A. Lambevska-Hristova, S. Bancheva, M. Karadelev & G. Hristov

New data on the diversity of lignicolous fungi in communities of Juniperus excelsa (Cupressaceae) in Bulgaria

Abstract


This paper provides information about the diversity of lignicolous fungi in communities of Juniperus excelsa in the Special area of conservation of NATURA 2000 “Kresna-Ilindentsi” in the Valley of Struma river floristic region, Bulgaria, where its associations is best developed. Six new species for the Bulgarian fungi are reported for the first time. New chorological data concerning three lignicolous fungi is presented too. Two of the recorded taxa, Antrodia juniperina and Pyrofomes demidoffii have high conservation value, assessed with the recent IUCN criteria, as dangerous parasites or saprobes on J. excelsa. Their role as indicators of the Greek juniper populations’ state was evaluated.

Key words: Greek juniper, Conservation, Mycological diversity, Kresna-Ilindentsi, SAC NATURA 2000, Wood-decay fungi.

Introduction

Juniperus excelsa M. Bieb. (Greek juniper, Cupressaceae) is a rare species in Bulgaria, protected by the Biological Diversity Act (2002). It is included in the Red List of Bulgarian Vascular plants (Petrova & Vladimirov 2009) as “Vulnerable” and is a key species for habitat with European importance, named “39G3 Forests of Grecian juniper”, included in the Council Directive 92/43/EEC (1992), the Bulgarian Biological Diversity Act (2007) and the Red Data Book of Republic of Bulgaria as “Critically Endangered” (Tzonev & Dimitrov 2015). J. excelsa occurs in Bulgaria only in areas with a pronounced Mediterranean climate – in the Rhodope Mts. and the Struma river Valley floristic regions. In the first locality, the population is small and strongly degraded, while the most representative communities in the country are situated in the Struma river Valley (Fig. 1).

In 2019 a study of the population state of Greek juniper in the Special area of conservation (SAC) of NATURA 2000 “Kresna-Ilindentsi”, in the Valley of Struma river was undertaken. During the fieldwork, the attention of the team was attracted by the large num-
ber of lignicolous fungi developing on the dead wood of *J. excelsa*, most of which proved to be new to the Bulgarian Mycota.

The purpose of this work is to announce the establishment of six new lignicolous fungi for the Bulgarian Mycota, and to provide information on the morphology and key features of species differentiation, their distribution, and role as indicators of the Greek juniper populations’ state. In addition, the paper aims to provide information on new chorological data for some lignicolous fungal species.

**Material and methods**

For this study, three major localities of distribution of *J. excelsa* in Struma river Valley were selected, which are the most representative in terms of numbers and density: 1. “Tisata” Nature Reserve in the Kresna Gorge and Stara Kresna region, 2. The Valley of Vlahi river and 3. “Moravska” protected place and Kamenitsa village. The route and stationary methods were used for selected objects.

The Kresna Gorge is one of the least areas with well-preserved forest of Greek juniper in Bulgaria, mainly between 100 and 300–400 m alt. The most representative communities are in “Tisata” Nature Reserve and in its buffer zone. During the field work a remarkable floristic diversity was established with predominance of xerophytes with mostly southern and eastern origin. The vegetation is composed by sclerophilic evergreen and deciduous...
shrubs and small trees with many open places covered by rich herbaceous vegetation. Some rare, endangered and protected plants are included in the composition, as *Anthemis auriculata* Boiss., *Prangos trifida* (Mill.) Herrm. & Heyn (*Cachrys alpina* M. Bieb.), *Colchicum lusitanum* Broth., *Delphinium balcanicum* Pawl., *Goniolimon tataricum* (L.) Boiss., *Ornithogalum oreoides* Zahar., *Sempervivum ciliops* Craib, *Campanula jacquinii* (Sieber) A. DC. (*Trachelium rumelianum* Hampe), *Verbascum roripifolium* (Halácsy) I. K. Ferguson, etc.

The specimens were identified used standard methods (in accordance with Еriksson & Ryvarden 1973; Jülich 1984; Breitenbach & Kränzlin 1986; Domański 1991; Gilbertson & Ryvarden 1986, 1987; Hansen & Knudsen 1997; Bernicchia & Gorjón 2010), implying microscopy and application of reagents (Melzer’s reagent and 5% KOH). Measurements and photographs were examined at magnification up to 1000×, with a LW scientific microscope and MiniVID camera.

The nomenclature follows Index Fungorum, while the nomenclature of vascular plants follow The Euro+Med PlantBase — the information resource for Euro-Mediterranean plant diversity (http://ww2.bgbm.org/EuroPlusMed [last accessed 19.02.2020]). The studied specimens were deposited in the Mycological Collection of the Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences (SOMF).

