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RESEARCH ARTICLE

The potential threats posed by the invasive bark beetle *Polygraphus proximus* (Coleoptera, Curculionidae: Scolytinae) to a natural park in the Middle Urals (Russia)

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Abstract

The four-eyed fir bark beetle *Polygraphus proximus* Blandford, 1894 (Coleoptera, Curculionidae: Scolytinae) is an invasive species that originates from the Far East. Since early 2000s, it became known as an aggressive pest causing massive dieback of Siberian fir (*Abies sibirica* Ledeb.) in Siberia and, to some extent, in the European part of Russia. Here, we report the first record of *P. proximus* in Sverdlovsk Region (the Middle Urals, Russia). In summer 2023, *A. sibirica* trees colonized by the pest were discovered on the territory of the "Olenyi Ruchyi" Natural Park. The dendrochronological analysis of the cores of fir trees killed by the beetle indicated that the majority (72%) of these trees died in 2021–2022, while the earliest tree death occured already in 2007 and 2009, meaning that the pest was present in the

Natural Park for at least two decades. Mycological analysis of bark and wood samples of infested trees revealed the fungus colonies assigned to *Grosmannia aoshimae* (Ohtaka, Masuya & Yamaoka) Masuya & Yamaoka. Associated with *P. proximus*, this Far Eastern fungus species has spread with the beetle to Siberia and the Urals, contributing to tree mortality. Siberian fir is present on 69% of the forested area in the "Olenyi Ruchyi". The colonization of fir trees by *P. proximus* recorded in the park in 2023 alarms about the high risk of the pest outbreak in the coming years, which may lead to subsequent tree death over a huge territory of the park affecting its ecological, cultural, and recreational values. To prevent the dramatic impact, it is highly urgent to take all the necessary measures to suppress the distribution and combat this alien pest.

Keywords
Xylophagous species, alien pest, fir forest stands, tree mortality, *Grosmannia aoshimae*

Introduction

According to the Federal Law «On Specially Protected Natural Territories» (no. 33-F3 of March 14, 1995), natural parks are specially protected natural areas of regional significance, playing important ecological, cultural, or recreational roles. According to this, biological invasions pose significant threats to the functions of natural parks.

The four-eyed fir bark beetle *Polygraphus proximus* Blandford, 1894 (Coleoptera, Curculionidae: Scolytinae) is an invasive bark beetle from the Far East. Since the 2000s, it became known as an invasive pest in Siberia and in some parts of the European part of Russia, causing the decline of Siberian fir (*Abies sibirica* Ledeb.) over huge territory (Krivets et al. 2015). The pest continues to move westward in Russia (Krivets et al. 2015, 2018). Furthermore, in 2023, it was documented for the first time in the Republic of Kazakhstan (Kirichenko et al. 2023). The success of rapid colonization of Siberian fir, a new host plant of the bark beetle, is largely due to both the structural features of the fir bark (Baranchikov et al. 2014a; Astrakhantseva et al. 2023) and the phytopathogenic activity of the ophiostomatoid fungus associated with the bark beetle, *Grosmannia aoshimae* (Ohtaka, Masuya & Yamaoka) Masuya & Yamaoka (Pashenova, Baranchikov 2013).

In some regions of Western Siberia, the pest kills up to 90% of mature firs in forests and up to 50% of the fir undergrowth (Krivets et al. 2018). According to a decision of the Council of the Eurasian Economic Commission (Decision… 2023), *P. proximus* was included in the list of quarantine pests, having limited distribution on the territory of the Eurasian Economic Union. Furthermore, it was listed as quarantine pests for the countries of the European and Mediterranean Plant Protection Organization (*Polygraphus proximus* 2023).

Several articles on the invasion of this species in various regions of the Russian Federation have been published, including several reviews of its biology and ecology in Siberia and the European part of Russia (Krivets et al. 2015, 2018; Chilakh-

saeva et al. 2019; Krivets et al. 2019). Although *P. proximus* caused the mortality of fir stands in Russia, until 2023, there was no information about its presence in the Middle Urals.

