



Elaphomyces anthracinus and *E. septatus*, two new hypogeous ascomycete records for Greek mycota

Vasileios KAOUNAS[✉]

Greek Mushroom Society, Sokratous 58, Artemis Attica, TK 19016, Greece
*bkaounas@gmail.com

Received : 01.12.2023
Accepted : 25.01.2024
Online : 23.02.2024

Elaphomyces anthracinus and *E. septatus*, Yunanistan mikotası için iki yeni toprakaltı askomiset kaydı

Abstract: *Elaphomyces anthracinus* Vittad. and *E. septatus* Vittad., two seemingly rare hypogeous ascomycete, are reported for the first time in Greece. This paper provides the descriptions of the recorded collections, accompanied by images of their macroscopical and microscopical features.

Key words: *Ascomycota*, mycodiversity, hypogeous macrofungi, new record

Özet: Nadir olduğu değerlendirilen iki toprak altı askomiset olan *Elaphomyces anthracinus* Vittad. ve *E. septatus* Vittad., Yunanistan'da ilk kez rapor edilmiştir. Bu çalışma kaydedilen örneklerin betimlemelerini, makroskopik ve mikroskopik özelliklerine ilişkin görüntüleriyle birlikte vermektedir.

Anahtar Kelimeler: *Ascomycota*, mikoçeşitlilik, toprakaltı mantarları, yeni kayıt

Citation: Kaounas V (2024). *Elaphomyces anthracinus* and *E. septatus*, two new hypogeous ascomycete records for Greek mycota. Anatolian Journal of Botany 8(1): 30-33.

1. Introduction

Elaphomyces species have a cosmopolitan distribution. They grow in forests ranging from tropical to temperate climates, as well as subarctic coniferous forests. They are formed on the roots of many trees and bushes, a symbiotic relationship typical of ectomycorrhizal fungi Castellano et al. (2018). Most of the species of this genus grow below the ground, but some of them are semi-hypogeous. Their ascomata range from spherical to sub-spherical, and can be either fleshy or leathery. They have a single chamber consisting of a powdery spore mass during maturity. Ascospores are primarily spherical or polyhedral such as the form presented in this paper and the asci are spherical or sub-spherical. Only two *Elaphomyces* species, *E. muricatus* Fr., (Diamandis and Perlerou, 2008; Konstantinidis and Kaounas, 2012) and *E. roseolus* Setkos, Kaounas, A. Paz, Lavoise & Fern. Rodr., Paz et al. (2017), were previously known to exist in Greece.

In this paper, a third *Elaphomyces anthracinus* Vittad. and a fourth *E. septatus* Vittad. member of the genus in Greece are introduced, based on collections from Zagori district of Ioannina province and Retine district of Pieria province respectively. According to the existing check-list of Greek ascomycetous macrofungi by Zervakis et al. (1999) and a number of recent studies reporting hypogeous ascomycetes (Diamandis and Perlerou, 2008; Kaounas et al., 2011; Gyosheva et al., 2012; Konstantinidis and Kaounas, 2012, 2014), these two species have not been previously reported in Greece. This study aims to make a contribution to the mycobiota of Greece

2. Materials and Method

Fruit bodies of *E. anthracinus* were collected from Zagori

district of Ioannina province while *E. septatus* ascoma was collected from Retine district of Pieria. As they were discovered by truffle hunters, no photographs of them in their natural habitat exist, although notes were taken about the morphological and ecological characteristics. The specimens were later carried to private fungarium, and dried in an air conditioned room. Microscopic studies were performed on dried specimens under a AmScope T120C-E5-3PL trinocular light microscope. The specimens were submerged in water and Melzer reagent. Spore dimensions were obtained from measurement of 30 random, mature spores using Piximetre 5.10 software. The specimens were identified with the help of Gori (2005), Arroyo et al. (2005), Montecchi and Sarasini (2000), Uzun and Kaya (2019), Uzun (2021) and Paz et al. (2017). The nomenclature follows mainly "Index Fungorum" (<http://www.indexfungorum.org>). Where VK is the initials of the author and the collection code. The samples included in this study are kept in the author's private fungarium.

