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Research article

First record of the fungus *Setomelanomma holmii* (Pleosporales, Phaeosphaeriaceae) on *Picea abies* in Bulgaria

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Abstract: Spruce needle drop disease of Norway spruce (*Picea abies*) associated with a fungal pathogen *Setomelanomma holmii* (Pleosporales, Phaeosphaeriaceae) was established for the first time in Bulgaria in 2023. Severe symptoms of the disease were observed in 12.4% of 6-year old Norway spruce saplings planted in the nursery of Training and Experimental Forest Range, Yundola village (Southwest Bulgaria). Fruiting structures of the fungus were found on the branches and twigs of *P. abies*. Only current year needles remained on severely affected samplings. Symptoms of the disease were not detected on the Colorado blue spruce (*Picea pungens*) young trees planted in the same nursery.

Keywords: pathogen, *Picea abies, Setomelanomma holmii,* spruce needle drop

Introduction

Spruce needle drop disease caused by the fungus *Setomelanomma holmii* M. Morelet, 1980 (Pleosporales, Phaeosphaeriaceae) was first reported on *Picea pungens* Engelm. in France in 1980 (Morelet, 1980). The genus *Setomelanomma* M. Morelet is a monotypic, containing the sole species *S. holmii* that is pathogenic on branches and twigs of *Picea* species. The fungus causes chlorosis, brown discoloration and needle drop of older spruce needles in the autumn. Rossman et al. (2002) reported the presence of the fungus in North America and conducted detailed studies including both morphology and phylogeny (Zhang et al., 2012). Subsequently, *S. holmii* was reported on *P. glauca* (Moench) Voss in North America (Canada and the United States) (Rossman et al., 2002), *P. abies* (L.) Karst in the United States (Plewa et al., 2012), *P. crassifolia* Kom. in China (Wu et al., 2014). In Europe (Lithuania), the fungus was observed on *P. abies* shoots in a study for assessment of the diversity and composition of fungal communities following the invasion of spruce bud scale (*Physokermes piceae* Schrank) (Menkis et al., 2015). According to the GBIF Secretariat (2023), *S. holmii* was found in the Europe (France, Estonia, Denmark and Finland), in North America (the United States and Canada), and in Asia (China and Russian Federation). While the pathogenicity of this fungus is still under investigation, it has been reported to cause disease on white spruce (*P. glauca*) and Colorado blue spruce (*P. pungens*) in Canada (Rossman et al., 2002).

The purpose of this note is to report the presence of *Setomelanomma holmii* on Norway spruce (*P. abies*) saplings that appeared for the first time in Bulgaria.

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Material and methods

In November 2023, severe symptoms of needle drop disease were observed on 6-year old Norway spruce (*P. abies*) saplings planted in the nursery of Training and Experimental Forest Range, Yundola Village (in the pass between Rila and the Western Rhodopes mountains, 42°03′37.63″N; 23°51′01.82″E; 1375 m a.s.l.). Symptomatic saplings were eradicated and transported to the Forest Protection Station in Plovdiv and to the Laboratory of phytopathology at Forest Research Institute in Sofia. Parts of dead shoots with and without needles were surface washed with tap water for 5 min, surface sterilised for 1 min in 96% ethanol, and placed in Petri dishes on moistened filter paper. The samples were incubated at 22 °C under artificial light in a Thermo Heraeus BK 6160 testing chamber. The shape and size of 100 fruiting bodies were examined with Zeiss GSZ stereo microscope. A number of 100 asci and ascospores were measured at magnification 125× using Olympus CH20 stereo microscope. Both stereomicroscopes were equipped with a digital camera DinoEye AM-423X.

Results

A total number of 485 Norway spruce (*P. abies*) seedlings was planted in the nursery of Training and Experimental Forest Range in Yundola Village in 2020. In November 2023, symptoms of spruce needle drop disease were observed on 60 young trees (12.4%). Forty young symptomatic saplings (66.7%) were completely defoliated and dead, and the rest ones (33.3%) were partially affected. On the severely affected saplings dry needles were left only on the tips of twigs that were hanging down (Fig. 1A, B). Symptoms of the disease were not detected on the Colorado blue spruce (*Picea pungens*) trees planted in the same nursery.

