

## ***Elaphomyces muricatus* (Ascomycota), a new record for hypogeous fungi in Armenia**

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**Key words:** *Ascomycetes*, *Elaphomyces muricatus*, *E. granulatus*. – Hypogeous fungi, mycorrhizal fungi, biodiversity, ecology. – Mycobiota of Armenia.

**Abstract:** *Elaphomyces muricatus*, new for Armenia, and *E. granulatus* have been found in the forests of Vanadzor and Dilijan in Armenia. Morphology, anatomy, as well as ecology and geographical distribution of *E. muricatus* are described. The ecological adaptation and geographical distribution of the genus *Elaphomyces* are wider than known before.

**Zusammenfassung:** *Elaphomyces muricatus*, neu für Armenien, und *E. granulatus* wurden in den Wäldern von Wanadzor und Dilijan in Armenien gefunden. Morphologie, Anatomie sowie Ökologie und geografische Verbreitung von *E. muricatus* werden beschrieben. Die ökologische Anpassungsfähigkeit und geografische Verbreitung der Gattung *Elaphomyces* ist größer als bisher bekannt.

**Аннотация:** *Elaphomyces muricatus*, новый для Армении гриб, и *E. granulatus* были обнаружены в лесах Ванадзора и Дилижана в Армении. Морфология, анатомия, а также экология и географическое распространение *E. muricatus* описаны. Род *Elaphomyces* имеет более широкую экологическую адаптацию и географическое распространение, чем было известно раньше.

The biocycle of the hypogeous fungi, including formation and maturing of fruitbodies, occurs underground and spores are dispersed passively after destruction of fruitbodies and via animals. Hypogeous fungi include species from *Ascomycota*, *Basidiomycota*, *Zygomycota*, *Glomeromycota* and mitosporic fungi. They mostly are mycorrhizal; the main symbionts are wide spread trees, such as species of *Quercus*, *Fagus*, *Corylus*, *Carpinus*, *Populus*, *Salix*, *Tilia*, and *Pinus*. Many hypogeous fungi are valuable edible species (e.g. *Tuber* spp., *Choiromyces meandriformis*, *Terfezia leonis*, *Rhizopogon* spp.). Some of them have medicinal features and other uses, for example *Elaphomyces muricatus*, the fungus discussed below, is noted to have sacral use in Mexico (GUZMÁN 2008) and was used as aphrodisiac in Europe (HAWKER 1954).

The following hypogeous fungi have been found in Armenia so far: *Tuber aestivum* VITTAD. (TASLAKHCHYAN & NANAGULYAN 1988, NANAGULYAN & TASLAKHCHYAN 1991); *T. rufum* PICCO, *T. rapaeodorum* TUL. & C. TUL., *T. scruposum* R. HESSE (BADALYAN & al. 2005); *Tirmania pinoyi* (MAIRE) MALENÇON, *Hydnотrya tulasnei* (BERK.) BERK. & BROOME, *Picoa juniperi* VITTAD., *Elaphomyces granulatus* FR. (NANAGULYAN & TASLAKHCHYAN 1991); *E. muricatus* FR. (only shortly noted in HOVSEPYAN 2006) (*Ascomycota*); *Hymenogaster griseus* VITTAD., *H. olivaceus* VITTAD., *Octaviania stephensii* (BERK.) TUL. & C. TUL. (HOVSEPYAN 2006); *Rhizopogon roseolus* (CORDA) TH. FR. (MELIK-KHACHATRYAN & NANAGULYAN 1983); *R. luteolus* FR. (MELIK-KHACHATRYAN & MARTIROSYAN 1971); *Cremeo-*

*gaster klikae* MATTIR. (NANAGULYAN 1987, NANAGULYAN & OSIPYAN 2000) (*Basidiomycota*); and *Glomus macrocarpum* TUL. & C. TUL. (HOVSEPYAN 2006) (*Glomeromycota*).

