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Research article

Data on the distribution of *Osmoderma barnabita* Motschulsky, 1845 (Coleoptera: Cetoniidae) in Bulgaria from 1904 to 2022

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Abstract: The hermit beetle, *Osmoderma eremita* s.l. (Scopoli 1763), is a species complex of scarab saproxylic beetles listed in the IUCN Red List of Threatened Species and protected in Bulgaria by Bern Convention, the Habitats Directive, and the Bulgarian Biodiversity Act. The taxonomic status of the species complex in Bulgaria is not fully known. It is believed that *Osmoderma barnabita* Motschulsky, 1845 is the main species present in the country, but it is also possible that *Osmoderma lassalei* Baraud and Tauzin, 1991 exists in the southernmost parts, where is the northern limit of its distribution. Data collected over the years on the presence of *O. barnabita* are very scarce due to its hidden lifestyle and lack of interest from the scientists. The present study aims to summarise all available data on *O. barnabita* in Bulgaria (literature, National Natural History Museum – Bulgarian Academy of Sciences collection, field work, and citizen science) for the period from 1904 to 2022. A map of the current species distribution was generated. The altitudinal distribution ranged from 0 to 1700 m, mainly in mountainous areas with well-preserved forests. Eighty-three percent of the records with exact coordinates were from forests aged above 50 years, and 35% were from forests aged above 100 years. There were also individual records from cities, showing the need to protect urban green spaces and the old trees within them. Based on the results obtained, *O. barnabita* is newly registered for six Natura 2000 sites, confirmed in 18 sites; and for the remaining 18, where it is included in the standard data forms based on modelling its habitat, additional studies are needed to confirm or exclude it.

Keywords: conservation, distribution, Natura 2000, *Osmoderma eremita*

Introduction

The hermit beetle, *Osmoderma eremita* s.l. (Scopoli, 1763), is a species complex of scarab saproxylic beetles associated with hollow veteran trees in the broadleaf forests of Europe (Maurizi et al., 2017). The complex consists of *Osmoderma eremita* s.str. in western Europe; Italian endemics *Osmoderma italicum* Sparacio, 2000 and *Osmoderma cristinae* Sparacio, 1994; *Osmoderma barnabita* Motschulsky, 1845 in eastern Europe; and *Osmoderma lassallei* Baraud and Tauzin, 1991 from Greece and European Turkey (Audisio et al., 2009). They are listed in the IUCN Red List of Threatened Species (Cálix et al., 2018) as priority species of community interest: *O. eremita* and *O. barnabita* have a Near Threatened status and decreasing population trend; *O. lassallei* has an Endangered status and decreasing population trend; *O. italicum* has an Endangered status and unknown population trend; and *O. cristinae* has an Endangered status and stable population trend (Nieto et al., 2010).The species complex is protected by the Bern Convention and the Habitats Directive (Council Directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora) under Annexes II and IV as a high-priority species. It is also a protected species under the Bulgarian Biodiversity Act (09.08.2002, latest amendment, State Gazette No. 102/23.12.2022), and it is included in the Red Book of Bulgaria (Guéorguiev, 2015). The main limiting factor for *O. eremita* s.l. populations is the disappearance of old

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hollow trees due to anthropogenic activities. As stated by Ranius et al. (2005), the conservation of *O. eremita* relates to three areas that are challenging for nature conservation in Europe: preserving natural woodlands, habitats associated with old agricultural landscapes and small pockets of nature in urban areas.

According to Jurc et al. (2008), *O. eremita* s.l. is a stenotopic, foliophilic, sylvicolous, xylodetriticolous, phytophagous and saproxylic species. It is a dendrobiont, that inhabits the hollows of old, still-living trees. It occurs in old deciduous forests, with preferred habitats being forest edges and riverbanks; it prefers oak (*Quercus* sp.), then linden (*Tilia* sp.), willow (*Salix* sp.), beech (*Fagus sylvatica* L.), cherry (*Prunus* sp.), pear (*Pyrus* sp.), apple (*Malus domestica* Borkh.) and other old fruit trees in orchards. The microhabitat of *O. eremita* s.l. is exclusively decaying and rotting old trees. Females lay their eggs in decaying wood in tree hollows, where the larvae develop. Larval development lasts 2–3 years. Adult insects occur from May to early September, but they are most commonly seen in June and July. In the autumn, the adults die (Tauzin, 1994a; 1994b; Oleksa et al., 2007; Dubois, 2009; Chiari, 2011; Chiari et al., 2015; Maurizi et al., 2017).

