Elaphomyces species (Elaphomycetaceae, Eurotiales) from Bartlett Experimental Forest, New Hampshire, USA

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Abstract: We describe five new species of Elaphomyces from Bartlett Experimental Forest, New Hampshire, USA (E. americanus, E. bartlettii, E. macrosporus, E. oreoides, and E. remickii) and revise the description of a sixth previously published species (E. verruculosus). Of the five new species, E. bartlettii and E. remickii are only known from New Hampshire whereas E. americanus, E. macrosporus, and E. oreoides are widely distributed in eastern North America. Elaphomyces verruculosus is the most widespread and abundant Elaphomyces species in eastern North America with a distribution extending from eastern Canada south to northeastern Mexico. All six Elaphomyces species are putatively associated with Tsuga canadensis, a tree species in regional decline. For five of the six Elaphomyces species, we report partially consumed ascomata or rodent fecal samples containing spores, indicating that small mammals play a key role in dispersing these Elaphomyces species and that the Elaphomyces are an important part of the small mammals’ diet.

Key words: Ascomycota, hypogeous fungi, mycophagy, sequestrate fungi, truffles, Tsuga canadensis

White Mountain National Forest

INTRODUCTION

The truffle genus Elaphomyces (Elaphomycetaceae, Eurotiales, Ascomycota) is characterized by a gleba composed of powdery ascospores encased in a thick peridium. The genus is ectomycorrhizal with many vascular plant taxa and occurs in both the Northern and Southern Hemispheres (Castellano et al. 2011, 2012a, b, c, 2016, Reynolds 2011, Trappe et al. 2009). In northeastern North America, the genus is ecologically important because it forms mycorrhizal associations with a diversity of tree species and comprises a substantial portion of the diets of mycophagous mammals (Vernes et al. 2004, Vernes & Poirier 2007). Recent truffle ascomata sampling conducted at Bartlett Experimental Forest, New Hampshire, USA, in stands dominated by eastern hemlock (Tsuga canadensis) and American beech (Fagus grandifolia) indicates a high diversity of Elaphomyces in the Northeast. Using a systematic sampling approach across forest types we sampled 768 4-m² plots, detecting six Elaphomyces species. Here we describe five of the Elaphomyces species as new, and provide a revised description of a sixth previously published species. Of the five new species, E. bartlettii and E. remickii are only known from New Hampshire whereas E. americanus, E. macrosporus, and E. oreoides are widely distributed in eastern North America. Elaphomyces verruculosus was previously described and is the most abundant Elaphomyces species in eastern North America with a distribution extending from eastern Canada south through the eastern USA to northeastern Mexico. All six Elaphomyces species are putatively associated with eastern hemlock, with E. bartlettii and E. macrosporus exclusively associated with that tree. Declines of eastern hemlock throughout the eastern USA, due to the introduced hemlock woolly adelgid, Adelges tsugae (Orwig et al. 2002), may reduce local diversity or abundance of Elaphomyces species. Additionally, we report partially consumed ascomata for four Elaphomyces species (E. americanus, E. bartlettii, E. macrosporus, and E. verruculosus). Moreover, these observations, coupled with rodent fecal samples containing spores of E. bartlettii and E. oreoides, suggest that small mammals and their predators play a key role in dispersing spores and increasing local diversity of Elaphomyces and that small mammals depend in part on the Elaphomyces as a food source.

MATERIALS AND METHODS

Ascomata were collected from 6 June to 5 October 2014 from Bartlett Experimental Forest, Northern Research Station, USDA Forest Service, New Hampshire, USA (44° 3’ 7.2" N, 71° 17’ 25.1” W; 250–435 m elevation). Additional ascomata were collected during directed searches in 2015 and ascomata that had been partially consumed by small mammals were collected opportunistically during 2014 and 2015. We sampled for ascomata on 12 established small mammal-trapping grids (105 x 105 m; 11 025 m²) stratified across hardwood (n = 4), softwood (n = 4), and mixed (n = 4) forest stands (R. Stephens, unpubl.). Within each 11 025 m² grid, we
sampled at 64 plots (4-m²; 16 plots each month) raked to a depth of 10 cm or until mineral soil was reached (Luoma & Frenkel 1991). In total, we sampled 788 4 m² plots across all grids (256 in each forest type). Hardwood forest stands were dominated by American beech, red maple (Acer rubrum), sugar maple (A. saccharum), yellow birch (Betula alleghaniensis), white birch (B. papyrifera), and white ash (Fraxinus americana), whereas softwood stands were dominated by eastern hemlock, red spruce (Picea rubens), and balsam fir (Abies balsamea). The coarse-loamy, well-drained soils are spodosols developed from glacial till and are underlain by granite (Schaller et al. 2010).

Colours are given in general terms, and not by reference to a colour chart. Ascomata were initially air dried in the field and then thoroughly dried for 24–48 h at 60 °C in the laboratory. Dried specimens were rehydrated and examined in 3 % KOH, Melzer’s reagent, and Cotton blue. Microscopic descriptions and micrographs are based on 3 % KOH mounts unless otherwise specified. Twenty ascospores were measured from the holotype collection of each species; dimensions include ornamentation. Dried ascospores from holotypes were mounted on aluminum pegs with double-sided tape and sputter coated with gold for scanning electron microscopy (SEM) with an AmRay 3300 FE field emission scanning electron microscope. Specimens were deposited in OSC (Oregon State University), FLAS (Florida State University), and BPI (US National Fungus Collection, Beltsville, MD).