*Elaphomyces muricatus* will be introduced below with details.

## Materials and methods

The fruitbodies of *Elaphomyces*, herbarium of host plants, and soil samples were collected, and notes of ecological conditions were recorded during field investigations at the forests of Armenia in 2003-2005. Objects for field observations were hypogaeal fruitbodies, vegetation and soil covers, host plants, geographical and environmental conditions of the localities for hypogaeal fungi. Fruitbodies of hypogaeal fungi were found by digging around of roots of the potential host plants. The affiliation to genetic horizon, soil layer, thickness of litter layer, vegetation cover and position (depth) of fruitbody were recorded for each soil sample. Types of soils were preliminarily identified in the field. Some geographical parameters (microrelief) also were recorded during field works.

Light microscopes were used for micromorphological and anatomical studies, identifications, description and biometry (magnification:  $\times 100$ -1350). Illustrations of fruitbodies were made with scanner or digital camera, microphotographs with digital camera and microscopes.

Identification of the fruitbodies was carried out with HAWKER (1954), LAWRYNOWICZ (1988) and PEGLER & al. (1993). Taxonomy and names of authors of species are given according to [www.indexfungorum.org](http://www.indexfungorum.org). Plant species have been identified with the "Flora of Armenia" (TAKHTAJAN 1954-2010).

The first stages of this study were done in the Scientific centre of soil science, agrochemistry and melioration MA of Armenia and in the Faculty of Biology, Yerevan State University.

**Specimens examined** (Herbarium acronyms are given in [ ]: ERHM = Yerevan State University Cryptogamic Herbarium, FBBL = Fungal Biology and Biotechnology Lab of Yerevan State University, CMI-Unibo = Center of Mycology of Bologna University):

***Elaphomyces muricatus*:** Armenia: district Vanadzor, locality Vanadzor-1, eastward from "Tsitsernak Children's Camp", 01. 06. 2003, leg. & det.: R. HOVSEPYAN, specimens no: V1a-q [FBBL], 2189 [CMI-Unibo]; - idem, 12. 07. 2004, specimen no: 10812 [ERHM]. – Dilijan, locality Dilijan-5, from right bank of Kalavan river, south from Kalavan village, broad-leaved forest with domination of *Carpinus*, 22.07.2005, leg. & det. R. HOVSEPYAN, specimens no: 10813 [ERHM], D35a-b [FBBL].

***Elaphomyces granulatus*:** Armenia: Vanadzor, locality Vanadzor-2, right bank of Vanadzor river, park of *Pinus*, 01. 06. 2003, leg. & det. R. HOVSEPYAN, specimen no: V2 [FBBL].

## Description of morphology and anatomy of the specimens found in Armenia

### *Elaphomyces muricatus* FR. forma *muricatus*

**Etymology:** *Elaphomyces*, from Greek ελαφος, a slag and μυκης, a fungus; from Latin "muricatus", roughened with short, hard points. English name: Rough-coated deer truffle.

**Ascomata:** hypogaeous cleistothecia, globose or subglobose, sometimes slightly depressed, commonly 5-20 mm in diam., firm, hard when dry, yellowish-ochraceous or yellowish-brown, usually darker and duller with age, the surface densely covered with warts (Fig. 1 a).

**Smell:** slight, not distinctive.

**Peridium:** firm, 1.2-2.0 mm thick, two-layered. External layer (cortex) with small pyramidal warts formed by densely interwoven hyphae, c. 0.5 mm thick, dark yellowish-brown. Warts in average 164  $\mu$ m high and (246-)287(-328)  $\mu$ m wide at base.