In the Balkan Peninsula as a whole, the species of the *Osmoderma* complex are not well studied, either taxonomically or ecologically. According to genetics studies, there are two well-defined clusters, each consisted by two species: the first cluster is restricted to western Europe (*O. eremita* and *O. cristinae*) and the second one to eastern Europe (*O. barnabita* and *O. lassallei*). The morphological characteristics used to distinguish *Osmoderma* species are not always clear, and they vary considerably. The lack of well-defined boundaries can lead to a high degree of uncertainty when studying populations living in areas of contact between the ranges of different taxa because of the presence of mixed morphological traits that make it difficult to assign individuals to one of the two neighbouring taxa. Furthermore, apart from *O. lassallei* and *O. barnabita,* a few poorly investigated taxa exist in the area between the Balkan Peninsula and the Caucasus Mountains. Although the nominative species *O. eremita* is relatively well studied, there is little information on *O. barnabita;* moreover, the ecology and distribution of *O. lassallei* are completely unknown (Audisio et al., 2007; Audisio et al., 2009; Landvik et al., 2017). The first records of *O. eremita* s.l. in Bulgaria were made by three authors between 1904 and 1909 (Markovich, 1904; 1909; Nedelkov, 1906, 1909; Yoakimov, 1904). Since then, data on *O. eremita*/*barnabita* from Bulgaria have been mentioned only in a few papers published in the period 1960–2005 (Anguélov, 1960; Nüssler, 1986; Palm, 1966; Ranius et al., 2005). In total, there are about 13 known findings, mainly from the south-western and south-eastern parts of Bulgaria. Reasons why so few data have been collected over the years have to do with the hidden lifestyle of this species and the lack of interest from scientists.

It should be noted that for practical conservation reasons, the species from the complex in Bulgaria is kept as *O. eremita;* this determination was made in line with EC recommendations (Council Directive 92/43/EEC). The species is included in 30 protected Natura 2000 sites in Bulgaria, based on published records and the availability of its potential habitat, assessed by modelling (Supplementary material 01 [*.xlsx]).

According to the Habitats Directive country reports under Article 17 in 2013 (for the period 2007–2012), the species has Favourable (FV) status in all parameters for Alpine, Continental and Black Sea biogeographical regions; in 2019 (for the period 2013–2018), the assessment of all status parameters was ‘Unknown - XX’ (https://eunis.eea.europa.eu/index.jsp). The present study aims to summarise all available data on *Osmoderma barnabita* in Bulgaria to date, in order to facilitate environmental conservation efforts to protect the species.

Material and methods

To generate a map of the known distribution of *O. barnabita* in Bulgaria, we compiled presence records from the following sources: published scientific literature and country reports; the collection of the National Museum of Natural History – Bulgarian Academy of Sciences (NMNHS – BAS); field data collected by the authors and other professional entomologists in 2012–2021 and by citizen science (voluntary provided data from amateurs, members of the Bulgarian Facebook group ‘The Insects and the Entomologists’) incorporated in the SmartBirds.org database (Popgeorgiev et al., 2015). For the forest age and Natura 2000 sites analyses, only records with exact coordinates (accuracy up to 20 m) were used; this resulted in 47 records.

Fig. 1. *Osmoderma barnabita* (male) – Sredna Gora Mts.

Results and discussion

We reported the distribution of *Osmoderma barnabita* in Bulgaria (Fig. 1), and 66 out of 79 records were new and unpublished (Fig. 2, Supplementary material 02 [*.xlsx]). Their altitude ranged from 0 to 1700 m, mainly in mountainous areas with well-preserved forests. The findings in the plains and in some towns (Smolyan, Sliven) were interesting as they showed a wider distribution than those only in the mountain forests. Eighty-three percent of the records with exact coordinates were from forests aged above 50 years, and 35% were from forests aged above 100 years.

Most likely, the hermit beetle was a more frequent species in Bulgaria in the past. In the early 20th century, Markovich (1909) mentioned that it was a common species in Razgrad Town. However, only single findings are evident nowadays, despite the efforts made and the use of more sophisticated methods for collection, such as pheromone traps. The difficulty in finding the species was also reflected in the preparation of the standard forms for the Natura 2000 sites in Bulgaria, where data were deficient (DD) in 19 cases and of medium quality (M) in the rest (Supplementary material 01 [*.xlsx]).