TAXONOMY

Key to the Elaphomyces species of Bartlett Experimental Forest

|   | Peridial surface yellow to yellow-brown................................................................................................................. | 2 |
|---|---------------------------------------------------------------------------------------------------------------|---|
|   | Peridial surface dark brown to nearly black......................................................................................................... | 4 |
| 2 (1) | Inner layer of peridium marbled............................................................................................................... | E. americanus |
|   | Inner layer of peridium homogen in colour........................................................................................................ | 3 |
| 3 (2) | Gleba medium brown at maturity ........................................................................................................................ | E. remickii |
|   | Gleba dark brown to nearly black at maturity.................................................................................................... | E. verruculosus |
| 4 (1) | Peridial surface with dark yellow-brown spots; spores 41–44 µm diam ........................................................ | E. macrosporus |
|   | Peridial surface an even dark brown to black colour; spores < 31 µm diam......................................................... | 5 |
| 5 (4) | Spore ornamentation a complete reticulum; odour sweet .................................................................................. | E. oreoides |
|   | Spore ornamentation incomplete and slight with scattered smooth areas; odour garlicy, acrid ...................... | E. bartlettii |

Elaphomyces americanus Castellano, sp. nov. MycoBank 816067

(Fig. 1)

Etymology: “americanus”, describing the North American, particularly the eastern portion, distribution of this species.

Diagnosis: Differs from all North American species in the marbled inner peridium and from E. muricatus of Europe by being slightly smaller in overall spore size, having a coarser ornamentation with more lines then rods, and having a taller ornamentation.

Type: USA: West Virginia: Pocahontas Co., Middle Mt., Iron Bridge, 25 July 2001, S. Stephenson 14815 (OSC 81113 – holotype).

Description: Ascomata to 16 mm broad, more or less globose, completely embedded in a pale mycelial mat which forms a poorly to well-developed husk around individual ascomata incorporating soil, ectomycorrhizal roots, and debris; mycelium not staining when handled. Peridium overall 1.5–1.6 mm thick when mature, epicutis 500–600 µm thick, of distinct, yellow-brown warts, most acutely pointed, some blunt, 4–5 sided, bases more or less contiguous, to 500 µm tall, 500–750 µm broad at the base, paler within and at the base, darker along the outline in section; inner layer ±1000 µm thick, marbled with veins which are white to off-white when young to pale brown when mature, matrix pale tan near surface grading to tan to brown to finally dark brown or black near the gleba, overall dark brown when mature, hyphal strands invaginating into the gleba from the inner peridium, pale tan to rose-tan to white to grey-brown, disintegrating at maturity. Gleba spore mass at maturity powdery, dark brown, with tan, spider web-like hyphae, when immature stuffed with bright white mycelium, then as spores form and mature the mycelium becomes off-white to pale grey. Odour indistinct, mild. Taste not recorded.

Peridium two-layered, outer (warty) layer of yellow-brown, compact, curly, contorted, agglutinated hyphae, walls ±2 µm broad, with a low wall of septate, hyaline, parallel hyphae between warts ±7 µm broad, walls ±3 µm thick, beneath the warts is a yellow-brown layer that rapidly grades into hyaline hyphae of a similar texture and size as the warts, then grading into a red-brown layer of similar texture except with amorphous pigmented granules scattered across the
Fig. 1. *Elaphomyces americanus*. A. Ascomata showing peridial surface, gleba, and peridium in section (OSC 150010). B. Ascoma that has been chewed upon by a small mammal (OSC 149877). C. Peridial warts. D. Outer peridial warts with stacked compact hyphae between warts. E. Ascospore in cross-section showing height and pattern of the ornamentation. F. Ascospores in surface view. G. Scanning electron micrograph of ascospores showing variable ornamentation pattern. H. Scanning electron micrograph of an ascospore showing the complex structure to the wart structure. C–H (OSC 8113 – holotype). Bars A = 1 mm, B = 5 mm, C = 500 µm, D–G = 10 µm, H = 5 µm.
layer, these dark areas in the inner layer matrix attributable to numerous pigment granules, macroscopically the entire area is dark making it difficult to determine the transition from white veins to dark veins. *Gleba* of spores and hyaline hyphae that are, smooth, septate, extensively branched, occasionally somewhat curly, loosely interwoven, 2–3 µm broad, walls <0.5 µm thick. Asc globose, hyaline, 42–46 µm broad, walls 3–4 µm thick, 4–8-spored, arising from acrogenous hyphae which are puzzle-like, ±5 mm broad; spores with ascus smooth at first then soon with tall spines. *Ascospores* globose, 28–34 µm (mean = 30.9 µm) diam, ornamented, walls ±1 µm thick, brown in KOH when mature singly and in mass, ornamentation of tufts consisting of convalesced rods, not reticulate, 1.5–2.5 µm tall; with small, apparently aborted, globose spores measuring 16–17.5 µm broad.