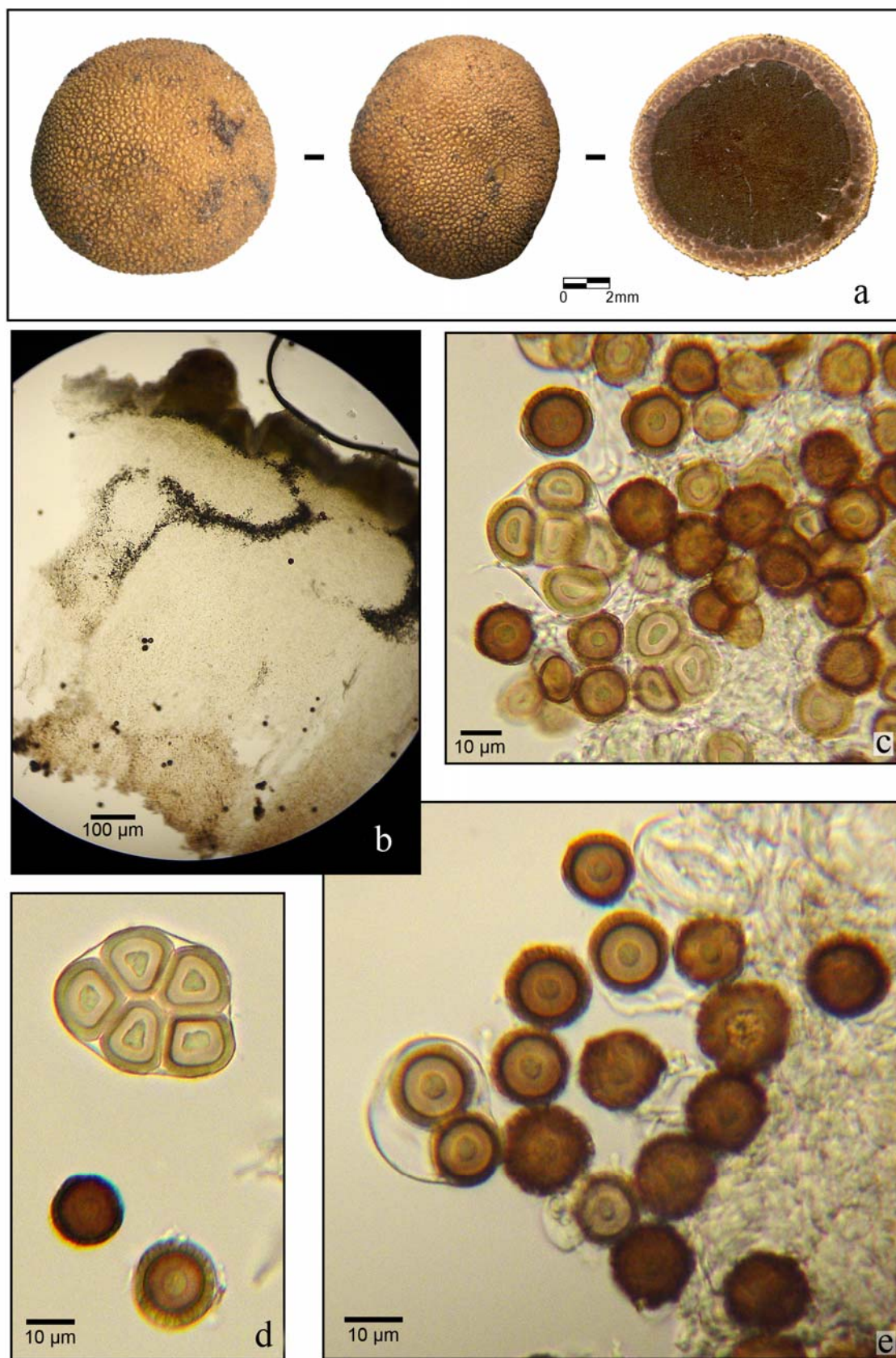


Fig. 1. *Elaphomyces muricatus* found in *Fagus* and *Carpinus* forest in the valley of Vanadzor river (Vanadzor-1, 01. 06. 2003); a ascocarp, b peridium, c-e asci and ascospores.



