of Wildwood, 4 Apr. 1947, L.E. Arnold & E. West (FLAS 48170); Wakulla Co., Apalachicola National Forest, Silver Lake Recreation Area, 3 Jan. 2007, D. Mitchell (OSC 150037); Mississippi, Hancock Co., Steenis Space Center, along upper Gainesville rd., 6 Aug. 2007, M.A. Castellano T32037 (OSC); Virginia, Montgomery Co., near Blacksburg, Estes farmland near City Golf course, 20 Nov. 1982, S. Miller 598 (RMS).

**Note:** *Elaphomyces ramulospinus* is placed in *Elaphomyces* section *Elaphomyces* subsection *Muricati* because of the brown warty peridial surface and the marbled inner peridium (Paz et al., 2017).

The brown, warty peridial surface and marbled inner peridium of *E. ramulospinus* resembles all the other species in *Elaphomyces* section *Elaphomyces* subsection *Muricati* except *E. decipiens* Vittad. from Europe. The small spores (20–25 µm, mean = 25.0 µm) of *E. ramulospinus* are clearly smaller than other *Elaphomyces* in subsection *Muricati* that occur in North America (*E. americanus*, *E. holstii*, *E. verrucosus*, and *E. wazhazhensis*). The spore ornamentation of tall, forked spines anastomosed to form a fine punctuation to the spore surface is unique in this subsection and clearly differentiate *E. ramulospinus* from other *Elaphomyces* species in this subsection. Some specimens were parasitized by *Tolypocladium ophioglossoides* (J.F. Gmel.) C.A. Quandt, Kepler & Spatafora.

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of Wyoming, D. Mitchell of West Virginia, C. Stihler of West Virginia, and J.M. Trappe of Corvallis, Oregon. I also appreciate the opportunity to study specimens from the following herbaria: CUP, FLAS, ITCV, OSC, and RMS.

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