Abundant small black dots were visible in November on the Norway spruce twig surface. Fruiting bodies (ascomata) were found on current and second year old host twigs. The observed perithecia were 100–275(187) µm in diameter, black, globose to subglobose, with black setae (Fig. 1 C, D). Asci were with broadly cylindrical shape, 8-spored, 66.9–83.5(75.3) × 12.1–13.4(12.8) µm in size (Fig. 1E). The size of measured ascospores was 17.5–21.1(19.0) × 6.4–7.9(7.3) µm, ellipsoidal, hyaline to pale yellowish, transversely septate with three-septate, slightly constricted at the septa (Fig. 1F).

Discussion

The size, shape and colour of fruiting bodies, asci and ascospores of *Setomelanomma holmii* were consistent with descriptions of the fungus made by Rossman et al. (2002).

In our study the fungus was identified on the basis of morphological characteristics as the causal agent of Spruce needle drop disease on *Picea abies* saplings in Southwest Bulgaria. Black flask-shaped fruiting bodies were found on the surface of the branches and twigs (Fig. 1C, D). According to Rossman et al. (2002), the ascomata of *S. holmii* develop in late May and early June, while needles are still present. O'Brien (2013) noticed that all of the needles on the affected branches drop off by the end of the summer, except the current needles on the tips of the branches. In our study symptoms of the disease were observed in November when gradual loss of needles were detected on 12.4% of severely affected trees.

Spruce needle drop disease is consistently associated with the fungus *Setomelanomma holmii*. Its fruiting structures on the twigs were often associated with spruce decline (needle browning and drop off) (Rossman et al., 2002; Wu et al., 2014; Jayasiri et al., 2015). Spruce needle drop disease affects stressed trees (Eilers et al., 2021). It is thought that the environmental stress (long period of drought and high temperatures) can trigger the pathogen growth and subsequently loss of needles. In 2019 the fungal species was listed in the EPPO Plant Quarantine Data Retrieval System (EPPO, 2019).

Since the spruce needle drop disease is a relatively new problem, not many details are known about its life cycle. It is known that the fungus overwinters in infected branches (Eilers et al., 2021). Kosawang et al. (2018) revealed that fungal endophytic communities of tolerant ash species including *Setomelanomma holmii* may protect them from ash dieback. The selected endophytes have the potential to act as biocontrol agents with high antagonistic activity against *Hymenoscyphus fraxineus* causing ash dieback that threatened ash trees in Europe for more than two decades (Pacia et al., 2023).

The fungus *S. holmii* was first reported in North America in 1998 caused discolouration and needle