3. Results

Ascomycota Caval.-Sm.

Elaphomycetaceae Tul. ex Paol.

Elaphomyces anthracinus Vittad., Monogr. Tuberc. (Milano): 66 (1831).

Syn: [*Elaphomyces anthracinus* f. *talosporus* A. Paz & Lavoise; *Lycoperdastrum anthracinum* (Vittad.) Kuntze]

Macroscopic and microscopic features: Ascoma hypogeous, globose to subglobose, at times compressed and broadly umbilicated, with a diameter of 1-2 cm (Fig. 1a). Hard and leathery, but also brittle. It is black with a thick peridium, consisting of two layers visible with naked

eye. The outer layer is carbon-black, hard, brittle, slightly granular or smooth, 0.3-0.7 mm thick. It consists of short hyphae of 20-40 µm long and up to 6 µm thick, with dark brown to black coloured walls of 1-2.5 µm thickness (Fig. 1b). The inner layer is whiteish or pale grey in colour, 1-2 mm thick, consisting of interconnected pale yellow or hyaline, septate, sometimes branched hyphae of 3-6 µm thick (Fig. 1c). Gleba is at first white and cottony, consisting of fine, branched hyphae 1.5-3 µm thick, black at maturity, consisting of spore powder. It gave a weak, indistinct odour. Asci were not observed due to maturity. Ascospores 18.4-24.6 × 17.6-24.3 µm, are initially hyaline, yellow and brown, almost black at maturity, globose to

almost polyhedric with up to six faces and rounded corners (Fig. 1d-e). With thick walls, surface decorated with an abundance of striations which are highly variable in morphology but of equal length (0.6-1.2 µm), leaving deep and irregular cracks between them, spore size based on n = 30 spores. Bears fruit in winter and spring.

Elaphomyces anthracinus forma *talosporus* grows in soil and decaying leaves under deciduous or coniferous trees, commonly under *Castanea sativa* Mill., *Fagus sylvatica* L., *Coryllus avellana* L., *Tilia cordata* Mill., *Quercus robur* L., *Betula pendula* Roth and *Pinus sylvestris* L. (Paz et al., 2017).