The monitoring and assessment of the population state of *J. excelsa* has been carried out in accordance with the approved methodologies of the National Biodiversity Monitoring System of the Executive Environment Agency of Bulgaria (http://eea.government.bg/bg/bio/nsmbr/praktichesko-rakovodstvo-metodiki-za-monitoring-i-otsenka/vishshi-rasteniya).

Results

The variety of lignicolous fungi is impressive. To date, no targeted studies have been conducted on *J. excelsa* communities.

As a result of this study, six new basidiomycetes for Bulgaria have been identified, as follows:

1. **Megalocystidium luridum** (Bres.) Jülich, Persoonia 10(1): 140 (1978) [Stereaceae].

   **Morphological description.** Basidiome resupinate, effused, adnate, hymenophore smooth, yellowish to pale cream-brown, margin indifferentiated, hyphal system monomitic, hyphae with clamps, thin-walled, smooth, 2-3 µm, gloeocystidia numerous, tubular, thin-walled, 60-120 × 6-10 µm, with granular contents, basidia clavate, 35-60 × 5-8 µm, with 4-sterigmata, and with a basal clamp, basidiospores ellipsoid, thin-walled, smooth, 7-9 × 4-7 µm, amyloid (Fig. 2, A-B). The presence of gloeocystidia and the amyloidity of the basidiospores make this species easy to recognize. The closely related species *M. leucoxanthum* (Bres.) Jülich, has clearly larger and allantoid spores.

   **Ecological remarks.** A widespread white-rot lignicolous species that occurs on decayed wood, mostly of deciduous tree, rarely on conifers.

**General distribution.** *M. luridum* has been reported from different parts of Europe, Turkey, and the Caucasus (Eriksson & Ryvarden 1975; Bernicchia & Gorjón 2010). The species has not been previously reported for Bulgaria.

**Distribution in Bulgaria.** Struma river Valley, “Moravska” protected place.

**Material examined.** Struma river Valley, “Moravska” protected place, 1,5 km W of Kresna town, mixed forest with domination of *J. excelsa*, fallen branches of *J. excelsa*, N 41.710916° E 23.142861°, 360 m, 03.V.2019, leg. A. Lambevska-Hristova & G. Hristov, det. A. Lambevska-Hristova & M. Karadelev (SOMF 30 224).

2. **Peniophora junipericola** J. Erikss., Symb. bot. upsal. 10(5): 52 (1950) [Peniophoraceae].

**Morphological description.** Basidiome resupinate, effused, closely adnate, but margin loosening from the substratum, hymenophore smooth to rimose when dry, pinkish or greyish red to violaceous, pale brownish red when old, margin whitish, hyphal system monomitic, hyphae with clamps, basal hyphae thick-walled, brown, 3-4 µm wide, subhyphal hyphae hyaline to brown, thin- to thick- walled, 2.5-4 µm wide, cystidia: lamprocystidia hyaline to brown, thick-walled, encrusted, 40-80 × 6-15 µm, gloecystidia...
fusiform. Basidia subcylindrical, 30-50 × 5-7 µm, with 4-sterigmata and with a basal clamp. Basidiospores allantoid, 7-12 × 2.2-4 µm, smooth, thin-walled, hyaline (Fig. 2, C-D). The species is very similar to *P. pithya* (Pers.) J. Erikss. which occurs on conifers, but differs mainly in colour of basidiome and the margins (Eriksson & al. 1978).

**Ecological remarks.** According to the Eriksson & al. (1978) ideal ecological conditions for this species are the combinations of dry and warm weather, alternating with periodic humidity.

**Host.** *P. junipericola* is considered a white rot saprobe that is apparently restricted only to species of genus *Juniperus* and on *Cupressus, Platycladus* (Ginns & Lefebre 1993; Boidin 1994; Isikov 1997; Minter & al. 2009; Bernicchia & Gorjón 2010; Lambevska & al. 2013).

**General distribution.** The species is known from Europe, Caucasus, Central Asia and North America (Parmasto & Parmasto 1992; Boidin 1994; Miller & al. 1994; Dämon & al. 2009). The new finding from Bulgaria widens the distributive area of such interesting species.