Based on the results obtained, the species is newly registered for the following six sites: BG0000134, ‘Choklyovo blato’; BG0000366, ‘Kresna–Ilindentsi’; BG0000382, ‘Shumensko plato’; BG0000608, ‘Lomovete’; BG0001023, ‘Rupite–Strumeshnitsa’; and BG0001043, ‘Etropole–Baylovo’. Moreover, for the first time, exact and up-to-date data are reported for the following sites: BG0000164, ‘Sinite kamani’; BG0000167, ‘Belasitsa’; BG0000301, ‘Cherni rid’; BG0000314, ‘Rebro’; BG0000494, ‘Tsentralen Balkan’; BG0000496, ‘Rilski manastir’; BG0001007, ‘Strandzha’; BG0001012, ‘Zemen’; BG0001013, ‘Skrino’; BG0001021, ‘Reka Mesta’; BG0001028, ‘Sreden Pirin–Alibotush’; BG0001030, ‘Rodopi Zapadni’; BG0001032, ‘Rodopi Iztochni’; and BG0001493, ‘Tsentralen Balkan–buffer’. In this way, the presence of *O. barnabita* has been confirmed in 18 protected areas; for the remaining 18, additional studies are needed to confirm or exclude it.

Although, not exactly validated within the boundaries, for such sites as BG0001011, ‘Osogovska

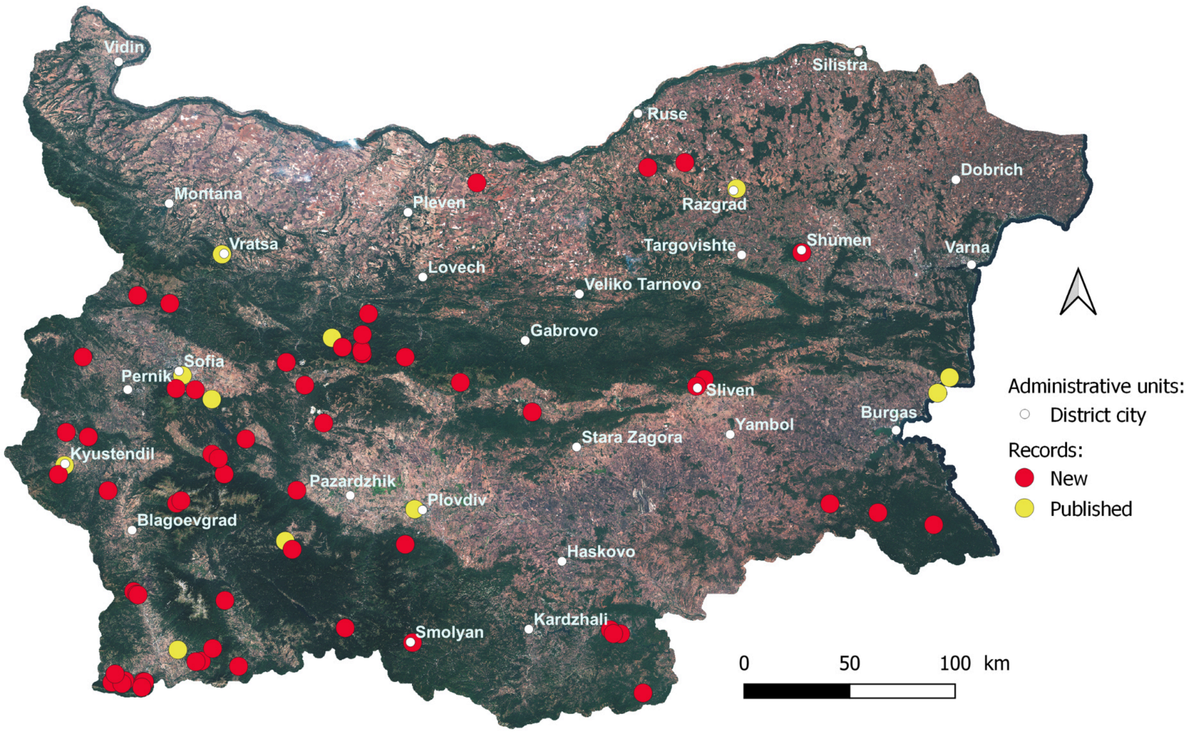

Fig. 2. Map of the current *Osmoderma barnabita* distribution in Bulgaria, based on a satellite map of Bulgaria from Sentinel 2 (Geopolymorphic Cloud, 2017).

planina', it can be argued that the species is certainly present there. We show recent findings that are quite close to the administrative boundary of the area, which is enough to credibly claim that it is also present in other places in the zone where potential habitats exist. Unfortunately, there are also sites where the species has not only never been established but where almost complete degradation of the existing forests and a lack of old trees were also observed, making the existence of *O. barnabita* impossible (e.g. BG0000271, 'Mandra–Poda' and BG0000198, 'Sredetska reka').