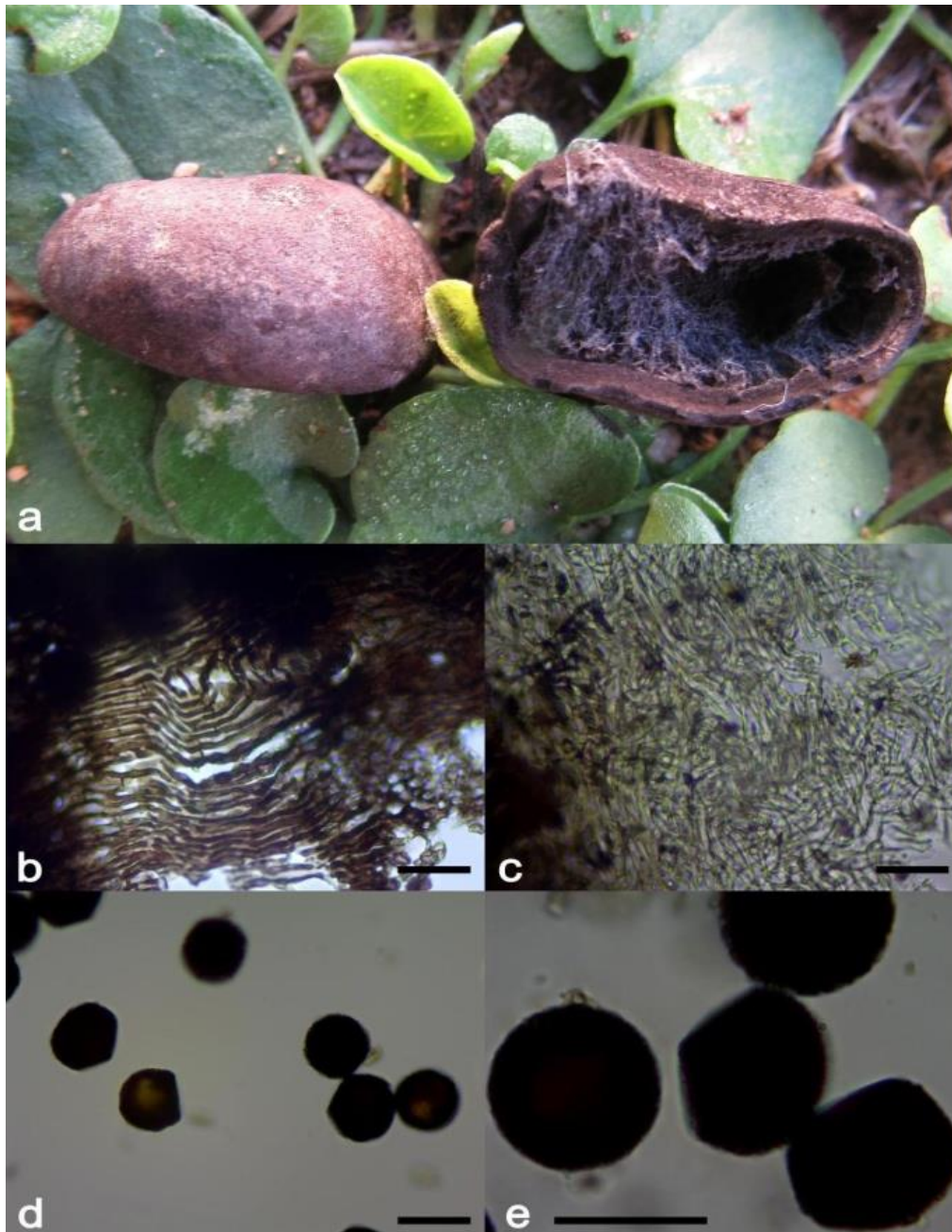


Figure 1. Dried ascocarp (a), outer peridium (b), inner peridium (c), and ascospores (d,e) of *Elaphomyces anthracinus* (bars: 20 µm)

Specimen examined: Greece, Ioannina, Zagori, in soil among decaying leaves under *Quercus* sp., *Carpinus orientalis* Mill. and *Pinus* sp., 03.11.2023, Leg: Niaros V, Det: Kaounas V, VK7360.

Elaphomyces septatus Vittad., Monogr. Tuberc. (Milano): 67 (1831).

Syn: [*Lycoperdastrum septatum* (Vittad.) Kuntze]

Macroscopic and microscopic features: Ascoma small, 20 mm in diameter, subglobose, almost smooth to slightly grainy, without basal protrusion, blackish brown. Surface without intertwined mycelial hyphae and with only very few remnants of the substrate. Hard and covered with pale brown septate hyphae of 2.5-7.5 μm thick, and readily severed when handled (Fig. 2a,b). Peridium approximately 2 mm thick, dual-layered. Outer layer 420-480 μm thick, concolorous with the surface, consisting of brown-blackish hyphae of 4-13 μm thick, and walls of 1-2.5 μm thick, arranged in multiple directions (Fig. 2c). Inner layer >800 μm thick, grey-black, consisting of hyaline hyphae of similar dimensions and arrangement (Fig. 2d). Gleba is white when young, cream with pink tones, and pulverized

powder at maturity. Odour weak, not distinctive. While asci were not observed, ascospores 29.2-33.7 \times 28.4-32.6 μm , including ornamentation, spherical, hyaline, pale grey at first, then greyish to brownish grey at maturity, spore size based on $n = 30$ spores. Yellow with Melzer (Fig. 2e), covered with quite thin spines of 2-3.5 μm long.

Elaphomyces septatus grows in deciduous montane forests from autumn to spring, under *Quercus* L., *Fagus* L., *Carpinus* Mill. (Vittadini, 1831; Vidal, 2000).

Specimen examined: Greece, Pieria, Retine, in soil among decaying leaves under *Carpinus orientalis* Mill., *Platanus orientalis* L. and *Ruscus aculeatus* L. 20.10.2020, Leg: Gougoulianis D., Det: Kaounas V, VK5940.

