

FIRST RECORD OF THE GENUS *EXARMIDIUM*
(ODONTOTREMATACEAE, ASCOMYCOTA) FROM
BULGARIA

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Abstract

This article presents the first record of *Exarmidium hemisphaericum* in Bulgaria. The genus is also reported for the first time from the country. The observed colour reaction of the excipulum to nitric acid is documented here for the first time. Detailed descriptions of the morphological structures and colour reactions are provided. A lichen-like interaction between the fungus and a green alga is described. Additional comments on the systematic position of the genus within the fungal systematics are included.

Key words: Bulgaria, coniferous wood, lichen-like interaction, lignicolous fungi

Introduction. The genus *Exarmidium* consists of species occurring on decorated withered wood, which often cause a white or pinkish discolouration giving a UV-positive whitish reaction. The members of the genus are distributed on coniferous and deciduous plants and have mostly subarctic or subalpine distribution [1, 2]. This group of ascomycetous fungi within the family Odontotremataceae is poorly known in Bulgaria. Only two species from this family are currently recorded

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in Bulgaria based on the available databases and literature data: *Odontura raphidospora* (Rehm) Clem. [3] and *Sphaeropezia vaccinii* (Rehm) Rehm. [4].

The taxonomic position of *Exarmidium* in the fungal systematics is still unclear. BARR and BOISE [1] revised the genus and placed several taxa in synonymy with *Exarmidium*. They suggested the inclusion of the genus in Hyponectriaceae mainly due to the occurrence of positive blue reaction with iodine in the ascus tips. However, the reaction is not constant among specimens from one species and is absent entirely from other species. A year later, studies in lignicolous Ascomycetes dealing with the genera *Xylopezia* Höhn. and *Mycowinteria* Sherwood, examined similar species to the members of *Exarmidium*, but placed them in *Xylopezia* (Odontotremataceae, Ostropales) [5]. The similarity between *Xylopezia* and *Exarmidium* has been noted by APTROOT [2] and *Xylopezia* was accepted as a synonym of *Exarmidium* together with the genera *Clypeothecium* Petrak and *Trematostoma* (Sacc.) Shear. Aptroot [2] retained the idea of the iodine reaction in the ascus, describing it as enigmatic, and maintained the placement of the genus within the Hyponectriaceae. WANG and HYDE [6] suggested exclusion from Hyponectriaceae, due to the occurrence of a positive blue reaction with iodine in the hymenium of the type species, *E. hysteriiforme* P. Karst., but did not propose where to be accommodated. However, the systematic position of the genus is retained in Hyponectriaceae in the most recent Outline of Fungi and fungus-like taxa [7].

A modern revision of the genus incorporating molecular evidence is lacking. The only available molecular data are from *E. hemisphaericum* and resolve its phylogenetic position among members of the Odontotremataceae [8]. Since no sequence data exist for the type species, the systematic position of the genus remains unresolved.

Materials and methods. During fieldwork in the Rila Mountains aimed at collecting lignicolous and corticolous lichens, a stump of *Abies alba* Miller was found covered with a pyrenocarpous fungus. Using most lichenological identification keys, the specimen keyed out to *Mycoglaena* Höhn. However, instead of the typical red reaction of the involucrellum to nitric acid (N) seen in *Mycoglaena*, the exciple of the studied specimen produced an emerald-green reaction. Additional examinations using the general key in SMITH et al. [9] led to the genus *Exarmidium* P. Karst. The specimen agrees with *Exarmidium hemisphaericum* (Fr.) Aptroot, which is a rare saprotrophic fungus on bleached coniferous wood. The locality is situated next to the road leading to Kirilova Polyana, within the Rila Monastery Nature Park, Rila Mountains (Fig. 1). The area is characterized by mixed forest of *Abies alba*, *Fagus sylvatica*, and *Picea abies* in a deep valley surrounded by summits reaching up to 2700 m. The average temperature in January is -2.7°C , while in August it is 16.3°C . Temperatures in the region can drop to -28°C in winter and reach up to 31°C in summer [10]. The studied specimen was deposited in the Mycological Collection of the Institute of Biodiversity and