Two main priority directions for species conservation in Bulgaria could be identified: the preservation of old-growth forests and the strengthening of systematic surveys on them. Considerable efforts are being made to identify and preserve old-growth forests and forest islands and to protect them and exclude them from forestry activities, both at the state level and by non-governmental organisations (NGOs) and the scientific community. There have been some successes, such as the declaration of areas with old-growth forests to be inaccessible for economic activities (Order RD-49-421/02.11.2016 of the Ministry of Agriculture and Food; http://www.iag.bg/docs/lang/1/cat/14/index), as well as the Forest Stewardship Council (FSC) certification of all state forest enterprises, resulting in at least 10% of the natural forest area in Natura 2000 sites being designated as old-growth forest protection areas. These areas with old-growth forests were considered in the forest management plans and in the orders for the designation of the Natura 2000 protected sites, thus ensuring their persistence. Attention also needs to be paid to the protection of old urban trees (Carpaneto et al., 2010; Kadej et al., 2016) in parks, gardens and other green areas, as it is obvious that vital populations of the species could also exist there (the records from Sliven, Smolyan, etc.).

Although there has been some increase in the amount of presence data in recent years, including from citizen science, there is still an urgent need for further research using a variety of methods (Chiari et al., 2012; Maurizi et al., 2017). Especially, such research can help to establish the distribution and population characteristics and status of the species.

Large-scale awareness-raising campaigns and data collection by volunteers (tourists, forest workers, schoolchildren, and students) can also help improve species presence data. At the same time, it is important that specific scientific studies, such as taxonomic composition, population dynamics and dispersal, be sufficiently funded by various projects (government, conservation, etc.) to ensure long-term and sustainable data collection and expert analysis. The combination of both approaches, citizen science and expert research, would greatly support the future preparation of management plans for this species and the areas it inhabits.

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Supplementary materials

01
Document title: Information from the Standard Data Form (SDF) for *Osmoderma eremita* s.l. in the national report under Article 17 of the Habitats Directive (2019) and validity of the information
Kind of document: Microsoft Excel (OpenXML)
MIME type: application/vnd.openxmlformats-officedocument.spreadsheetml.sheet
Document name: 000516000452023-01.xlsx
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02
Document title: Records of the presence of *Osmoderma barnabita* in Bulgaria in the period 1904–2022
Kind of document: Microsoft Excel (OpenXML)
MIME type: application/vnd.openxmlformats-officedocument.spreadsheetml.sheet
Document name: 000516000452023-02.xlsx
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Research article

First records of *Sirococcus conigenus* causing shoot blight on *Pinus peuce* in Bulgaria

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Abstract: Macedonian pine (*Pinus peuce*) is a southern Balkan Peninsula endemic, growing in high mountains of Bulgaria, between 1400 and 2100 m a.s.l. Stands of *P. peuce* form the upper tree line forest areas. During a survey in 2020–2022 on Macedonian pine natural stands and plantations in Bulgaria, symptoms of shoot blight were observed in the Pirin Mts, the Rila Mts and Mt Vitosha. The fungal pathogen *Sirococcus conigenus* was identified as the causal agent of the disease that appeared for the first time on *Pinus peuce* in Bulgaria and Balkan Peninsula. Incidence of blighted shoots on individual trees varied, but was as high as 70–80% in the Rila Mts and Mt Vitosha.