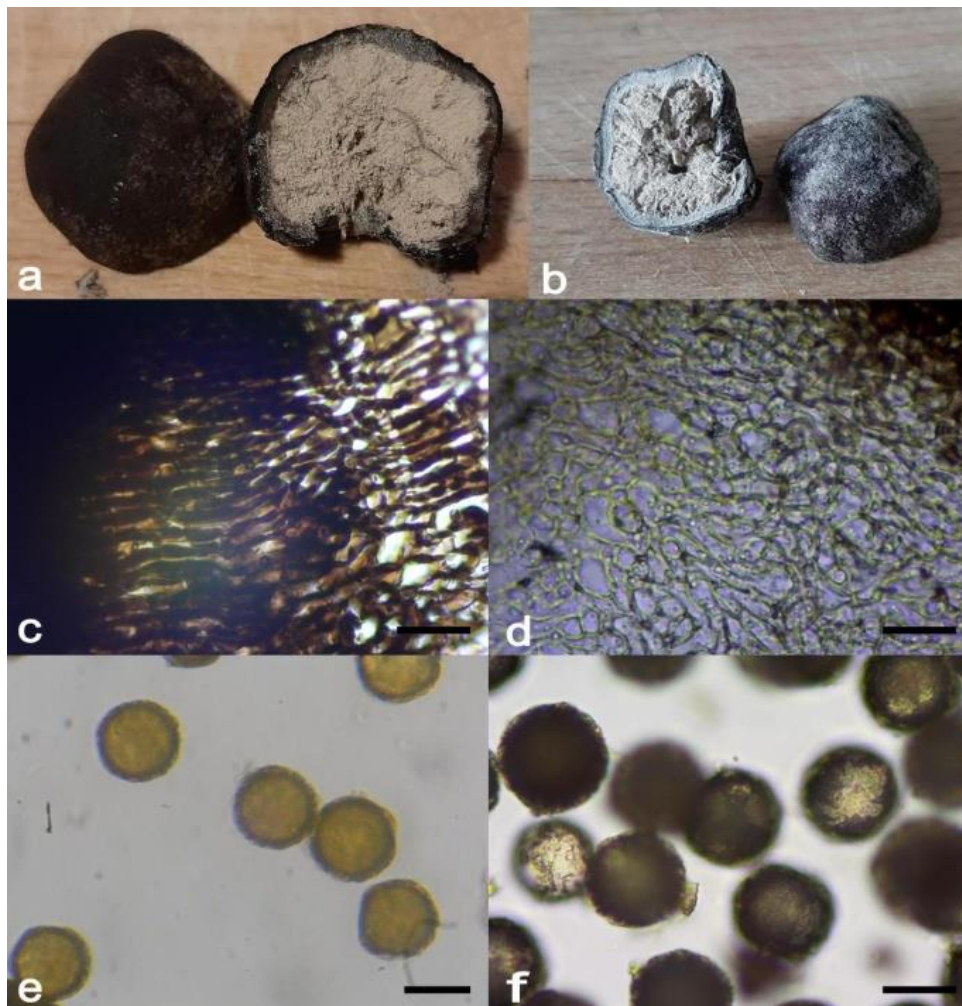


Figure 2. Fresh (a) and dried (b) ascocarp, outer peridium (c), inner peridium (d) and ascospores (e,f) of *Elaphomyces septatus* (bars: 20 μm) foto: Gougoulianis

4. Discussions

The polyhedric shaped ascospores (Fig. 1d-e), leaves no doubt that the specimen is an *E. anthracinus* forma *talosporus*, as described by Paz et al. (2017). The collections, cited as forma *talosporus*, that is quite easily distinguished from typical *E. anthracinus* by several microscopical differences are not distinguishable by either ITS or 28S (Paz et al., 2017). Asci were not observed due to maturity of the specimen, but Paz et al. (2017) report that the asci of forma *talosporus* are spherical, thin-walled and transient with 2-5 ascospores.