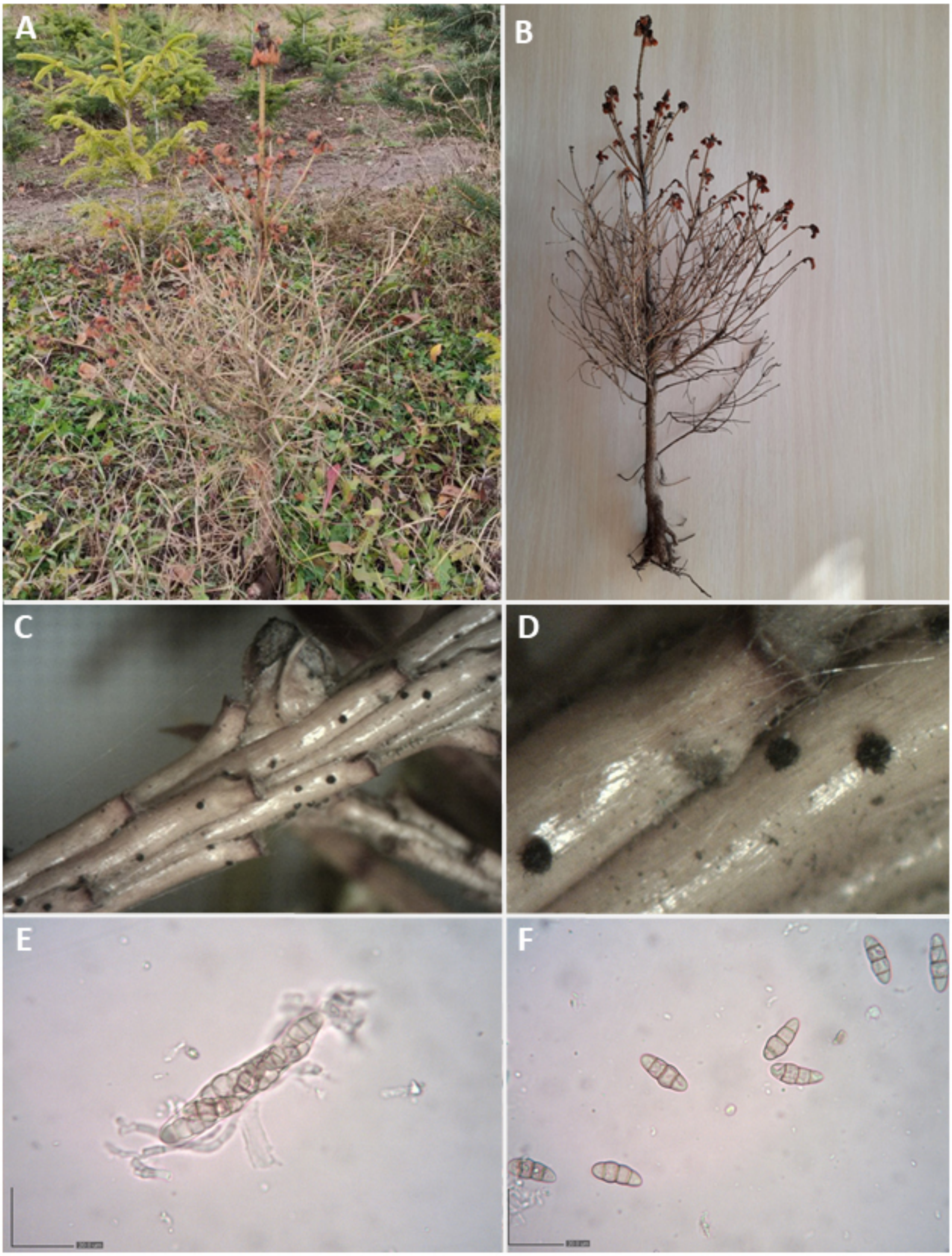

Fig. 1. *Setomelanomma holmii* on *Picea abies* saplings: A and B – infected saplings of *Picea abies;* C and D – ascomata on twigs; E – asci; F – ascospores. Scale bars: E and F = 20 μm.

drop of older needles on *Picea glauca* and *P. pungens* (Rossman et al., 2002). On the herbarium material examined of *P. pungens* branches from the United States (Kansas, Shawnee County, collected in May 1959), black fruiting bodies of *S. holmii* were found (Jayasiri et al., 2015), which is an indication that the pathogen most likely has a North American origin. No anamorph is known for *S. holmii* on natural substrata or in culture on agar (Rossman et al., 2002).

The current study of pathogen *S. holmii* on Norway spruce saplings is the first report for presence of the fungus in Bulgaria. It probably has the potential to cause intense problems on stressed and physiologically weakened young spruce plants of different species planted in the nurseries and plantations. In 2016, the fungal pathogen *Sirococcus conigenus* (Pers.) P.F. Cannon & Minter was identified for the first time on Norway spruce in the same nursery (Dobreva et al., 2017). It is necessary to monitor for the presence of the pathogens not only in the studied area, but also where spruce species are planted in forests or as an ornamental trees in urban green ecosystems.

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Research article

Lizards in the dark: first records of nocturnal activity of the eastern green lizard (*Lacerta viridis*) (Reptilia: Lacertidae) in Bulgaria

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Abstract: We present the first in situ observations of nocturnal activity in *Lacerta viridis* from two different regions in Southern Bulgaria. All three cases involved adult males observed on the surface in an artificial light-free environment between 41 and 103 minutes after sunset. A possible explanation for this unusual behaviour is discussed.

Keywords: Balkan Peninsula, lacertids, unusual activity timing, Sauria

Introduction

While some lizards, such as geckoes, are mostly nocturnal, the majority – especially those found in Eurasia – are primarily active during the day (Vidan et al., 2017). Species may extend their activity in response to extreme thermal conditions or artificial lighting (Perry et al., 2008; Amadi et al., 2021). Novel food resources and decreased predation may favour opportunistic nocturnal activity in some species (Hódar et al., 1996; Gordon et al., 2010).