Keywords: Balkan Peninsula, Bulgaria, Macedonian pine, shoot blight, *Sirococcus conigenus*

Introduction

Macedonian pine (*Pinus peuce* Griseb.) is endemic to the southern Balkan Peninsula, growing in high mountains of Albania, Bosnia and Herzegovina, Bulgaria, Greece, Kosovo, Montenegro, North Macedonia and Serbia. In Bulgaria the species is distributed in the Rila Mts, the Pirin Mts, the Western Rhodopes, the Central Balkan Range, Mt Slavyanka and Mt Vitosha, between 1400 and 2100 m a.s.l. Stands of *Pinus peuce* form the tree line forest areas in the mountains, where play an important ecological role for the stability of the forest ecosystems. Its restricted range, coupled with the effects of limited exploitation and its potential susceptibility to climate change mean that it is currently assessed as 'near threatened' (Alexandrov & Andonovski, 2011; Thomas, 2019).

In the temperate zone, the main fungal pathogens causing damage to forest tree crowns are *Gremmeniella abietina* (Lagerb.) M. Morelet, *Diplodia sapinea* (Fr.) Fuckel, *Sirococcus conigenus* (Pers.) P.F. Cannon & Minter (Capretti et al., 2013), *Dothistroma septosporum* (Doroguine) Morelet (Drenkhan et al., 2016) and *Lecanosticta acicola* (Thümen) Syd. (Tubby et al., 2023). These pathogens are among the best known agents of serious epidemics on pine species (*Pinus* spp.) (Tubby et al., 2023).

Sirococcus Preuss is a genus of asexually reproducing fungi that includes important pathogens causing shoot blight and tip dieback of conifers. *Sirococcus conigenus* is an anamorphic fungus responsible for shoot tip blight on seedlings, saplings and mature trees of several species of conifers in the temperate and boreal forests of the northern hemisphere (CABI, 2023). Subsequently, the disease was reported on a wide range of conifer hosts from genera *Picea, Pinus, Larix, Tsuga, Pseudotsuga* in Europe and North America (Peace, 1962; Sutherland et al., 1987; Butin, 1995; Smith et al., 2003; Sinclair & Lyon, 2005; Dobreva et al., 2017), and on *Picea*

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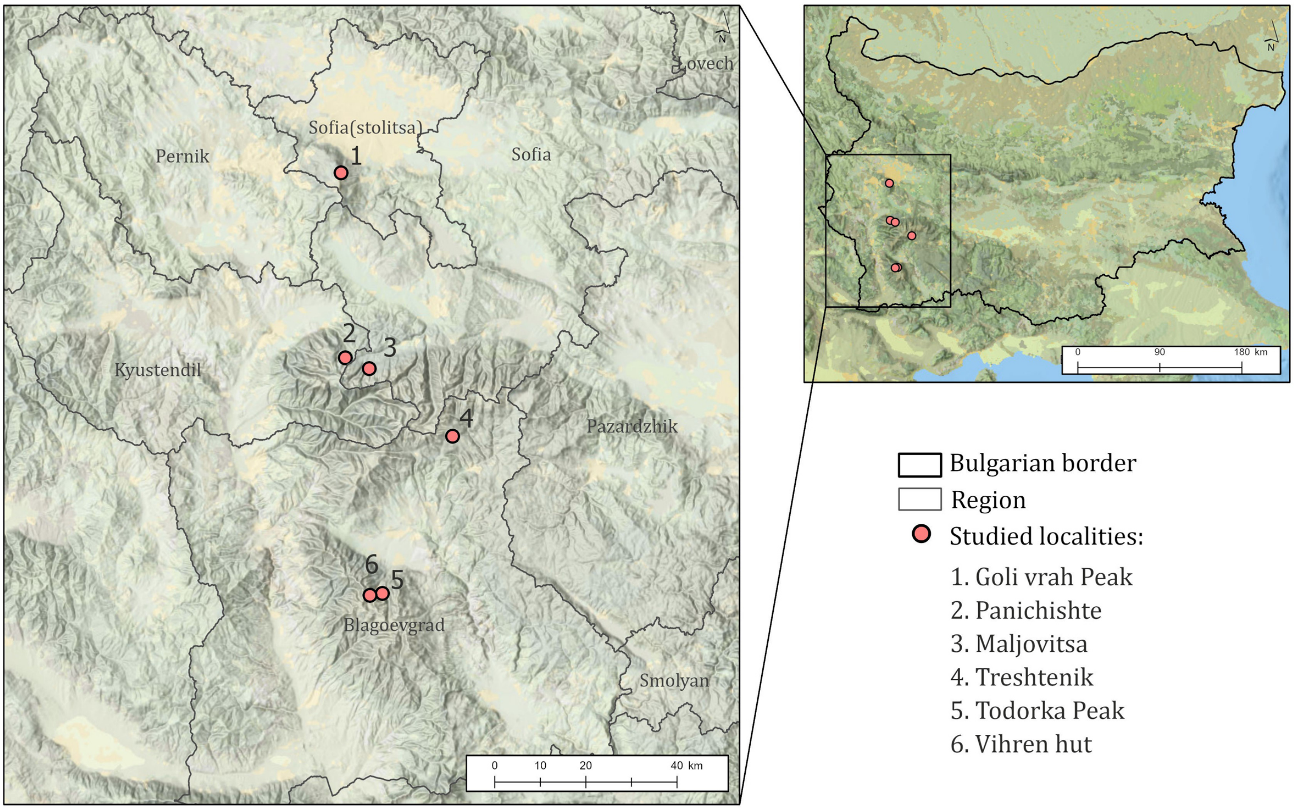