**Distribution, habit, habitat and season**: Known from Canada (New Brunswick, Ontario, and Quebec) and the USA (Connecticut, Maine, Massachusetts, Michigan, New York, North Carolina, Tennessee, Virginia, and West Virginia); hypogeous in sandy or clay soils; under Abies balsamea, Picea rubens, Pinus banksiana, P. strobus, and West Virginia); hypogeous in sandy or clay soils; under Abies balsamea, Picea rubens, Pinus banksiana, P. strobus, and West Virginia). montmorency Co., White Rocks Recreation Area, 10 June 1983, S. Miller 666 (OSC 149885); Virginia: Fairfax Co., Oakton, corner of Jermtown rd. and Chain Bridge rd., 12 July 2015, M. Castellano (OSC 149918); Gilles Co., north fork of Stoney Creek, 25 Oct. 1983, S. Miller 714 (OSC 149884); West Virginia: Pocahontas Co., near Cranberry Glades, along Scenic Hwy, 20 Sept. 1981, S. Miller 485 (OSC 149883); Cromer Run, 23 July 2001, S. Stephenson 14822 (OSC 81119); Three Forks of the Williams River, 3 Oct. 1981, S. Miller 0503 (OSC 149886); Randolph Co., Stuart Knob, 30 Oct. 1995, D. Mitchell (OSC 149038); 7 miles from Ekins, Shavers Fork of the Cheat River, 22 Aug. 2007, T. Elliott (OSC 149247); Tucker Co., Moore Run, 6 Aug. 2001, S. Stephenson 14819 (OSC 81116).

- **Canada**: Quebec: St Anaclee de Rimouski, 14 July 1981, C. Godbout (OSC 40569); Montreal, 1 Oct. 1992. F. Marzitelli (OSC 51100); Rawdon, 13 Sept. 1992, F. Marzitelli (OSC 51037); Beaufort, 18 Aug. 1991, J. Trappe (OSC 44265); Baie St. Paul, 19 Aug. 1991, J. Trappe (OSC 40572); Duchesnay, Las Jaune, 24 Aug. 1938, R. Cain (BPI 840813); New Brunswick: Fundy National Park, Upper Salmon River, 3 June 1999, K. Vernes (OSC 62335, 149046, 149047, 149048, 149049, 149051, 149053, 149052); Point Wolfe, 14 July 1999, K. Vernes (OSC 149053, 149045); same data except 24 June 1999 (OSC 149055, 149054); Kinnie Brook, 23 June 1999, K. Vernes (OSC 149056, 149057); same data except 10 July 1999 (OSC 149059, 149058); same data except 12 Aug. 2000 (OSC 149069); Devil's Half Acre, 25 June 1999, K. Vernes (OSC 149065, 149064, 149063, 149062, 149061, 149060); same data except 10 Aug. 2000 (OSC 149066); same data except 12 July 1999 (OSC 149067); same data except 8 Sept. 2000 (OSC 149068); Ontario: Holland River Marsh, 6 May 1936, R. Cain (BPI 840812); Gull Lake, 9 Sept. 1935, R. Cain (BPI 840808).

**Discussion**: The marbled inner peridium separates this species easily from all other *Elaphomyces* species in North America. *Elaphomyces americanus* resembles *E. muricatus* from Europe, but the spores of *E. americanus* have a coarser ornamentation with more lines then rods, are slightly smaller, and have a taller ornamentation. The spores of *E. americanus* enlarge as they mature due to maturation of the ornamentation. The Canadian ascomata examined were younger and the spores in many specimens only 24–28 µm broad. Ascomata with a loose powdery gleba (the most mature specimens) have spores 28–32 µm broad.

Two specimens collected at Bartlett Experimental Forest on 11 Sept. 2015 (OSC 149823) and 21 July 2014 (OSC 149877) were partially consumed by small mammals. Both specimens had been excavated and discarded after consumption of most of the peridium, leaving the gleba exposed. Characteristics of the incisor marks on OSC 149877 indicate consumption by a red squirrel (*Tamiasciurus hudsonicus*) from Europe, but the spores of *E. americanus* have a coarser ornamentation with more lines then rods, are slightly smaller, and have a taller ornamentation. The spores of *E. americanus* enlarge as they mature due to maturation of the ornamentation. The Canadian ascomata examined were younger and the spores in many specimens only 24–28 µm broad. Ascomata with a loose powdery gleba (the most mature specimens) have spores 28–32 µm broad.

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**Elaphomyces species from New Hampshire**

*Elaphomyces* species from New Hampshire

**Description:** Distinguished from *E. leveillei* and *E. morettii* of Europe by the dark brown hyphae that envelope the ascoma and spores which are coarse in ornamentation and 26.5–30 \( \mu m \) in size.

**Type:** USA: New Hampshire: Carroll Co., White Mountain National Forest, Bartlett Experimental Forest, 1.5 km south and 0.25 km east of the intersection of Bear Notch rd. and Hwy 302, 22 June 2015, *R. Stephens & T. Remick* (OSC 149816 – holotype; BPI 893241 – isotype).

**Distribution, habit, habitat and season.** New Hampshire; hypogeous in humic soils, scattered to gregarious; under *Tsuga canadensis*; June through October.

*Other collections examined:* USA: New Hampshire: Carroll Co., White Mountain National Forest, Bartlett Experimental Forest, 1.5 km south and 0.25 km east of the intersection of Bear Notch rd. and Hwy 302, 8 July 2014 (OSC 149820); Strafford Co., Durham, East Foss Farm, 1 km south of the intersection of Mill rd. and Foss Farm rd., 2 Oct. 2016, *R. Stephens & K. Kwasnik* (OSC 150062).

**Discussion:** Immature ascomata of *Elaphomyces bartlettii* have a bright white inner peridium which grades to off-white towards the centre at maturity. The interior is initially hollow, filling with white cottony hyphae, becoming black and powdery as the spores develop and mature. *Elaphomyces bartlettii* bears some resemblance to *E. leveillei* and *E. morettii* from Europe. The ascomata of *E. leveillei*, however, are covered with a greenish mycelium and a green mycelium patch persists at the base after handling. *Elaphomyces leveillei* has similar sized spores but the ornamentation appears smoother in cross-section and under SEM (not shown). The ascomata of *E. morettii* have a brownish red mycelium and spores which are much smaller (17–22 \( \mu m \)) and have a coarser ornamentation.