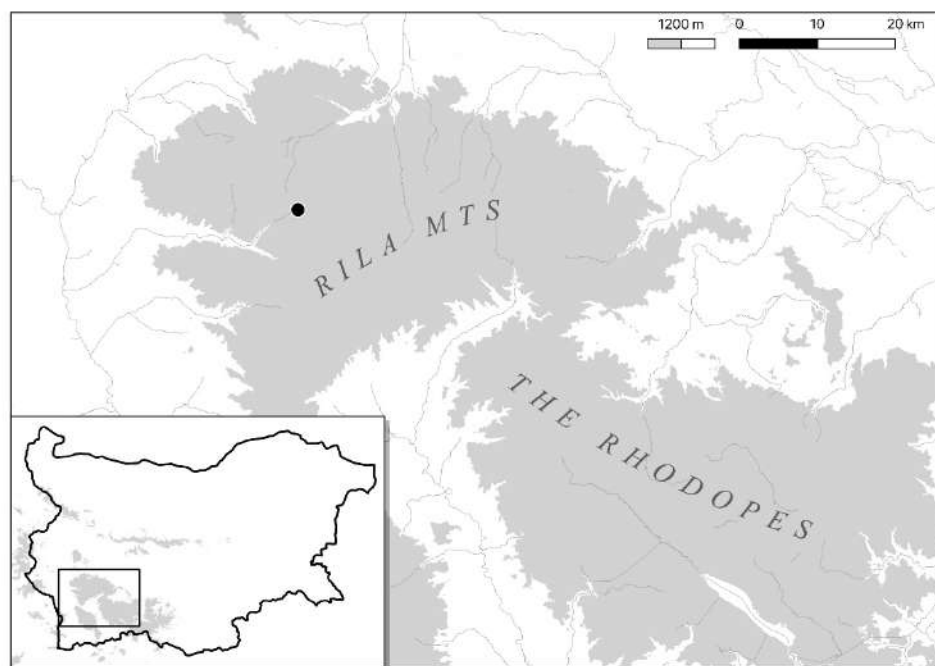


Fig. 1. The location of *Exarmidium hemisphaericum* in Rila Mountains, Bulgaria

Ecosystem Research, Sofia (SOMF). The length and width of the ascospores are given as: $(\min -)\{\bar{x} - SD\} - \bar{x} - \{\bar{x} + SD\}(-\max)$, where min and max are the extreme values, $\bar{x}$ the arithmetic mean, and SD the corresponding standard deviation; n represents the number of measurements. The measurements of all microstructures were made in water. The observations and photographs were made under a Windaus Labortechnik D-38678 dissecting microscope (Windaus-Labortechnik GmbH & Co., Germany) equipped with a Canon PowerShot A630 digital camera (Canon Inc., Japan) and a Boeco MB-180/T/SP microscope with a digital camera Boeco B-CAM10 (Boeckel GmbH & Co., Germany). Nikon D7200 (Nikon Inc. Japan) was used for photographs in the field. Taxonomically important colour reactions of lichen substances (I – Lugol's solution, K – 10% potassium hydroxide, K/I – pretreatment with 10% potassium hydroxide and subsequently applied Lugol's solution, N – nitric acid) were made following ORANGE et al. [11]. Additionally citric acid was used to test the colour reaction in the excipulum. For staining fungal structures, we used Cotton Blue and Congo Red [11].

The identification of the algae is based on the observation of free-living cells at the base of the ascomata, following the photobiont key in WIRTH [12].

The distribution of the species is given by data from literature and preserved specimens in collections available in GBIF – the Global Biodiversity Information Facility [3].

Results. In this article, we report the genus *Exarmidium* from Bulgaria for the first time. The colour reaction of the excipulum with acid, as observed here, is described for the first time. The reaction with nitric acid has not been previously reported in the mycological literature. We designate the pigment as “Aspicilia green”, a pigment known from lichenised fungi.

Taxonomic description

Exarmidium hemisphaericum (Fr.) Aptroot, Nova Hedwigia 66(1–2): 138, 1998, (Fig. 2–4).