Fig. 1. Map of the studied sample plots.

spinulosa (Griff.) Henry in Bhutan, South Asia (Kirisits et al., 2007).

Sirococcus shoot blight was reported mostly from temperate and alpine forests, but also from the Mediterranean basin on Aleppo pine (*Pinus halepensis* Mill.) in Spain (Muñoz-Lopez, 1997), Italy (Danti & Capretti, 1998), and in Morocco, northern Africa (Morelet, 1972). In Bulgaria, damage on Norway spruce, *Picea abies* (L.) Karst. seedlings infected by *S. conigenus,* were reported for first time in 2016 in a forest nursery in Yundola Village (Southwest Bulgaria) (Dobreva et al., 2017).

The present note reports *Sirococcus conigenus* as a new fungal pathogen on *Pinus peuce,* causing shoot blight, which appeared for the first time in Bulgaria on this host species.

Materials and methods

In 2020, six permanent sample plots (three in natural stands in Rila, two in natural stands in Pirin and one plantation in Mt Vitosha), were selected to study the structural-functional characteristics and health status of Macedonian pine (*P. peuce*) (Fig. 1; Table 1).

In the period 2021–2022, symptoms of shoot blight were observed on Macedonian pine trees in all sample plots. The extent of the crown that was affected was estimated once a year during the period July – August for forty trees in each sample plots and recorded as follows: none (1–10%), slight (11–25%), moderate (26–60%), severe (61–99%), dead (100%) (Eichhorn et al., 2006, 2020). Records of damage caused by abiotic and biotic factors were also conducted.

Samples (symptomatic shoots and cones) were collected and transferred to Forest Research Institute, Sofia. Symptomatic needles and cone scales were surface washed with tap water for 5 min, surface sterilised for 1 min in 96% ethanol, subsequently rinsed once in sterile distilled water. Sterilised parts were placed in Petri dishes on moistened filter paper and incubated for 4–5 days at 22±2°C under artificial light. Isolations of fungal pathogen were made by placing pycnidia onto malt extract agar Difco (MEA: 20 g malt extract; 16 g agar-agar; 1000 ml tap water),

Table 1. Main characteristics of studied Macedonian pine stands.

N	Sample plots	Mountains	Origin	Latitude	Longitude	Altitude, m	Age, years
1	Goli Vrah	Vitosha	plantation	42.591972	23.292611	1814	90
2	Panichishte	Rila	natural stand	42.228167	23.325194	1948	150
3	Maljovitsa	Rila	natural stand	42.208972	23.390028	1760	110
4	Treshtenik	Rila	natural stand	42.082167	23.618028	1915	100
5	Todorka Peak	Pirin	natural stand	41.767306	23.449167	1993	150
6	Vihren Hut	Pirin	natural stand	41.761917	23.416944	1969	150

Table 2. Assessment of the degree of affected tree crowns in studied Macedonian pine stands in 2021 and 2022.

N	Sample plots	Mountains	Damaged part of crowns, %			Defoliation class (by Eichhorn et al., 2006)
			Average values		Range	
			2021	2022		
1	Goli Vrah	Vitosha	37.7	41.3	20–70	slight to severe
2	Panichishte	Rila	43.5	47.5	30–70	moderate to severe
3	Maljovitsa	Rila	53.8	48.0	30–80	moderate to severe
4	Treshtenik	Rila	23.8	26.3	10–40	none to moderate
5	Todorka Peak	Pirin	38.8	33.7	20–60	slight to moderate
6	Vihren Hut	Pirin	17.5	14.5	10–30	none to moderate

supplemented after autoclaving with 100 mg streptomycin sulphate to suppress bacterial growth. The shape and size of pycnidia were measured at magnification 40× using an ocular micrometer of Zeiss and conidia at 125× using Carl Zeiss NU2 light microscopes equipped with a digital camera DinoEye AM-423X. The identification of size and shape of conidia was made according to Butin (1995) and (Rossmann et al., 2008).