One specimen collected at Bartlett Experimental Forest on 30 June 2014 (OSC 149818) was partially consumed by a small mammal. The mature ascoma was left in situ at a depth of approximately 7 cm. Soil was excavated to reach the ascoma and animal activity had left the gleba partially exposed. Additionally, spores of *E. bartlettii* were detected in fecal samples from a southern red-backed vole (*Myodes gapperi*) and *T. striatus* live-trapped at Bartlett Experimental Forest. The *M. gapperi* sample was collected on 3 Aug. 2014 just 17 m from OSC 149817, and the *Tamias striatus* sample was collected on 1 July 2014 approximately 90 m from OSC 149820.
Fig. 2. *Elaphomyces bartlettii*. A. Ascomata showing peridial surface, gleba, and peridium in section. B. Ascoma showing small warts on peridial surface. C. Epicutis with the darker outer area grading into a paler inner area. D. Subcutis with abundant amorphous, crystalline particles scattered across the layer. E. Thick-walled, pedicellate ascus. F. Ascospores in surface view showing the clumpy ornamentation. G. Scanning electron micrograph of ascospores showing the labyrinthine pattern to the clumpy ornamentation. H. Scanning electron micrograph showing the fine detail of the ornamentation. A–H (OSC 149816 – holotype). Bars A, B = 10 mm, C–E = 20 μm, F, G = 10 μm, H = 5 μm.
**Elaphomyces species from New Hampshire**

***Elaphomyces macrosorus*** Castellano & T.F. Elliott, sp. nov.

**MycoBank** 816070

(Fig. 3)

**Etymology:** "macrosorus" macro – large, sporus – spore, in reference to the large spores.

**Diagnosis:** This species differs from other *Elaphomyces* species by its large spores which are ornamented with rods and clumps of rods that form broad based cones or ridges and the black hyphae that cover the brown peridial warts.

**Type:** **USA:** West Virginia: Tucker Co., Canaan Heights, along Hwy 32, south of Davis, 6 July 2015, M. Castellano (OSC 149891 – holotype).

**Description:** Ascomata to 2 cm broad, irregular, often completely enveloped in a dark sordid yellow mycelium, organic debris, and ectomycorrhizal roots, occasionally ascomata are dislodged from the substrate without adherent mycelium, debris and roots; mycelium not staining when handled; base with yellow mycelial tuft. **Peridium** to 1000 µm thick, outer surface of yellow-brown warts ±200 µm tall by 160–200 µm broad at the base, warts embedded in a black mycelium to give the appearance of a black ascoma with dark yellow-brown dots that are the tips of the warts emerging from the black mycelium, subcutis ±800 µm thick, uniform, grey, leathery, homogeneous (not marbled). **Gleba** spore mass powdery, dark brown to black at maturity, with dark spider web-like hyphae, when immature gleba of stuffed bright white hyphae and pale brown tissue. **Odour** initially a faint hint of citrus, increasing as ascomata begin to decay underground. **Taste** not recorded.

**Peridium** two-layered, epicutis, ±200 µm thick, of compact, septate, yellow-brown, disorganized, compact hyphae, ±5 mm broad, walls ±1 µm thick; subcutis ±800 µm thick, composed of hyaline, septate, compact, interwoven hyphae, to 10 µm broad, walls ±1 µm thick; also an ill-defined inner layer of pale tan, loosely interwoven hyphae, to 4 mm broad near the gleba. **Asci** subglobose to globose with a stipitate base, 48–53 µm broad, walls ±2.5 µm thick, hyaline, 4- or 8-spored, stipitate base to 11 µm long. **Ascospores** globose, (39.5–) 41–44(–45.5) µm (mean = 42.2 µm) diam., ornamented, walls ±1 µm thick, in KOH singly and in mass dark brown to red-brown when mature, ornamentation of rods and clumps of rods that form broad based cones or ridges, to 3 µm tall, immature spores abundant, dense, dark red-brown, 20-30 mm broad.

**Distribution, habit and habitat and season:** Known from Maine, Massachusetts, New Hampshire, North Carolina, and West Virginia; hypogeous, usually clustered; under *Tsuga* canadensis; June through November.