Elaphomyces septatus has ascomata similar to *E. maculatus* Vittad. but differs in the smaller size and pale coloration of the ascospores until maturity. Two other characteristics that distinguish it from *E. maculatus* are the absence of green mycelium and of basal protrusion. Another species with pale ascospores is *E. leucosporus* Vittad., however it is smaller than 1 cm, covered with a greenish layer of mycelium and has smaller ascospores of 18-20 μm in diameter.

The macro and micromorphological characters of our samples fit in with the literature. Therefore *Elaphomyces anthracinus* and *Elaphomyces septatus* are new records for

Greek mycobiota. As far as the previous Greek collections of *Elaphomyces* are concerned, *E. muricatus* has a macroscopic difference in the peridium while *E. roseolus* has much smaller ascospores.

Acknowledgments

The author would like to thank the truffle hunters, Gougoulanis Dimitris and Niaros Vaggelis for providing the samples for study.

References

- Arroyo BM, Fernández JG, Calmaestra EP (2005). Tesoros de nuestro montes. Trufas de Andalucía. Córdoba: Consejería de Medio Ambiente, Junta de Andalucía.
- Castellano MA, Elliott TF, Trappe JM (2018). Three new black *Elaphomyces* species (*Elaphomycetaceae*, *Eurotiales*, *Ascomycota*) from eastern North America with notes on selected European species. *Fungal Systematics and Evolution* 1: 1-12.
- Diamandis S, Perlerou C (2008). Recent records of hypogeous fungi in Greece. *Acta Mycologica* 43(2): 139-142.
- Gori L (2005). Funghi Ipogei della Lucchesia di Altre Province Italiane e dell'Estero. Lucchessa: Pacini Fazzi.
- Gyosheva M, Assyov B, Konstantinidis G, Stoykov D (2012). Collections of *Tuber macrosporum* from the Balkan Peninsula (Bulgaria and Greece). *Ascomycete.org*, 4(4): 75-78.
- Index Fungorum. <http://www.indexfungorum.org/names/Names.asp> / [accessed 29 November 2023].
- Kaounas V, Assyov B, Alvarado P (2011). New data on hypogeous fungi from Greece with special reference to *Wakefieldia macrospora* (*Hymenogastraceae*, *Agaricales*) and *Geopora clausa* (*Pyronemataceae*, *Pezizales*). *Mycologia Balcanica* 8: 105-113.
- Konstantinidis G, Kaounas V (2012). *Elaphomyces muricatus* and *Fischerula macrospora*, two interesting hypogeous fungi from Greece. *Ascomycete.org* 4(5) : 95-98.
- Konstantinidis G, Kaounas V (2014). *Hydnotrya tulasnei* and *Mattiolomyces terfezioides* (*Pezizales*) two hypogeous fungi that rarely appear in Greece. *Ascomycete.org* 6(1): 1-4.
- Montecchi A, Sarasini M (2000). *Funghi Ipogei D'Europa*. Vicenza: Fondazione Centro Studi Micologici dell'AMB.
- Paz A, Bellanger JM, Lavoise C, Molia A, Ławrynowicz M, Larsson E, Ibarguren IO, Jeppson M, Læssøe T, Sauve M, Richard F, Moreau PA (2017). The genus *Elaphomyces* (*Ascomycota*, *Eurotiales*): a ribosomal DNA-based phylogeny and revised systematics of European 'deer truffles'. *Persoonia* 38: 197-239.
- Uzun Y (2021). *Elaphomyces anthracinus*, a new hypogeous ascomycete record for Turkish mycota. *Anatolian Journal of Botany* 5(1): 29-31.
- Uzun Y, Kaya A (2019). A new *Elaphomyces* record for Turkey. *The Journal of Fungus* 10(1): 40-43.
- Vidal JM (2000). Dos hipogeos de Carlo Vittadini a menudo confundidos: *Elaphomyces maculatus* y *Elaphomyces septatus*. *Bulletin Semestriel de la Federation des Associations Mycologiques Mediterraneennes* 18: 119-126.
- Vittadini C (1831). *Monographia Tuberacearum*. Milano: Rusconi.
- Zervakis G, Lizoň P, Dimou DM, Polemis E (1999). Annotated check-list of the Greek macrofungi: II. *Ascomycotina*. *Mycotaxon* 72: 487-506.