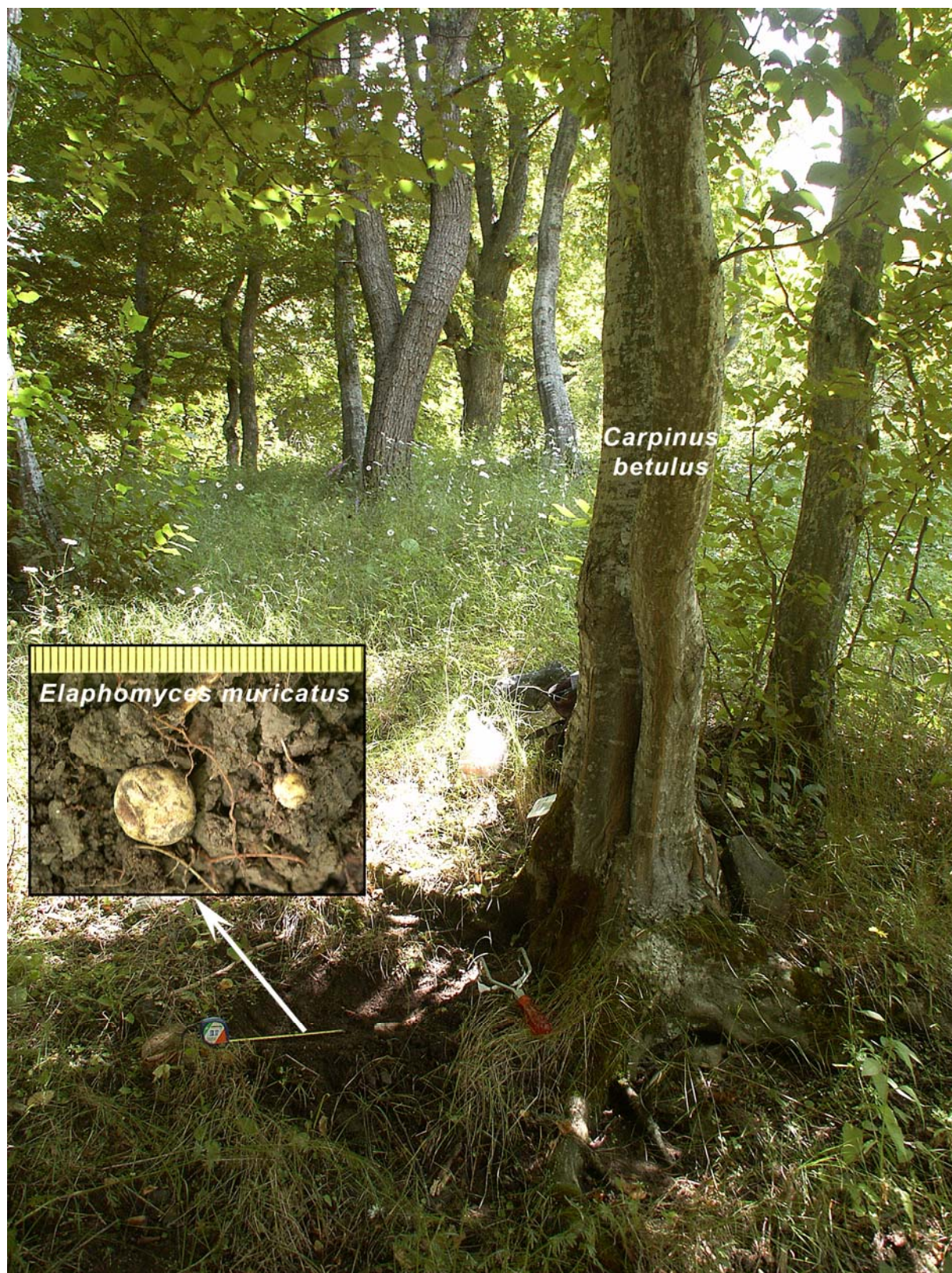


Fig. 2. Habitus of *Elaphomyces muricatus* in the valley of Kalavan river (Dilijan-5, 22. 07. 2005).