**Collections examined:** **USA:** Maine: Cumberland Co., Bradbury Mountain State Park, at campground, 17 Oct. 2015, R. Stephens (OSC 149827); **Massachusetts:** Hampshire Co., University of Massachusetts campus, near Brown Dormitory, 10 Aug. 1986, M. Castellano (OSC 149882); Worcester Co., Wachusett Mountain, 16 Nov. 1984, J. Trappe (OSC 149112); **New Hampshire:** Carroll Co., White Mountain National Forest, Bartlett Experimental Forest, 1.5 km south and 0.25 east of the intersection of Bear Notch rd. and Hwy 302, 17 June 2014, R. Stephens & T. Remick (OSC 149795); same data except 2.25 km south and 0.75 east of the intersection of Bear Notch rd. and Hwy 302, 20 June 2014 (OSC 149796); same data except 3 Aug. 2015 (OSC 149826); same data except 19 June 2014 (FLAS-F59192); same data except 2.8 km south and 0.06 west of the intersection of Bear Notch rd. and Hwy 302, 14 Sept. 2014 (OSC 149803); same data except 2 Aug. 2015 (OSC 149825); same data except 2 Aug. 2015 (BPI 893243); same data except 2.25 km south and 0.8 west of the intersection of Bear Notch rd. and Hwy 302. 4 Aug. 2014 (OSC 149802); same data except 2 km south and 1.4 km west of the intersection of Bear Notch rd. and Hwy 302, 28 Sept. 2014 (OSC 149800); same data except 3 km south and 1.7 km west of the intersection of Bear Notch rd. and Hwy 302, 31 July 2014 (OSC 149799); same data except 21 Aug. 2014 (OSC 149801); Strafford Co., Durham, College Woods, 13.5 metres north of the intersection of Mill rd. and Hemlock Way, 29 Sept. 2015, R. Stephens & T. Remick (OSC 149828); **North Carolina:** Haywood, Co., Nantahala National Forest, Buckeye Creek, 13 Aug. 1996, S. Loeb & F.H. Tainter (OSC 149251); Mitchell Co., Pisgah National Forest, Carver’s Gap, 19 Sept. 1995, S. Loeb & F.H. Tainter (OSC 149252); Transylvania Co., Pisgah National Forest, near Bold, trail to Pink Beds, 27 Nov. 2011, T. Elliott (OSC 149894, 149895); Pisgah National Forest, Devil’s Courthouse, 31 Aug. 1995, S. Loeb & F.H. Tainter (OSC 149258); **West Virginia:** Pocahontas Co., Monongahela National Forest, along FS rd. 44, 30 July 2013, C. Diggins (OSC 149113); Randolph Co., Kumbraw Officer State Forest, Oxley Creek, 18 July 2013, C. Diggins (OSC 149200); same data, 22 July 2013 (OSC 149115); same data, 16 July 2013 (OSC 149114); Tucker Co., Blackwater State Park, near lodge, between parking lot and road, 6 July 2015, M. Castellano (OSC 149889, 149890); Canaan Heights, along Hwy 32, south of Davis, 6 July 2015, M. Castellano (OSC 149892, 149893).

**Discussion:** “Elaphomyces macrosorus nom. prov.” was listed by Beug et al. (2014) and had been previously identified as *E. leveillei* by Loeb et al. (2000) from North Carolina. The peridial surface of *Elaphomyces macrosorus* resembles that of *E. aculeatus* from Europe which has much smaller spores (21–24 µm broad). The peridial surface and spore ornamentation of *E. spinoreticulatus* (Zhang & Minter 1989) is also similar to *E. macrosorus* but *E. spinoreticulatus* spores are demonstrably smaller (35–38, mean = 36.1 µm) than *E. macrosorus* spores (41–44, mean = 42.2 µm). *Elaphomyces macrosorus* is also similar (macroscopically and in spore characteristics) to *E. verruculosus* and both occur in eastern North America. *Elaphomyces verruculosus* lacks any black mycelium surrounding the brown peridial warts and on average the spores are smaller (36–45 µm, mean = 41.1 µm broad) and have a more coarse appearance. Immature ascomata of *E. macrosorus* have a bright white inner peridium which grades to pale tan towards the gleba at maturity, often creating a two-tone appearance. The gleba of immature ascomata is solid and white, becoming black and powdery as the spores mature.

Two specimens collected at Bartlett Experimental Forest on 2 Aug. 2014 (OSC 149825) and 3 Aug. 2015 were partially consumed by small mammals. The peridium of OSC 149825 (maturing specimen) was partially consumed. The soil was...
Fig. 3. *Elaphomyces macrosporus*. A. Ascomata showing peridial surface, gleba, and peridium in section. B. Ascoma showing peridial warts embedded in dark hyphae. C. Microscopic view of sectioned outer peridium showing the pale, stacked hyphae between the darker wart tissue. D. Subcutis showing the thick-walled, interwoven hyphae. E. Ascospores in surface view showing the ornamentation pattern. F. Ascospores in cross-sectional view showing the height and pattern of the ornamentation. G. Scanning electron micrograph of ascospores. H. Scanning electron micrograph of ascospores showing the fine detail of the ornamentation. A, B (OSC 149890); C–F (OSC 149891 – holotype); G, H (OSC 149802). Bars A = 1 cm, B = 5 mm, C–G = 20 µm, H = 10 µm.
excavated to reach the ascoma at a depth of approximately 3 cm; the ascoma was left in situ. The peridium and gleba of OSC 149826 (immature specimen) were consumed and left in situ at approximately 3 cm.

**Elaphomyces oreoides** Castellano, sp. nov.
MycoBank 816071
(Fig. 4)

_Etymology._ “oreoides”— resembling Oreo, in reference to the resemblance of the profile of an Oreo cookie with three inner peridial layers, dark/light/dark, in addition to the slightly sweet odor.

**Diagnosis:** Differs from other eastern North American species of the genus by having yellow-brown spores with a complete reticulum ornamentation and the inclusion of ectomycorrhizas within the lower portion of the inner peridium. It differs from the _E. persoonii_ of Europe by the brown mycelium surrounding the ascoma.

_Type_ **USA:** _North Carolina:_ Buncombe Co., milepost 385.5 along Blue Ridge Parkway, 4 Sept. 1994, M. Castellano, Z. Miller & W. Miller (OSC 58219 – holotype).