Thallus absent. Ascomata 0.3–0.6 mm in diam., ascohymenial, hemiangio-

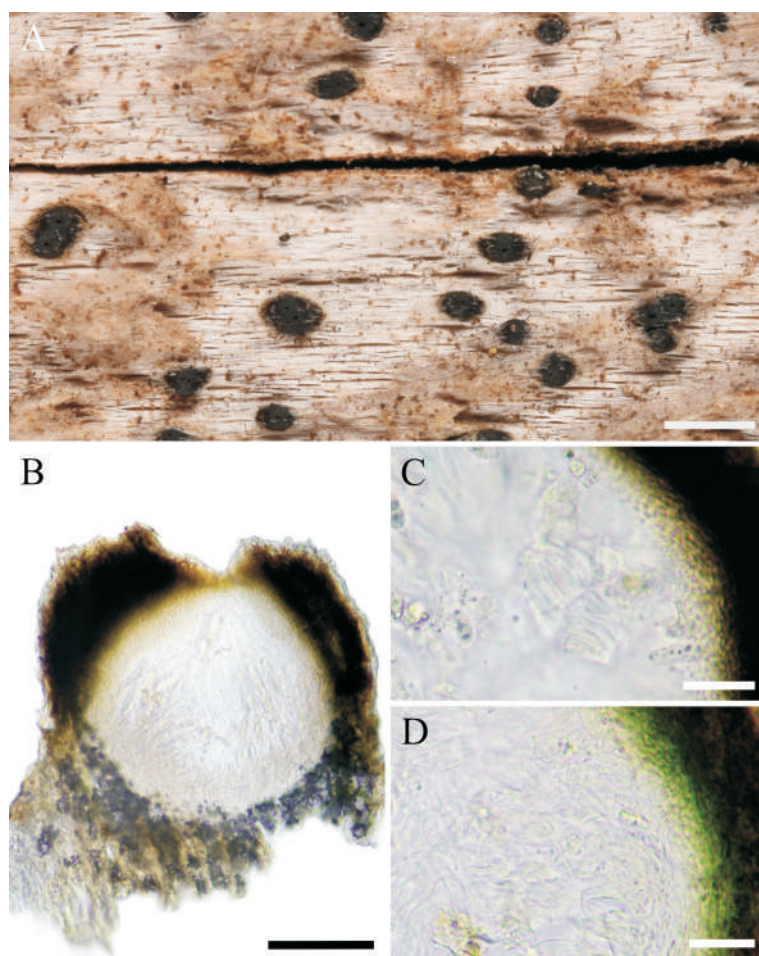


Fig. 2. Specimen (SOMF 32496) of *Exarmidium hemisphaericum* from Bulgaria: A – general habit, B – cross-section of perithecia like ascomata, C – excipulum in water, D – emerald green colour reaction of the excipulum with nitric acid. Scale bars: A = 1 mm; B = 60 µm; C, D = 30 µm

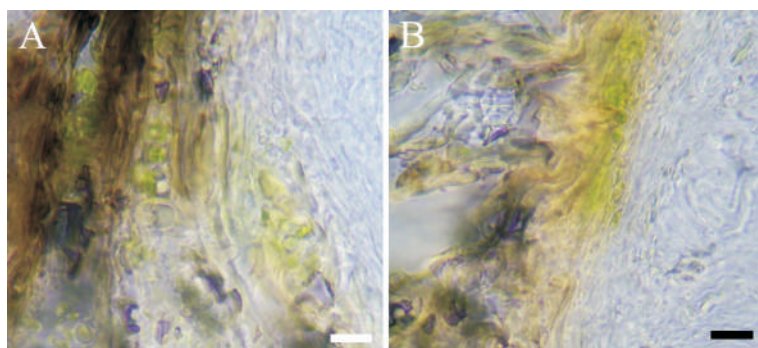


Fig. 3. A – free-living algae in the involucellum, B – modified algal cell integrated into the exciple. Scale bars = 10 μ m

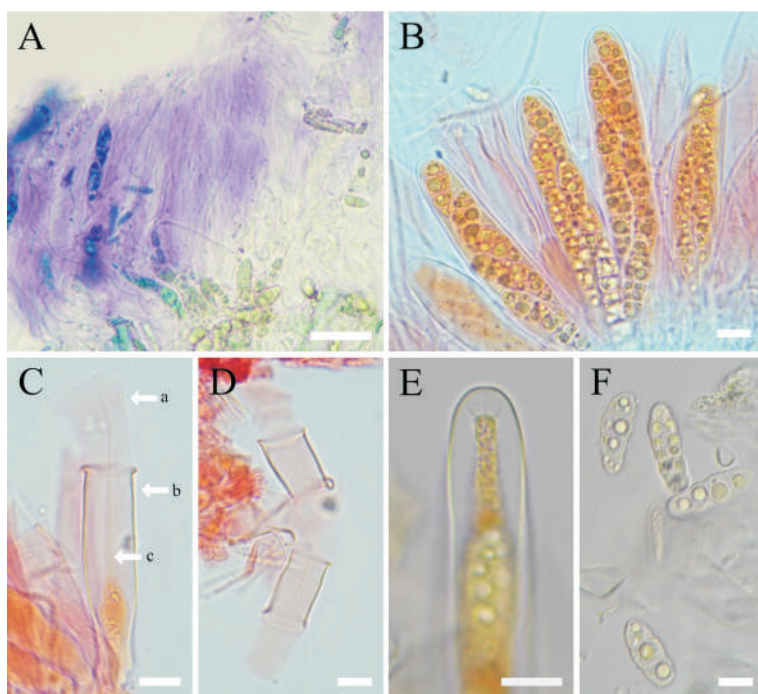


Fig. 4. A – hymenial gel showing a violet colour with Cotton Blue, B – mature asci stained in Congo Red, C – immature ascus in Congo Red and KOH showing separation of endotunica and exotunica (a – endotunica, b – exotunica, c – ascoplast), D – typical cracking of the exotunica, E – ascus in Lugol's solution, with ascoplast giving an I+ red, and a typical crown-like invagination in the apical region, F – ascospores with three and five septa. Scale bars: A = 30 μ m; B–F = 10 μ m

carpic, initially perithecioid, black, single or occasionally two to three beneath a clypeus. Involucellum dark brown, cover the upper $\frac{3}{4}$ of the exciple, K+ brown,