As strictly heliothermic species that rely on sunlight for thermoregulation, lacertids are widely considered diurnal (Avery, 1982; Castilla et al., 1999). They persist in their circadian rhythms even in different laboratory conditions (Molina-Borja et al., 1986), and are well adapted for diurnal activity, evolving multiple anatomical and physiological traits that allow them to thrive in diurnal environments (Hall, 2008; Pérez i de Lanuza & Font, 2014; Martin et al., 2015). However, there are anecdotal reports of nocturnal activity of lacertids (e.g., Carretero et al., 2012; Afsar et al., 2018), and those related to wild and undisturbed areas are a few (e.g., Carretero & Rato, 2013). Recently nocturnal activity was recorded for a typically diurnal lizard species, the snake-eyed skink, *Ablepharus kitaibelii* (Bibron & Bory de St-Vincent, 1833), occurring on the Balkan Peninsula (see Tzoras et al., 2025).

The eastern green lizard, *Lacerta viridis* (Laurenti, 1768) is one of the largest representatives of its family in Eastern Europe, reaching up to 150 mm snout-vent length (Vacheva et al., 2022). It occurs on the Balkan Peninsula, parts of Central Europe, southern Ukraine and northern Turkey (Sillero et al., 2014; Marzahn et al., 2016). In Bulgaria, *L. viridis* is distributed throughout the country, usually found up to about 1200 meters above sea level, with some observations at altitudes up to 1800 m a.s.l. (Stojanov et al., 2011). It inhabits open and semi-open habitats, such as meadows with sparse shrub vegetation and forest edges, but can also be found in areas that are densely covered with shrubs or

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sparse forests (Stojanov et al., 2011; Vacheva et al., 2020).

To our knowledge, there have been no reports of nocturnal activity of *L. viridis* in nature so far; here we present the first such cases in Bulgaria.

Material and methods

Data on the nocturnal activity of *Lacerta viridis* presented below were collected during field trips aimed at finding nocturnally active snakes. We conducted visual searches using head lights between sunset and midnight. The first site was the Kresna Gorge (SW Bulgaria; N41.76070° E23.15200°, 200 m a.s.l.; N41.76930° E23.15631°, 220 m a.s.l.), sampled on 21 and 22 June 2023, 21 May 2024, and 11 June 2024. The only human infrastructures in the vicinity are a major two-lane international road E79 and a railway line that pass through the gorge. There is no artificial lighting in the area. The second site was 500 meters north-west from the nearest village of Meden Buk, at the foot of a hilly area (Eastern Rhodopes Mts, N41.38000° E26.02667°, 120 m a.s.l.), where we conducted night surveys 29–31 July 2024. The observation was made next to a building without electricity. The individuals from Kresna were captured by hand. Their cloacal body temperature was measured immediately using a digital probe thermometer with a resolution of 0.1 °C. Snout-vent length was measured using a plastic ruler with an accuracy of 1 mm. Substrate and air temperatures were measured at ca. 20 cm above the ground. Relative air humidity was measured using a digital hygrometer with a resolution of 1%. All individuals were measured at the capture site and released shortly after that. Furthermore, the following parameters were taken into account: maximum air temperature on the relevant date (measured by us at noon); meteorological conditions at the time of lizard registration and for the relevant date as a whole (wind strength and rainfall based on our estimation); exact time of the sunset, dusk duration and moon phase (according to Anonymous, 2023, 2024).

Results and discussion

Three male *Lacerta viridis* were found active between 41 and 103 minutes after sunset (Table 1). The observations in May 2024 and June 2023 referred to the Kresna Gorge, and the observation in July 2024 – to the area of Meden Buk Village. All three cases should be considered as behaviour of nocturnal activity, as the lizards were registered after twilight [the dusk lasted between 33 and 39 minutes during the corresponding months (Anonymous, 2023, 2024)].

