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Elaphomyces appalachiensis and E. verruculosus sp. nov. (Ascomycota Eurotiales, Elaphomycetaceae) from eastern North America

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Introduction

Elaphomyces Ness is characterized by having a single, large, empty chamber in youth that soon becomes stuffed with cottony hyphae. At maturity, the chamber is filled with yellow to brown, olive-brown to black or blue to black powdery spore mass (Castellano et al., 1989; Trappe et al., 2009). Elaphomyces species are ecologically important because they form part of the diet of animal mycophagists and are widespread ectomycorrhizal partners to a vast variety of large woody plants (Maser et al., 2008; Trappe et al., 2009).

In Mexico, a variety of species from various sequestrate genera such as Elaphomyces, Gautieria, Genea, Geopora, Glomus, Hydnangium, Hydnobolites, Hymenogaster, Hysterangium, Leucogaster, Macowanites, Melanogaster, Octaviania, Pachyphloehus, Radiigera, Rhizopogon, and Tuber have been reported for the states of Nuevo León, Coahuila, Durango, Tamaulipas and Mexico (Guzmán, 1971; Trappe and Guzmán, 1971; Trappe et al., 1979; Hosford and Trappe, 1980; Guzmán, 1983; Cázares et al., 1992, 2008; Guevara et al., 2008a, 2008b, 2011; Healy et al., 2009). Recently, we examined Elaphomyces collections from northern Mexico deposited at Oregon State University herbarium (OSC) and fresh material and discovered an undescribed species and one Elaphomyces species previously described from a single locality in the eastern United States. This paper is a contribution to the knowledge of the sequestrate mycota of North America.
Materials and methods

Methods of collection and macroscopic and microscopic study were generally those of Castellano et al. (1999, 2003). Colors of fresh fruiting bodies are in general terms by the authors. Dried specimens were hand-cut and mounted in 5% KOH or water for microscopic observation. Twenty spores were measured to estimate range and average size. Spore dimensions are with ornamentation. Herbaria are abbreviated according to Index Herbariorum (2011).

Results and discussion

*Elaphomyces appalachiensis* Linder, J. Elisha Mitchell Sci. Soc. 55: 133. 1939. Figure 1

Ascomata up to 5-8 x 6-10 mm, subglobose to irregular, completely embedded in a lilac to red mycelial mat which forms a husk around individual ascomata and incorporating much soil, ectomycorrhizal roots, and debris; mycelium not staining. Peridium ±1 mm broad, outer surface 200-250 µm broad, a felty layer of pale purple hyphae with embedded ectomycorrhizal roots scattered within, the mesocutis and subcutis pale gray to pale purple. Glebal spore mass, powdery, blue-green, with white, spider-web-like hyphae. Odor not recorded. Taste not recorded.

Peridium three-layered, outer felty layer, 200-250 µm broad, of pale purple to violet, septate, loosely interwoven, thin-walled hyphae 3-5 µm broad, embedded within this layer are scattered ectomycorrhizae; mesocutis ±350 µm broad, of hyaline, septate, compact, thin-walled, interwoven hyphae 8-10 µm broad in bundles or strands; subcutis 150-300 µm broad, of pale brown to pale green-brown, septate, thick-walled (±2 µm broad), compact cells, 10-15 µm broad. Gleba constituted by spores and thin-walled, hyaline, septate, somewhat branched, sinuous hyphae, 3-4 µm broad. Asci globose, hyaline, thick-walled (±1 µm broad), 20-25 µm broad, 8-spored, arising from knots of short, irregular hyphae. Spores globose, (8-) 10-13 µm broad (mean = 10.95 µm); walls ±1 µm broad, in KOH pale olive to yellow-brown singly and in mass when mature, ornamentation of compact rods and short ridges or clumps, ±1 µm tall.

Etymology: “appalachiensis” appalach – Appalachia, *ensis* – pertaining to origin or place, in reference to the locality of the original collection from the Appalachian Mountains of eastern United States of America.
Habit, habitat and season: Known from Iowa south to northern Florida and northeastern Mexico under *Carya glabra*, *C. ovata*, *Pinus pungens*, *P. virginiana*, *Quercus ovata*, or *Q. prinus*; July through early October.


Discussion: Overall the specimens in the holotype collection appear to be somewhat immature. Linder's (1939) specimens were parasitized with *Cordyceps intermedia* S. Imai which would be in keeping with the young age of specimens. Linder also reports the spores as 7.5-9 µm broad. Our spore measurements of the holotype material are (8-) 10-13 µm broad (mean = 10.95 µm) with ornamentation, 7-9 µm broad without ornamentation.

*Elaphomyces appalachiensis* resembles *E. atropurpureus* Vitt. in the enveloping, purple-colored mycelium surrounding the sporocarp. The slightly larger-sized spores of *E. atropurpureus* have a much less dense ornamentation that is readily apparent in surface-view using a light microscope with oil immersion at 1000x.

*Elaphomyces verruculosus* Castellano sp. nov. Figure 2

Mycobank – 563163.