Internal layer also consisting of interwoven hyphae, c. 1 mm thick, relatively light coloured, yellowish, distinctly marbled when mature (Fig. 1 b).

**Gleba:** with single cavity, at first empty, later permeated with ascogenous hyphae, and at maturity filled with a powdery mass of blackish-brown spores. Ascogenous hyphae visible as thin and fine white loosely woven veins originating from periphery and going to the centre (Fig. 1 a).

**Asci:** subglobose, widely ellipsoid or irregular, 30-60 µm, thin-walled, evanescent, 2,3,5-spored (Fig. 1 c-e).

**Ascospores:** globose, (8-)10-20(-26) µm in diam., at first hyaline, yellowish-brown, becoming dark brown to blackish and almost opaque in transmitted light at maturity, thick-walled, ornamented with closely spaced blunt spines or rods 2-3 µm high, irregularly arranged into blocks and variable in appearance (Fig. 1 c-e).

### ***Elaphomyces granulatus* FR.**

One old and partly decomposed fruitbody of *Elaphomyces* was found in a *Pinus* park at Vanadzor river valley (Location: Vanadzor-2; Sample: V2 [FBBL]), which seems to be *E. granulatus*. The fruitbodies of *E. muricatus* are similar in appearance to those of *E. granulatus* but may be readily distinguished by the marbled peridium, smaller spores (*E. muricatus* - 10-25 µm, *E. granulatus* - 20-35 µm) and different number of spores in the asci (*E. muricatus* - 2-6, *E. granulatus* - 6-8; HAWKER 1954, PEGLER & al. 1993; LAWRYNOWICZ 1988).

### **Description and ecology of the *Elaphomyces* localities in Armenia**

Fruitbodies of *Elaphomyces* were found at three places (named Vanadzor-1, Vanadzor-2 and Dilijan-5) in the middle mountainous zone forests of North-Eastern Armenia. Type of soil corresponded to the desaturated subtype of mountainous-sylvan gray type soils, see Soils map of Republic of Armenia (EDILYAN 1990); also verified by pedological analyses (HOVSEPYAN 2004, 2006). All localities are in the moderate cold forest climatic zone. Winter is long and snowy with frequent frosts. In Vanadzor the middle air temperature in winter is from - 5.0 °C to -0.4 °C, and in Dilijan it is from - 2.1 °C to +1.2 °C. Spring also is long and humid. Summer is mild with dominance of warm weathers. The middle air temperature in summer varies from +14.7 °C to +19.3 °C in Vanadzor and from +15.3 °C to +19.6 °C in Dilijan. Autumn is cold and rainy in the second half. In Vanadzor and Dilijan annual precipitations are c. 570 mm. Average monthly precipitation in summer is 72 mm in Vanadzor and 68 mm in Dilijan (BAGHDASARYAN 1962).

Vanadzor-1 locality is in the south of Vanadzor town, in the valley of Vanadzor river, which is a tributary of Debed river (tributary of Kura river). Altogether 19 fruitbodies of *Elaphomyces muricatus* in different states of maturity were found on 01.06.2003 and 12.07.2004 on a 30-60° inclined slope of northern orientation of the left bank of the river, about 1520 m s. m., c. 50 m higher than the river. The territory is covered with beech forest; the dominant species are *Fagus orientalis*, *Acer platanoides*, *Cerasus avium*, and *Sambucus nigra*. The fruitbodies were found near *Fagus orientalis*, from the surroundings of its roots, where grass cover was extremely scarce. Litter layer was about 5 cm thick. Fruitbodies were found from A<sub>0</sub>A genetic horizon of soil, from 10-15 cm depth.