_Description:_ Ascomata to 22 x 27 mm diam, subglobose, often with broad irregular pits, flattened to somewhat turbinate, with a persistent, large basal tuft, to 19 mm broad where attached, 6–7 mm long, composed of a tangle of dark yellowish grey to brown mycelium, ectomycorrhizal roots, soil particles, and debris; dense pale brown to dark brown to greyish black mycelium adherent to the entire peridial surface; mycelium not staining when handled, unchanged when dried. _Peridium_ subcartilaginous, 1.2–3.0 mm thick, four-layered, somewhat zonate with a very dark outer layer underlain by a medium-dark layer grading into a more or less hyaline layer with occasional pale tan streaks, underlain by another dark hyphal layer adjacent to the gleba; outer surface 200–250 µm thick, very dark brown, carbonaceous, of mostly flattened, irregularly shaped or plate-like warts, to 0.5 mm broad at the base, warts simplex in structure, warts larger at the ascoma apex, smaller along the sides and near the base, often nearly smooth near the base; three inner layers 1–2.75 mm thick combined, inner layers uneven in thickness, zonate to somewhat mottled, the middle layer off-white to pale tan, exterior of the inner layers brown; numerous ectomycorrhizas embedded within the inner two layers closest to the gleba, concentrated in the lower half of the specimen, more numerous at the base, absent at the ascoma apex. _Gleba_ spore mass powdery, very dark blue-grey, off-white, spider web-like hyphae invaginating from the inner peridial layer into the gleba towards the centre, thicker patches of off-white hyphae scattered around the gleba-peridium interface from which the spider web-like hyphae emerge. Odour slightly sweet. Taste not recorded.

_Peridium_ four-layered, outer layer 200–250 µm thick, of dark brown, short-segmented, compact hyphae, 3–7 µm broad, to 20 µm long, walls 1–2 µm broad, grading into the first inner layer, 250–500 µm thick, composed of brown, compact hyphae, 3–7 µm broad, arranged in a _textura intricata_, walls ±1 µm thick; middle inner layer, 250–1000 µm thick, composed of hyaline, compact hyphae, 2–3 µm broad, arranged in a _textura porrecta_, walls <0.5 µm thick, occasional streaks of pale brown hyphae present in this middle inner layer; third inner layer (closest to the gleba) 500–2000 µm thick, composed of brown, compact hyphae, to 10 µm broad, arranged in a _textura epidermoidea_, walls ±1 µm broad, somewhat restricted in diameter at the septa. _Gleba_ composed of spores and thin-walled, hyaline, septate, sinuous to curly, slightly encrusted hyphae, ±4 µm broad. _Asci_ globose to irregularly globose (from pressure of enlarging spores), 65–80 µm broad, 0.5 µm thick, 8-spored. _Ascospores_ globose, (27–)28–30(–31) µm (mean = 29.3 µm) diam, ornamented, walls ±1 µm thick, in KOH singly and in mass yellow-brown when mature, ornamentation a complete reticulum with coarse projections at the top of the alveoli, to 2 µm tall, alveoli to 5 µm broad.

_Distribution, habit, habitat and season:_ Known from New Hampshire, North Carolina, and West Virginia; hypogeous, single to clustered; under _Betula alleghaniensis_, _Carya sp._, _Fagus grandifolia_, _Gymnocladus dioicus_, _Pinus resinosa_, _P. strobus_, _Quercus prinus_, _Q. ruber_, _Q. virginiana_, and _Tsuga canadensis_; June, September, and October.

_Collections examined:_ **USA:** _New Hampshire:_ Carroll Co., White Mountain National Forest, Bartlett Experimental Forest, 2.25 km south and 0.8 west of the intersection of Bear Notch rd. and Hwy 302, 8 June 2014, R. Stephens & T. Remick (OSC 149798, FLAS-F59191); _North Carolina:_ Buncombe Co., Mountain to Sea trail, 1 Sept. 1991, W. Sturgeon (OSC 149105); milepost 383.5 along Blue Ridge Parkway, 4 Sept. 1994, M. Castellano, J. Roberts, G. Semlak, J. Shubzda & P. Perret (T13589); Haywood Co., Great Smokey Mountain National Park, near summit of Purchase Knob, 22 Sept. 2012, T. Elliott (OSC 149100); Rutherford Co., off Painters Gap rd., 23 Sept. 2007, T. Elliott (OSC 149248); _West Virginia:_ Barbour Co., Laurel Mountain, 16 Oct. 1997, D. Mitchell 597 (OSC 149102).

_Discussion:_ _Elaphomyces oreoides_ resembles _E. persoonii_ from Europe in the possession of a basal tuft of mycelium, a dark brown to black, warty peridium and reticulate spores. _Elaphomyces persoonii_ has a green mycelium covering the ascomata whereas _E. oreoides_ has a brown mycelium.

_Spores of _E. oreoides_ were detected in the fecal samples of two woodland jumping mice ( _Napaeozapus insignis_ ) live-trapped at Bartlett Experimental Forest. These _N. insignis_ were trapped on 4 June 2014 and 30 July 2014 on the same grid where the ascomata of _E. oreoides_ (OSC 149798, FLAS-F59191) was collected. One additional collection, OSC 149405, is parasitized by an unidentified _Tolypocladium_ species.