N–. Exciple hyaline to yellowish; outer layer with olive green pigment, N+ emerald green, K+ light brown (see the discussion below). Hamathecium of paraphyses and periphyses; paraphyses unbranched, thick at the base and becoming thinner in the upper part, long and surrounded by a gel; hymenial gel I–, with Cotton Blue gives violet colour, instead of the typical blue dyeing of the fungal tissue. Asci 8-spored, cylindrical, 60–120 × 14–16 µm, multilayered, functionally unitunicate, initially thick walled then reduced; ascus wall I–, K/I–; apical region initially exhibited a distinctive invagination of the wall resembling crown-like structure, then reduced and truncate, without structure, I–, K/I–; ascoplast I+ red. Ascospores (20.1–)22.3–24.5–26.7(–27.6) × (8.2–)8.7–9.4– 10.1(–10.5) µm, $n = 25$, hyaline, narrowly ellipsoid, usually 3 septate, rarely with 5 septa, constricted at the septa; the second cell usually wider. Conidiomata known only from living culture (see Aptroot [2]).

Specimen examined. BULGARIA, Rila Mts, Rila Monastery Nature Park, on decorticated wood of *Abies alba*, N:42.15431°, E:23.39168°, alt.: 1475 m, 16 October 2025, S. Popova, V. Shivarov (SOMF 32496).

Ecology and distribution. On dry weathered wood of conifers and occasionally deciduous species. It is typically found in forests and boreal regions. Common in clearcut areas [5]. Known from Europe: Austria, Czechia, Finland, Germany, Great Britain, Italy, the Netherlands, Norway, Slovakia, Spain, Sweden, Switzerland; Asia: Pakistan; North America: Canada, US; Australia [3, 8, 13, 14].

Discussion. The taxonomic position of the genus *Exarmidium* is still unclear due to the absence of modern molecular study. Based on our observations, this species is inconsistent with Hyponectriaceae due to the absence of a typical refractive ring and a negative iodine reaction in the ascus tip. The asci observed in the Bulgarian specimens have a distinct structure, with a thick endotunica at early stages and a thin, rigid exotunica. In mature asci, the endotunica becomes highly reduced, and the spores are released through cracking of the exotunica, without any extension of the endotunica. The two wall layers can be distinguished only in immature asci after treatment with KOH (Fig. 4C, D). Although the ascus wall consists of more than one layer, the asci function as unitunicate. Here we provisionally classify the species in Odontotremataceae.

We call the genus enigmatic due to the reported variations in iodine reactions, morphology of the ascomata, unique conidiomata, and our observation of the emerald green reaction in the exciple with nitric acid. The reaction is reversible and rare as a colour. The emerald green colour disappears when lowering the acidity. We have observed also the reaction with citric acid, but it appears slower and less vivid. These reactions with acids are not routinely studied by mycologists, but they are commonly studied by lichenologists. The reactions with nitric acid if positive usually fall in the red spectrum [9, 15]. An exception is the colour reaction in the epithecium of the genus *Aspicilia* A. Massal., which is caused by a pigment known as “*Aspicilia* green”. It is characterized by olive green colour in

water, which becomes emerald green in nitric acid and fades to brown in potassium hydroxide [9]. The observed green pigment in *E. hemisphaericum* seems identical to the pigment in *Aspicilia*. It is of particular interest that should *Exarmidium* be placed within Odontotremataceae, Ostropomycetidae, the genus will share a lineage with *Aspicilia*, Megasporaceae, providing a context for the occurrence of “*Aspicilia* green”.

We observed green algae (*Trebouxia*) at the base of all examined ascomata. The algae occur in cavities within the involucrellum, beneath the excipulum, or fully integrated into the excipular tissue. The algal cell shown in the example appears modified compared to the free-living algae surrounding the ascomata (Fig. 3A, B). This suggests a lichen-like interaction, although no defined thallus is present. It has already been reported that *E. hemisphaericum* possesses abundant single-celled algae around the ascomata [8]. Moreover, Ostropales, where we expect *Exarmidium* to be placed, includes lineages in Stictidaceae and Odontotremataceae that exhibit optional lichenisation. These species may be saprotrophic or lichenised depending on environmental conditions and available substrates [16–18].

Conclusion. The diversity of lignicolous fungi and lichens in Bulgaria remains poorly studied, and high species richness can be expected.

The newly observed colour reaction in this group of fungi may represent a useful taxonomic character, but its application should be supported by molecular data. The position of the genus within the fungal classification will become clear once DNA sequences from the type species, *E. hysteriiforme*, are available. The variation of characters among members of the genus may indicate that it represents a polyphyletic group.

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