It should be noted that in the period 1987–2024, dozens of night visits to the Kresna Gorge have been conducted by other colleagues and us (e.g., Dyugmedzhiev et al., 2020), but nocturnal activity of *L. viridis* has only been recorded in the last years (in 2023 and 2024). Considering the environmental parameters recorded, we argue that the lizard nocturnal activity observed was not caused by extreme weather conditions. However, it should be borne in mind that the temperatures we have indicated are the maximum measured for the day, but we do not have systematically collected data on the temperatures for the entire day. For the observations in the Kresna Gorge, the measured body temperature of the captured lizards were higher than the environmental temperatures (see Table 1). Therefore, likely at the time of capture these individuals had not yet entered their night shelters; we do not think they had come back to the surface after retreating for the night by accident (e.g., by being frightened by something in their shelters). We note the following about the observation at Meden Buk: presumably the lizard was first seen at 20:44 h (i.e., 7 minutes after sunset) chasing a large grasshopper (*Decticus* sp.), then lost from sight, but at 21:18 h (i.e., 41 minutes after sunset) it was seen again in the same place. Based on this, we suggest that the lizard's presence on the surface at dusk may have been related to recovering energy spent during hunting. The daily activity period of lacertids depends on numerous endogenous factors in addition to the exogenous parameters of light and temperature (Nettmann & Rykena 1984). Although several observations from different locations may indicate a probable cause, our observations are not sufficient to provide an explanation for the reasons for the nocturnal activity of *L. viridis* and purposeful research in this direction is needed. In contrast to most of the other records of crepuscular or nocturnal activity in strictly diurnal species, where light pollution was involved and that often lead to thermal and trophic benefits (Carretero et al., 2012; Afsar et al., 2018; Amadi et al., 2021; Baxter-Gilbert et al., 2021), such observations of lizards in undisturbed, natural habitats are scarce (e.g., Carretero & Rato, 2013).

Table 1. Registrations of nocturnal activity in *Lacerta viridis* by location. *yes/no refers to presence/absence of visible moon in the sky at the time of lizard registration and, in brackets, the percentage of illuminated moon on the date.

	Kresna Gorge		**Meden Buk**
Observation date	May 21, 2024	June 21, 2023	July 30, 2024
Observation time (UTC +3)	22:00 h	22:50 h	21:18 h
Lizard snout-vent length	119 mm	83 mm	n/a
Lizard body temperature	25.8 °C	28.4 °C	n/a
Substrate temperature	24.2 °C	24.8 °C	n/a
Air temperature	18.4 °C	26.8 °C	25.0 °C
Relative humidity	66%	53%	47%
Wind strength	weak	weak	weak
Rainfall	no	no	no
Visible moon*	no (97%)	yes (11%)	no (27%)
Sunset time (UTC +3)	20:48	21:07	20:37
Dusk duration	33 min	39 min	34 min
Maximum T (at noon)	27.4 °C	32.8 °C	33.5 °C
Wind strength (during the day)	weak to moderate	weak to moderate	weak
Rainfall (during the day)	no	no	no

Nocturnal activity, associated with reproduction, is known for the closely related species *L. bilineata* Daudin, 1802, distributed in the Apennine Peninsula and parts of Western Europe (Sillero et al., 2014). For example, Weber (1957) observed in terrarium conditions copulation at dusk, and egg laying only at night, while Baron (1996) reported egg laying at 0:30 and 1:30 in the wild. Our observations are probably not related to breeding since we observed only males and at least two of them occurred after the period of mating, which in Bulgaria lasts from mid-April to mid-May (Stojanov et al., 2011).

We speculate that the most probable explanation of this behaviour is high daytime temperatures. Lacertid lizards in hot and dry environments may shift their activity to cooler periods, such as dawn or dusk, to maintain optimal body temperature. This would explain the observed cases during the warm summer months. Still the recorded daytime temperatures do not indicate extreme values (Table 1), therefore we cannot categorically state that. However, extended activity during the evening and night might provide alternative feeding opportunities. Considering that some insects are active around dusk or at night, such as the Decticus sp. preyed by one of the individuals we observed, we could have observed opportunistic nocturnal foraging (Baxter-Gilbert et al., 2021). Nevertheless, extending activity period may benefit lizards and the nocturnal activity of *L. viridis* in South Bulgaria is likely driven by a combination of various ecological factors like thermal constraints and foraging opportunities.

The data presented so far on nocturnal activity in entirely diurnal species is not sufficient to formulate a specific hypothesis, but it provides a good basis for future and purposeful research in this direction, aiming to clarify the possible reasons for the extended activity.

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