**Morphological description.** Basidiome resupinate, effused, hymenophore smooth or slightly tuberculate, beige ochraceous or brown, margin indifferentiated, hyphal system monomitic, hyphae with clamps, hyaline to brown, 2-5 µm wide, cystida: gloeocystidia, 35-50 × 4.5-5 µm, cylindrical, thin-walled, lamprocystidia subcylindrical, thick-walled, encrusted, 30-55 × 8-20 µm, dendrohyphidia projecting with branched part, encrusted, brownish, basidia subclavate, 25-45 × 4.5-5.5 µm, with 4-sterigmata and a basal clamp, basidiospores cylindrical to allantoid, 7-10 × 2.5-3.5 µm, smooth, thin-walled, hyaline (Fig. 3, A-B). Easily recognized microscopically, *P. meridionalis* is characterized by the presence of three kinds of cystidia: gloeocystidia, lamprocystidia and dendrohyphidia.

**Ecological remarks and host.** It is a thermophilous species which occurs mainly on various deciduous trees and conifers (Bernicchia & Gorjón 2010), often on *Cupressaceae* (*Cupressus sempervirens* L. and *Juniperus oxycedrus* L.).

**General distribution.** The species is generally distributed in Europe, Asia, Africa, the Canary Islands, Australia (Boidin 1994; Andreasen & Hallenberg 2009). It has not been previously reported for Bulgaria.

**Distribution in Bulgaria.** Kresna Gorge, “Tisata” Nature Reserve, near Peyo Yavorov railway station.


   **Morphological description.** Basidiome resupinate, effused, adnate, hymenophore smooth, orange-white to pale orange or greyish orange, margins thinning, paler concolorous, hyphal system monomitic, hyphae with clamps, hyaline, thin-walled, 3-3.5 µm wide, cystidia absent, hyphal ends present, with obtuse apex, often with protuberances and branches; basidia clavate to cylindrical, 25-40 × 5-10 µm, with 4-sterigmata and a basal clamp, with oil drops, basidiospores broadly ellipsoid to subglobose, thin-walled, 6-9 µm, smooth or minutely warted. Among the *Radulomyces* species, it is characterized by the warted globose basidiospores but also in the broadly cylindrical basidia (Fig. 3, C-D). The very similar *R. confluens* (Fr.) M.P. Christ. differs in having slight but significant difference in spore size and form and also a small difference in basidiome size (Ghobad-Nejhad & Kotiranta 2007).

   **Ecological remarks and host.** The species grows on decayed, mostly decorticate wood from both conifers and broadleaved trees (Bourdot & Galzin 1928; Malençon 1952; Christiansen 1960; Cunningham 1963; Boidin & al. 1988; Legon & al. 2005; Gorjón & al. 2006 and Bernicchia & Gorjón 2010).

   **General distribution.** The species is reported from Europe, Australia, North Africa (Morocco), Western Asia (Iran), South America (Argentina, Brazil, Colombia) and New Zealand (Ghobad-Nejhad & Kotiranta 2007). It has not been previously reported for Bulgaria.


5. Xylodon asperus (Fr.) Hjortstam & Ryvarden, Syn. Fung. (Oslo) 26: 34 (2009) [Schizoporaceae].


Morphological description. Basidiome resupinate, effused, adnate, white, cream and even ochraceous colour, hymenophore odontoid with scattered aculei and with smooth hymenium between them, hyphals ends in the aculei usually smooth or with few crystals, hyphal system monomitic, hyphae with clamps, slightly thick-walled, 2-3 µm wide, cystidia absent, hyphal ends frequently capitate, basidia cylindrical, constricted, 18-25 × 4-5 µm, with 4-sterigmata and a basal clamp, basidiospores ellipsoid, 5-6 × 3.5-4 µm, smooth, thin-walled (Fig. 4, A-B). According to the data published by Breitenbach & Kränzlin (1986), this lignicolous saprotroph is very closed to X. brevisetus (P. Karst.) Hjortstam & Ryvarden and differ from it only by somewhat larger spores and which occurs preferentially on spruce.

Ecological remarks and host. The species within the genus Xylodon are primarily wood decomposers, cause decaying of white rot type on dead wood of conifers, but often also on deciduous trees. The species in this widespread genus commonly occur in temperate to tropical areas (Wang & Chen 2017). In Bulgaria it was recently collected on fallen branches of Greek juniper, a substrate that has not previously been reported in the literature.

General distribution. Asia (Hjortstam & Ryvarden 2004; Dai 2011; Ghobad-Nejhad 2011; Gafforov & al. 2017), Europe (Langer 1994; Bernicchia & Gorjón 2010); North America (Gilbertson 1980; Tapia & al. 2016); South America (Langer 1994; Hjortstam & Ryvarden 2008). The species is distributed in temperate and tropical regions with cold and hot seasonable climate (Gafforov & al. 2017). Very common species in all European countries, but it has not been previously reported for Bulgaria.