Soil sample analyses showed that it is a heavy sandy-clay soil (i.e. ratio of particles between 1.00-0.01 mm and smaller particles respectively is 54.3% and 45.7%) with a high stoniness level (i.e. stones, particles larger than 3 mm, 10.4%; gravel, particles between 1-3 mm, 3.0%; and fine soil, particles smaller than 1 mm, 86.6% of the total weight), rich in organics (23%) and humus rich (14.5%). There are no carbonates in that soil and it has a slightly acid reaction ( $\text{pH}_{\text{H}_2\text{O}} = 5.95$ ) (HOVSEPYAN 2006).

Vanadzor-2 locality is several hundred meters north of Vanadzor-1, on the right bank of Vanadzor river. One old fruitbody of *Elaphomyces* was found on 01. 06. 2003 from a 30° inclined slope of north-eastern orientation, c. 1400 m s. m. The slope is covered with pines. The fruitbody was found under *Pinus kochiana*. Grasses were practically absent under the trees.

Dilijan-5 locality is several hundred meters south of Kalavan village, in the valley of Kalavan river, which is a tributary of Getik, and Getik is a tributary of Aghstev (tributary of Kura). The locality is in the territory of the Palaeolithic archaeological site of Kalavan-1. One mature and one immature fruitbody of *Elaphomyces muricatus* were found amongst roots of *Carpinus betulus*, on 22. 07. 2005 on a 30° inclined slope of south-western orientation of the right bank of the river, at 1650 m s. m., several ten meters higher than the river. The slope is covered with a hornbeam-oak forest, where *Carpinus betulus*, *Quercus macranthera*, *Fagus orientalis*, *Ulmus laevis*, *Acer campestre* and *A. platanoides* are the main species and also species of *Salix*, *Fraxinus*, *Rosa*, *Crateagus*, *Lonicera*, *Malus*, *Pyrus*, *Cerasus*, *Viburnum* and *Prunus* are present. The fruitbodies were found directly near *Carpinus betulus*, between its roots (Fig. 2). Fruitbodies were found in the soil genetic horizon A, in 10 cm depth.

Soil sample analyses from 5-20 cm depth of genetic horizon A showed that it is a middle sandy-clay soil (ratio of particles between 1.00-0.01 mm and smaller particles respectively was 55.2% and 44.8%) with middle level stoniness (stones, 8.1%, gravel, 5.6%, and fine soil, 86.3% of the total weight). The soil was also rich in organics (15%) and had middle humus content (7.1%). There were no carbonates and it had a neutral reaction ( $\text{pH}_{\text{H}_2\text{O}}=6.60$ ) (HOVSEPYAN 2006).

## Discussion

*Elaphomyces muricatus* and *E. granulatus* very often occur in the same territories and can be considered as two of the most widely distributed species of the genus. Those species occur throughout Europe, and are also known from Asia and North America. The finding of *Elaphomyces muricatus* in Armenia along with recent findings of this and other *Elaphomyces* species from other countries (KONSTANTINIDIS & KAOUNAS 2012, LACHEVA 2012, WANG 2011, LÆSSØE & al. 2009, KUTORGA & KATARŽYTĖ 2008) shows that geographical distribution and ecological adaptation of these fungi are wider than known so far. Recent publications show that *Elaphomyces*, particularly *E. muricatus* and *E. granulatus*, grow also in northernmost countries (e.g., Norway), can reach up to middle and high mountainous zones (e.g. *E. muricatus* and *E. granulatus* in Armenia and *E. granulatus* in Taiwan) and that the preference of host plants and soil conditions are wider (for details see also PEGLER & al. 1993, LAWRYNOWICZ 1988, HAWKER 1954, KONSTANTINIDIS & KAOUNAS 2012, CAZARES 1992, WANG 2011).

In Armenia hypogean fungi are rare and have a very limited distribution thus they need protection. We suggest including *Elaphomyces muricatus* in the Red List of Fungi of Armenia.

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