The purpose of this study was to explore fir stands in the "Olenyi Ruchyi" Natural Park located in the Middle Urals (Russia) to determine the presence of *P. proximus*, date its presumable occurrence and discuss the potential threats that this species can pose to the forest stands of the Natural Park.

Materials and methods

The study was carried out in the Natural Park "Olenyi Ruchyi" in March-October 2020 and 2023. The following tasks were targeted: the detection and identification of the beetle and the assessment of demographic characteristics of the newly discovered population; the detection and identification of phytopatogenic fungi in the beetle galleries; the dendrochronological analysis to date the pest invasion in the park.

The general characteristics of the Natural Park

The Natural Park "Olenyi Ruchyi" was established on October 29, 1999 (Decree… 1999). The total area of the Natural Park is 18871 hectares. The territory consists of forest lands, lands of specially protected areas and objects, agricultural lands, reserve lands, and water area. The Natural Park is located in the southwestern part of Sverdlovsk Region (Fig. 1), in the Middle Urals low-mountain (Kolesnikov 1974). Thus, the "Olenyi Ruchyi" is located in a typical forested area. In the past, this area was covered almost entirely by broadleaf-fir-spruce forests with linden and, in some localities elm and maple were present in the undergrowth. Currently, a vast area of the Natural Park is occupied by mixed forest. Forest lands include the territories of Nizhne-Serginsky forestry, and parts of sites in the Mikhailovsky and Bardymsky forest districts: Mikhailovsky, Buysky, and Byrdymsky. The administrative center of the Natural Park is located in the village of Bazhukovo (56.52° N, 59.22° E).

The Natural Park includes a section of the Serga River valley and the western slope of the Bardymsky ridge from the city of Nizhnie Sergi to the village of Arakaevo. The Serga River serves as the border between the Sredneuralsk mountain taiga landscape and the Krasnoufimsk forest steppe. The variety of landscape conditions and the borderline character of the Serga River valley in its downstream supports a wide diversity of animals and plants. It is the most visited natural park in Sverdlovsk Region, with up to 3000 visitors per day (Natural Park… 2024).

The detection, identification, and study of the demographic characters of the alien beetle

In early March 2020, three trap fir trees were placed in coniferous stands of the Natural Park about 150-200 m from the "Nizhniye Sergi" train station. The 20 cm bark strip was removed on the three trap trees at a height of 1.3 m. Trees were placed 10-15 m away from each other. The inspection of these trees for beetle attacks was carried out in mid-August 2020. Furthermore, live trees with intense resin flaw on their stems discovered by employees at the Natural Park in the end of July 2023, were also inspected.

When surveying fir trees, we searched for *P. proximus* damage signs; the species identification was done based on morphological characteristics (Krivets et al. 2015; Kirichenko et al. 2023). The beetles were collected in tree stands near the village of Bazhukovo on August 21, 2023. Overall, 10 beetles were sampled from five dead but still standing trees, in which the exit holes were found. Independently, the Phytosanitary Surveillance (Department of Sverdlovsk Region) collected samples of this alien pest species for identification. The number of entry and exit holes per dm^2 of bark of the species was estimated following Krivets et al. (2018). The health category of trees was accessed according to the classification given in Krivets et al. (2015a). The proportion of fir trees in the forest stands of the "Olenyi Ruchyi" was calculated using forest inventory materials. Forest inventory data was done in 2015 on an area of 9.8 hectares of the park. The work was carried out by Ural Forest Inventory Expedition LLC at the request of Forestry Department of Sverdlovsk Region. The calculations of the proportion of fir in forest stands were based on updated forest inventory materials within the forested area of the Natural Park (Taxation description 2016a, 2016b, 2016c).