Morphological description. Basidiome resipinate, closely adnate, white-cream coloured, hymenophore smooth and with some scattered small granules, white, margin not especially distinct, but well defined, hyphal system monomitic, hyphae with clamps, thin-walled, 2-5 µm wide, encrusted, cystidia as subulate hyphal ends, 20-30 × 2-5 µm wide, encrusted, basidia subclavate to subcylindrical, 15-25 × 4-5 µm, with 4-sterigmata, thin-walled and with a basal clamp, basidiospores ellipsoid, thin-walled, smooth, 5-6 × 3-4 µm, non-amyloid (Fig. 4, C-D). It is very similar to *X. crustosus* (Pers.) Chevall., differs in having ellipsoid spores and odontoid hymenophore.

Ecological remarks. Yurchenko, Xiong & Wu (2013) mentioned that it occurs in boreo-nemoral, nemoral and mediterranean types of biomes. As shown in its distribution, *X. juniperi* prefers to grow in warm and moderately dry regions, more often on low mountains or stony areas. Species of *Xylodon* are wood-decaying saprobes, causing a white rot in coniferous and deciduous wood and therefore participating in carbon recycling of forest ecosystems (Eriksson & Ryvarden 1976).

Host. According to the available literature, the taxa prefers juniper species, but is known also on hard deciduous wood (Bernicchia & Gorjón 2010). Here we report it for the first time on Greek juniper.
General distribution. The species is known from North America, South America, Asia, Africa and Europe. According to Yurchenko, Xiong & Wu (2013) this species has predominantly temperate-holarctic distribution. It has not previously been reported in Bulgaria.


During this study, new chorological data for three lignicolous fungi were also identified:

   Bas.: Thelephora laevigata Fr., Elench. Fung. (Greifswald) 1: 224 (1828).

A. laevigatum is a key element of boreal ecosystems, due to their wood-decomposing abilities. The species cause decaying of white rot type in conifers (Lambevska-Hristova & Karadelev 2019). Till now, it is known from Cupressus sempervirens, Juniperus communis, J. communis subsp. nana Syme, J. excelsa, J. foetidissima Willd., J. oxycedrus, Taxus baccata L. and Thuja occidentalis L. (Eriksson, Hjortstam & Ryvarden 1981; Karadelev 2000; Bernicchia & Gorjón 2010; Lambevska-Hristova & Karadelev 2019). Here we report it for the first time on fallen branches of J. excelsa.

In Bulgaria the species was known, so far, only from Western Rhodopes Mts. (Reid & Vanev 1984). The species is known from the temperate regions of Eurasia and North America.


H. fuliginosa is a wood saprophyte which grows preferentially on fallen trunks and branches of conifers, such as Abies, Juniperus, Larix, Picea, Pinus and Taxus, but also on different deciduous substrates, such as Alnus, Populus and Salix (Corfixen & Parmasto 2017). It causes a white rot on coniferous and deciduous wood. In Macedonia, it generally grows on J. excelsa (Karadelev 1993; Karadelev & Rusevska 2004). Here we report it for the first time on fallen branches of Greek juniper.

This species has been reported previously only once from Bulgaria as Hymenochaete subfuliginosa Bourd. Et Galz., from Eastern Stara Planina Mts., more than 30 years ago (Kuthan & Kotlaba 1989). The general distribution covers North America, Europe, and
temperate Asia. According to data provided by Kriegelsteiner (2000), in Europe it is distributed from southern France through Central Europe to Northern Europe.


According to Gafforov & al. (2017) this saprotrophic odontoid species is well adapted to the arid and semi-arid environments. It occurred on dead wood of conifers, such as *Abies alba* Mill., *J. communis, J. oxycedrus, J. phoenicea L., Pinus pinea L.* and *Taxus baccata*, but also on different deciduous tree (Bernicchia & Gorjón 2010). In “Tisata” Nature Reserve, we found it on dead fallen branches of *J. excelsa*. This host has not previously been published for the species in the literature.


**Fungi of high conservation value**

During the recent study two lignicolous species of high conservation significance were registered, the saprobe *Antrodia juniperina* (Murrill) Niemelä & Ryvarden and the parasite *Pyrofomes demidoffii* (Lév.) Kotl. & Pouzar. They are included in the Red Data Book of the Republic of Bulgaria (Denchev & al. 2015) and the Red List of Fungi in Bulgaria (Gyosheva & al. 2006), as Endangered EN [B2ab(i,ii,iv)].

*Antrodia juniperina* (Murrill) Niemelä & Ryvarden. (Brown rot) [Fomitopsidaceae].