Ascomata are irregularly subglobose to reniform, up to 22 x 27 mm. Peridial outer surface of low, semi-rounded to angular or elongate warts, up to 500 µm wide and 300 µm tall, rounded to acute or even flattened at apex, contiguous with each other at base. Pale yellow-brown when young then mottled brown and yellow-brown when mature with much pale brown, brown or yellow-brown hyphae, soil and debris covering the warts. Warts often obscured by inter wart hyphal structures to make the appearance of the surface nearly smooth or papillate; in section warts are outlined with a layer ±140 µm thick of red-brown to dark red-brown cells underlain
with paler yellow-brown to off-white tissue, 300-350 µm thick. Below at the basis of the warts there is an off-white layer sometimes tinged pale gray-blue; subcutis up to 2000 µm broad, off-white to pale gray, sometimes zoned off-white above and pale gray or pale gray-tan below, this is more readily apparent on mature specimens; often a distinct, brown layer, ±100 µm thick at interface with gleba. This layer is contiguous and concolorous with the dissepiments that engaginate into the gleba. Gleba off-white and cottony when immature then spore mass powdery, dark brown to nearly black when mature, numerous pale gray to brown mycelial strands (dissepiments) arising from inner peridial wall and traversing gleba. Odor indistinct, mild. Taste not recorded. Peridium interwart spaces filled with stacked parallel, hyaline, pale yellow to pale yellow-brown, thick-walled (2-3 µm broad), septate hyphae 3-8 µm broad, also at times covering wart apex; Overall the peridium is two-layered, epicutis is a warty layer, ±450 µm broad, with a red to red-brown layer near wart surface then yellow-brown then grading to off-white near wart base, composed of compact, interwoven hyphae 3-8 µm broad, similar in structure to interwart hyphae and sometimes contiguous in organization but not in color; subcutis up to ±2000 µm broad, also similar in structure to outer interwart layer in structure except hyphae are hyaline with amorphous, hyaline granules or pigments interspersed across this layer, these granules scattered are much less dense near gleba and are dark gray or dark red-brown. Gleba constituted by spores and thick-walled (1-1.5 µm broad), hyaline, septate, smooth or slightly encrusted, sinuous, loosely, interwoven hyphae, up to 5 µm broad. Asci globose, hyaline, thick-walled (±2 µm broad), 42-50 µm broad, (2-) 4-8-spored, arising from ascogenous hyphae of clustered knots of hyaline, thick-walled (±1 µm broad), short-segmented hyphae, ±3 µm broad. Spores globose, (35-) 36-45 (-46) µm broad (mean = 41.1 µm), aborted spores present and are much darker and 23-28 µm broad, mature spores are brown to red-brown to dark red-brown in KOH singly and in mass, ornamentation of tall, hyaline spines or rods when immature (in asci), of dense rods and tufts of rods when mature, 2-3 x 2-3 µm, giving the spore surface a more or less coarse appearance, appearing fuzzy in section, as spores mature they darken and the tufts become darker and more distinct, when mature spores are dark red-brown they appear much more coarse.

Similar to Elaphomyces granulatus Fries of Europe is overall macromorphology with a brown, leathery peridium ornamented with low warts, an inner peridium of off-white to gray-colored tissue and spores ornamented with dense rods and tufts of rods. Elaphomyces granulatus has larger, more coarsely ornamented spores and a uniform, gray inner peridium.

Holotypus: Florida, Walton Co., Eglin Air Force Base, 1 mile west of junction of rd. 403 and rd. 382, 30 m along unnumbered road, 8 Aug. 2007, M. Castellano, Trappe 32038 (OSC 130866).

Etymology: “verruculosus” verruca—warty, losus—low, in reference to the low warts of the peridial surface.

Habit: habitat and season: Known from Quebec, Canada south to Florida and northeastern México; hypogeous under Picea abies, P. rubens, Pinus elliottii, P. palustris, P. pungens, P. resinosa, P. strobus, P. taeda, P. virginiana, and Tsuga canadensis; June through October.


Discussion: Probably the Elaphomyces granulatus of Trappe & Guzmán (1971) and Cázares et al., (1992) is Elaphomyces verruculosus. Elaphomyces verruculosus resembles E. granulatus of Europe in sporocarp characteristics but differs by its smaller spore size (36-45 µm broad, mean = 41.1 µm with spines 2-3 µm) and less coarse ornamentation compared to E. granulatus and by the uniform gray inner peridium of E. granulatus. Elaphomyces granulatus described by Montecchi & Sarasini, (2000) is actually E. leucocarpus Vitt. characterized by the uniform, white inner peridium and smaller, spiny-reticulate spores they describe and illustrate.

Key to the studied and related Elaphomyces species (spore size includes ornamentation)

1a. Ascoma completely embedded in a lilac to red mycelial mat, spores (8) 10-13 (means 10.95 µm), parasitized by Cordyceps intermedia ............... \textit{E. appalachiensis}

1b. Ascoma no embedded in a lilac to red mycelial mat ....... 2

2a. Peridium cross section no marbled ................. 3

2b. Peridium cross section marbled ................. 5

3a. Peridium yellow brown to brown ...................... 4

3b. Peridium with black warts or spines ............. \textit{E. aculeatus}

4a. Spores (35-) 36-45 (-46) µm broad (means 41.1 µm) .......................................................... \textit{E. verruculosus}

4b. Spores 39-40 µm broad .................. \textit{E. granulatus}

5a. Peridium pale, warts on cortex rather flat, spores 19-28 µm diam ........................................ \textit{E. reticulatus}

5b. Peridium dark, warts prominent, spores 18-25 µm diam ........................................ \textit{E. muricatus}

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