Results and discussion

During a survey on the structural and functional characteristics, and perspectives for diverse use of endemic relict coniferous forest communities in Bulgaria, symptoms and signs of the disease resembled those described for Sirococcus shoot blight were noticed on Macedonian pine (*Pinus peuce*). In the period 2021–2022, the health status of six stands of *Pinus peuce* was assessed in the Pirin Mts, the Rila Mts and Mt Vitosha. Records of damage caused by abiotic and biotic factors were conducted. Abiotic damage by strong wind, wet snow and ice-break were registered in studied stands. In the springs of 2021 and 2022, symptoms of wilting and dieback of the current year shoots were noticed in all studied stands.

In 2021, the tree crowns in different sample plots were affected by Sirococcus shoot blight on average between 17.5% and 53.8%, and in 2022 – between 14.5% and 48.0% (Table 2). Incidence of blighted shoots on individual trees varied widely, but was as high as 70–80% in Panichishte and Maljovitsa (Rila Mts), and in Goli Vrah (Mt Vitosha). In Treshtenik (Rila Mts) and Vihren Hut (Pirin Mts) none to moderate damaged crowns were observed.

Infections on shoots caused characteristics droop downward of fully-expanded needles (Fig. 2A).

Fig. 2. *Sirococcus conigenus* on *Pinus peuce:* A – typical symptoms of shoot blight disease; B – pycnidia on cone scales; C – pycnidia on needles; D – conidia.

Pycnidia (0.3–1.0 mm) long abundantly at the base of dead needles and cone scales (Fig. 2B, C), dark brown at maturity. Under humid conditions conidia oozed from pycnidia to form spore horns. Conidia with fusiform shape, one-septa, often slightly curved, 11.0–14.4 (–12.7) × 2.5–2.9 (–2.7) µm (Fig. 2D).

Based on morphological characteristics of the symptoms and reproductive structures, the pathogen *Sirococcus conigenus* (Ascomycota, Diaporthales, Gnomoniaceae) was observed as the causal agent of the disease. The current identification of this pathogen on Macedonian pine trees is the first report for Bulgaria and the Balkan Peninsula. In 2016, damage of *S. conigenus* were identified on Norway spruce (*Picea abies*) seedlings in a nursery in Yundola Village, Southwest Bulgaria (Dobreva et al., 2017).

In the present study, the pathogen was established in stands in high altitude between 1760 m and 1993 m a.s.l. The fungus was encountered on cone scales and needles of infected trees in all studied sample plots. The large numbers of spores produced on cones can contribute to infection and branch mortality of older trees or infection of seedlings and young trees growing near the older infected trees (Rossman et al., 2008). The fungus particularly affects stands in cold, humid and low light conditions in early spring (Butin, 1995). Infection in nurseries is often related to the physiological stress of seedlings in early spring when significant evapotranspiration occurs and they cannot be compensated by the absorption of water by roots.

In recent years, damage caused by the fungal pathogens were established on *Pinus peuce* trees in the Rila Mts and Mt Vitosha (Georgieva & Marković, 2018; Mirchev et al., 2021). Among the biotic factors, the main damage was caused by fungal pathogens *Heterobasidion annosum* (Fr.) Bref., *Diplodia sapinea, Cenangium ferruginosum* Fr., *Cytospora pinastri* Fr. and *Lophodermium conigenum* (Brunaud) Hilitzer. and bark beetles *Ips sexdentatus* (Börner), *I. amitinus* (Eichhoff).

In conclusion, the fungal pathogen *Sirococus conigenus* was reported for the first time on *Pinus peuce* in Bulgaria and the Balkan Peninsula as whole. Sirococcus shoot blight appeared to cause severe damage on Macedonian pine stands in high mountains in Bulgaria. The fungus has the potential to cause intense infections on other conifer species not only in nurseries and young plantations, but also in mature stands and ornamental trees in urban areas (Halmschlager et al., 2000).

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