The species has been reported, till now, only from Struma river Valley (“Tisata” Nature Reserve) and Central Rhodopes Mts. (Stoichev 1990; Denchev & Assyov 2010, Tzonev & Dimitrov 2015). It is worth to mention also that no specimens were found in SOMF to support this record. We found the species in addition to the “Tisata” Nature Reserve (19 samples) also in following 4 localities: “Moravska” protected place (20 samples), Kamenitsa village (3), Stara Kresna region (2) and along Vlahi river Valley (4). This is the first indication for the occurrence of this species in “Moravska” protected place, Stara Kresna region and along Vlahi river Valley. The protected sites “Tisata” and “Moravska” are the best place for development and conservation of this taxon compared to other areas. According to the published data by Doğan & Karadelev (2006) it grows exclusively as a saprobe, rarely as a parasite, on dry branches and old trunks of *J. excelsa* and *J. foetidissi-
ma. In Europe, it is a very rare species with restricted range that follows the spread of the juniper host (Fig. 5, A). It is particularly frequent on old trunks of Greek juniper in the Balkans (Macedonia, Bulgaria and Albania).


**Pyrofomes demidoffii** (Lév.) Kotl. & Pouzar (Juniper pocket rot) [*Polyporaceae*].

It has been previously recorded from Struma river Valley (“Tisata” Nature Reserve) and Central Rhodopes Mts. (Stoichev 1982 as *Phellinus demidoffii*; Denchev & Assyov 2010;
Alexov & al. 2012; Tzonev & Dimitrov 2015). It occurrence is now confirmed with new material from “Tisata” Nature Reserve (3 samples). In addition we found it also in “Moravska” protected place (4) and Stara Kresna region (11). This species was found for the first time in “Moravska” protected place and Stara Kresna region. It is a dangerous parasite that causes a considerable damage in Juniperus associations (Fig. 5, B). It parasites on old juniper trees usually present in a good developed forests. The species is registered on J. excelsa and J. foetidissima (Doğan & Karadelev 2006). Juniper pocket rot is a rare species in Europe with restricted area of distribution which following the host distribution. It is particularly frequent on old trunks of Greek juniper in the Balkans (Macedonia, Bulgaria and Albania).


The distribution map of species of A. juniperina and P. demidoffii in the SAC “Kresna-Ilindentsi” is shown in Fig. 6.

Discussion

The territory of Bulgaria falls in the northern part of the distribution of J. excelsa and the conservation of its populations is of great importance for the preservation of their specific gene pool. The presence of such a wide variety of saprobic fungi in the Greek juniper forests is evidence of their good functional condition, especially in “Tisata” Nature Reserve, followed by the “Moravska” protected place. This is because the prerequisite for their good development is the presence of old trees and dead wood. In this sense, the presence or absence of saprobic fungi in the juniper forests is a good indicator and can be used in assessing their conservation state.

The Greek juniper forests are an outstanding biodiversity hotspot for rare and specific fungal species with a restricted distribution in Europe. Part of them, such as the endangered Antrodia juniperina and Pyrofomes demidoffii, are characteristic taxa for the habitat of J. excelsa, which are rare for Europe and with priority for conservation. Some of the established taxa, such as A. laevigatum, A. juniperina, P. junipericola, P. demidoffii and X. juniperi grow exclusively on juniper species, including on J. excelsa. A part of them, such as A. juniperina and P. demidoffii are rare, and their protection in the studied area is invaluable for the mycodiversity of the country.

In our estimation, the presence of the parasite P. demidoffii on Greek juniper does not currently have a significant negative impact on the forests, but in the future it is necessary to monitor the extent of its spread and impact.

**Conclusion**

The results of this study provide the evidence that the Grecian juniper forests in SAC “Kresna-Ilindentsi”, possess an extraordinary richness of plants and fungi, some of which are of high conservation importance. The fungal and plant diversity there must be monitored periodically and strictly protected for future generations.
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Addresses of the authors:
Aneta Lambevska-Hristova¹, Svetlana Bancheva¹, Mitko Karadelev² & Georgi Hristov³,
¹ Department of Plant and Fungal Diversity and Resources, Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, 23 Acad. G. Bonchev St, 1113 Sofia, Bulgaria, E-mail: aneta.lambevska@gmail.com
² Institute of Biology, Faculty of Natural Sciences and Mathematics, University Ss Cyril and Methodius, Arhimedova 5, 1000 Skopje, Republic of Macedonia.
³ Department of Animal Diversity and Resources, Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, Tsar Osvoboditel Boulevard 1, 1113 Sofia, Bulgaria.