Beug _et al._ (2014) list “ _E. fallax_ nom. prov.” which is actually this species.
Fig. 4. Elaphomyces oreoides. A. Ascomata showing peridial surface, gleba, and peridium in section (OSC 149248, photo T.F. Elliott). B. Surface of the peridium showing the warts. C. Ectomycorrhizas embedded within the inner peridium. D. Outer peridium showing the gradation of the dark-colored outer hyphae into the paler colored inner hyphae. E. Ascospores with ornamentation in surface view showing the reticulate pattern of the ornamentation. F. Ascospores in cross-sectional view showing the height and pattern of the ornamentation. G. Scanning electron micrograph of ascospores. H. Scanning electron micrograph of ascospores showing the fine detail of the ornamentation. B–H (OSC 149979). Bars A = 20 mm, B, C = 500 µm, D = 20 µm, E–G= 10 µm, H = 5 µm.
Elaphomyces remickii Castellano & R.B. Stephens, sp. nov.
MycoBank 816072
(Fig. 5)

Etymology: Named for Tyler J. Remick, undergraduate student who aided R. Stephens immensely with collection and preparation of mycological samples at Bartlett Experimental Forest.

Diagnosis: Distinguished by the medium brown gleba, unusual in the genus, at maturity, tall pyramidal warts on peridial surface, and the hyaline to pale tan spore colour at maturity.

Type: USA: New Hampshire: Carroll Co., White Mountain National Forest, Bartlett Experimental Forest, 1.5 km south and 0.25 km east of the intersection of Bear Notch rd. and Hwy 302, 15 July 2014, R. Stephens & T. Remick (OSC 149814 – holotype).

Description: Ascomata subglobose, to 20 mm broad x 12 mm tall. Peridium overall 1250–1850 µm thick when mature, in surface view wart tips variably-shaped, emerging from dense, brown hyphal layer between warts, warts acute, brown, pyramidal-shaped overall, bases contiguous, base to 250–300 µm broad, many smaller, in section warts red-brown in outline, yellow at the base, with abundant, brown hyphae packed between the warts; inner layer homogeneous (not marbled), 1.0–1.5 mm thick in places, mostly off-white but occasionally somewhat tan near gleba, pale tan hyphal strands invaginating into the gleba from the inner peridium. Gleba spore mass powdery, medium brown, scattered, white, hyphal strands invaginating from the inner peridium into the gleba and reaching the centre. Odour not recorded. Taste not recorded.

Peridium two-layered, outer (warty) layer of yellow-red-brown, compact, septeate, agglutinated, stacked hyphae forming a textura prismaticà, 6–8.5 µm broad, walls 2–3 µm broad, hyphae between the warts arranged in a tightly stacked manner, sometimes perpendicular to the hyphae within warts, of hyaline, septeate, compact hyphae, 3–4 µm broad, walls ±1 µm thick, inner layer a textura epidermoidea, of hyaline, compact, tangled hyphae, 4–5 µm broad, walls ±1 µm broad. Gleba of spores of hyaline, smooth, septeate, extensively branched, loosely interwoven hyphae, 2–4 µm broad, walls 0.5 µm thick. Ascii not seen. Ascospores globose, 31–35 µm (mean = 33.2 µm) diam, ornamented, walls ±1 µm thick, in KOH hyaline to pale tan at maturity singly and in mass, ornamentation to 1 µm tall, appearing as a compact undulate surface, in cross-sectional view lumpy; in SEM appearing as a coarse, rugose spore surface.

Distribution, habit, habitat and season: Known only from a single locality in New Hampshire; hypogeous in humic soils, clustered; under Abies balsamea, Betula alleghaniensis, or Tsuga canadensis; July.

Discussion: Macromorphological characteristics of Elaphomyces remickii are unusual in the genus due to the medium brown, powdery spore mass and the tall, pyramidal warts of the peridial surface.

Elaphomyces verruculosus Castellano, Rev. Mex. de Micol. 35: 19 (2012).
(Fig. 6)

Description: Ascomata irregularly subglobose to reniform, to 22 x 27 mm. Peridial outer surface of semi-rounded to angular or elongate warts, to 500 µm wide and 300 µm tall, rounded to acute or even flattened at the apices, contiguous with each other at the 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 making the surface appear nearly smooth or papillate; in section warts are outlined with a ±140 µm thick layer of red-brown to dark red-brown cells underlain with paler yellow-brown to off-white tissue, 300–350 µm thick. Below at the base of the warts there is an off-white layer sometimes tinged pale grey-blue; subcutis to 2 mm broad, off-white to pale grey, uniform (not marbled) or sometimes zoned off-white above and pale grey or pale grey-tan below, more readily apparent on mature specimens; often a distinct, brown layer, ±100 µm thick present at the interface with the gleba. This layer is contiguous and concolorous with the dissepiments that invaginate into the gleba. Gleba off-white and cottony when immature, then the spore mass becoming powdery, dark brown to nearly black when mature, numerous pale grey to brown mycelial strands arising from the inner peridial wall and traversing the gleba. Odour indistinct to musky or skunky. Taste of mild cultivated mushrooms.

Peridium inter-wart spaces filled with hyaline, pale yellow to pale yellow-brown, septeate, parallel hyphae 3–8 µm broad, walls 2–3 µm thick, also at times covering the wart apices; overall the peridium is two-layered, epicutis a warty layer, ±450 µm broad, with a red to red-brown layer near the wart surface then yellow-brown then grading to off-white near the wart base, composed of compact, interwoven hyphae 3-8 µm broad, similar in structure to the inter-wart hyphae and sometimes contiguous in organization but not in colour; subcutis to ±2 mm broad, also similar in structure to the outer inter-wart layer except that the hyphae are hyaline with amorphous, hyaline granules or pigments interspersed across this layer, the scattered granules much less dense near the gleba and dark grey or dark red-brown. Gleba constituted of spores and hyaline, septeate, smooth or slightly encrusted, sinuous, loosely interwoven hyphae, to 5 µm broad, walls 1–1.5 µm thick. Ascii globose, hyaline, 42–50 µm broad, (2)–4–8-spored, walls ±2 µm thick, arising from acrogenous hyphae of clustered knots of hyaline, short-segmented hyphae, ±3 µm broad, walls ±1 µm thick. Ascospores globose, (35–)36–45(–46) µm broad (mean = 41.1 µm) diam, ornamented; in KOH brown to red-brown to dark red-brown singly and in mass; walls ±1 µm thick; 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, mature spores dark red-brown and appearing much more coarse, aborted spores (23–28 µm broad) present and much darker.
Fig. 5. *Elaphomyces remickii*. A. Ascomata showing gleba and peridium in section. B. Outer peridial layer showing the dark coloured tissue of the warts embedded within pale stacked hyphae. C. Pale, thick-walled, stacked hyphae occurring between wart tissue. D. Inner peridial tissue forming a *textura epidermoidea*. E. Ascospores with ornamentation in surface view showing the undulate pattern of the ornamentation. F. Ascospores in cross-sectional view showing the height and pattern of the ornamentation. G. Scanning electron micrograph of ascospores. H. Scanning electron micrograph of ascospores showing the fine detail of the ornamentation. A–H (OSC 149814 – holotype). Bars A = 10 mm, B = 50 µm, C = 10 µm, D–G = 20 µm, H = 10 µm.
Fig. 6. Elaphomyces verruculosus. A. Ascomata showing peridial surface, gleba, and peridium in section (OSC 150012, photo T.F. Elliott). B. Ascomata embedded in ectomycorrhizas, soil, and debris; shown in surface and cross-section view (OSC 150013). C. Ascoma showing the peridial surface in finer detail. D. Outer peridium showing the pale, stacked hyphae between the darker coloured wart tissue. E. Ascospores with ornamentation in surface view showing the rods and spines of the ornamentation. F. Ascospores in cross-sectional view showing the height and pattern of the ornamentation. G. Scanning electron micrograph of ascospores showing the fine detail of the ornamentation. H. Scanning electron micrographof ascospores. D–F (OSC 49670); C, G, H (OSC 48377). Bars A = 20 mm, B = 15 mm, C = 5 mm, D = 30 µm, E–F = 20 µm, G = 1 µm, H = 10 µm.
Distribution, habit, habitat and season: Known from Quebec, Canada south to Florida and northeastern Mexico; hypogeous, single to clustered; under Fagus grandifolia, Picea abies, P. rubens, Pinus elliottii, P. palustris., P. pungens, P. resinosa, P. strobus, P. taeda, P. virginiana, and Tsuga canadensis; June through October but also found once in December.

Collections examined: USA: New Hampshire: Carroll Co., White Mountain National Forest, Bartlett Experimental Forest, 2.5 km south and 1.7 km west of the intersection of Bear Notch rd. and Hwy 302, 4 Oct. 2014, R. Stephens & T. Remick (OSC 149806, 149807, 149808); same data except (OSC 149879); same data except 2.8 km south and 0.06 km west of the intersection of Bear Notch rd. and Hwy 302, 2 Aug. 2014 (OSC 149809); same data except 6 June 2014 (BPI 893249); same data except 2.5 km south and 0.5 km east of the intersection of Bear Notch rd. and Hwy 302, 15 Aug. 2014 (OSC 149810); same data except 2.8 km south and 0.35 km west of the intersection of Bear Notch rd. and Hwy 302, 31 July 2014 (OSC 149831); same data except 2.25 km south and 0.8 km west of the intersection of Bear Notch rd. and Hwy 302, 8 July 2014 (FLAS-F59193); Rockingham Co., Kwaks Sanctuary, Audubon Society land on Route 152, 1.5 miles west of intersection with Route 108, in Newmarket, 26 Dec. 2015, R. Stephens & K. Kwasnik (OSC 150009).

Discussion: This species was described by Castellano et al. (2012b) from Connecticut, Georgia, Louisiana, Massachusetts, Mississippi, New York, North Carolina, Vermont, Virginia, West Virginia, northeastern Mexico, and Quebec in Canada. This is the most commonly encountered and abundant Elaphomyces species in eastern North America.

On a sampling grid dominated by old growth Tsuga canadensis at Bartlett Experimental Forest, 80 % (51 of 64) of the 4 m²-plots had ≥ 1 ascomata (collections including: OSC 149806, 149807, 149808, 149879). On another grid, one sampling plot had 173 sporocarps in 4 m² (BPI 893249). The abundance of this species may make it an important food source for small mammals. Vernes & Poirier (2007) found E. verruculosus cashed by a Tamias Hudsonicus in a bird’s nest in New Brunswick, Canada. We found an air-drying ascoma (OSC 149831) on a broken-off snag which was putatively placed there by a T. hudsonicus based on the incisor marks. Additionally, we found a single ascoma on 21 June 2014 (OSC 149879) with incisor marks from a vole or mouse-sized small mammal.

The warty, brown, leathery peridial surface and off-white to pale grey, homogeneous inner peridial layer (occasionally tinged bluish) in cross-section clearly distinguish E. verruculosus from other Elaphomyces species in North America. It is similar to E. granulatus from Europe, but E. granulatus has somewhat smaller spores (35–41µm, mean = 37.9 µm) and a coarser pattern to the ornamentation of agglutinated rods and spines that form larger patches. The statements about spore size in E. granulatus in Castellano et al. (2012b) are erroneous.

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