Detection and isolation of phytopatogenic fungi

In the middle of October 2023, the bark and underlying wood samples of Siberian fir trees infested with *P. proximus* in the "Olenyi Ruchyi" were examined for the presence of pathogenic fungi. Bark and wood (2-3 × 3-5 cm) containing the bark beetle galleries or necrotic spots, which occured after unsuccessful penetration of beetle adults through the bark, were removed. Individual samples of bark and the wood were quickly flamed and separately placed in Petri dishes (90 mm in diameter) on a layer of 2% malt extract agar with lactic acid added (4 ml per liter of medium). The bark beetle galleries and necrotic spots on the bark and wood samples were facing the Petri dish lids. The observations and isolation of fungi in pure culture were carried out every week for one month. To examine the samples, a MBS-9 stereomicroscope was used. 2% malt extract agar was used to isolate, cultivate and maintaining the fungal cultures in the collection. Primary fungus identification was carried out based on cultural and morphological characteristics (Pashenova and Baranchikov 2013).

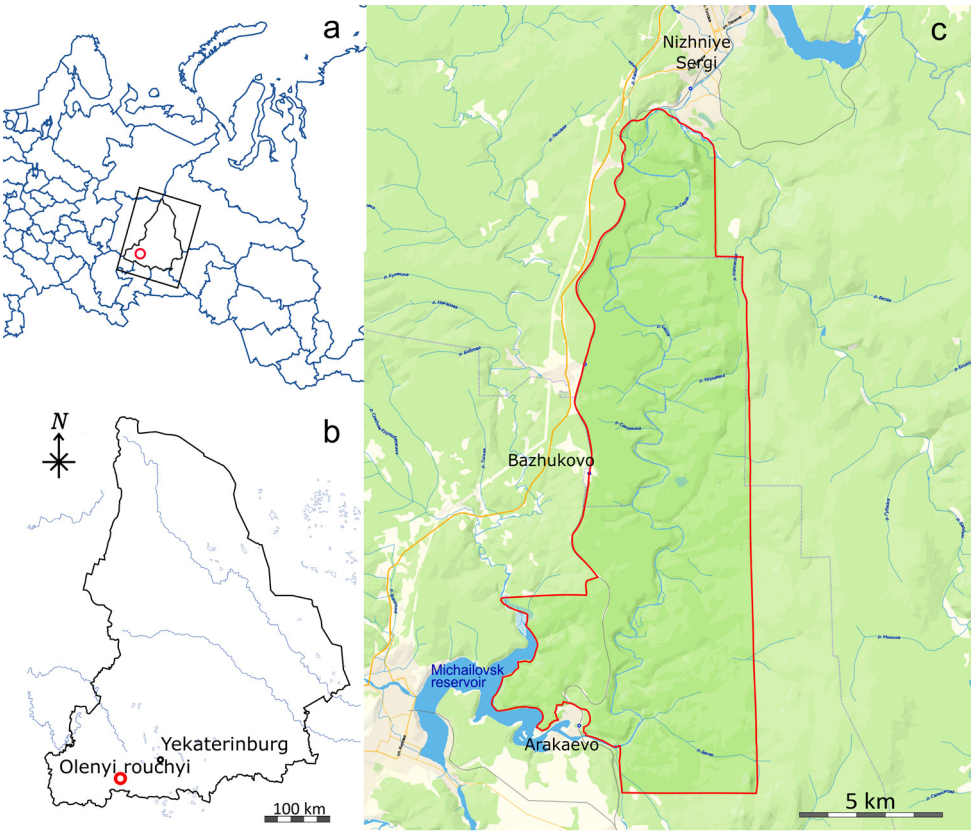

Figure 1. The location of the Natural Park "Olenyi Ruchyi": Sverdlovsk Region, the east of the European part of Russia (a); the boundary of Sverdlovsk Region, indicated by black line (b); the map of the Natural Park, with the park boundary shown by read line (c).

The dating of the beetle invasion

The tree cores were sampled from the firs killed by *P. proximus* for dating the beetle invasion to the territory of the Natural Park, and from the live intact firs for control. The cores were collected at several locations nearby the tourist trails on October 16, 2023. We took transverse cores, whenever possible, at 1.5 m height from the ground. Overall, 54 cores were sampled from 37 trees (i.e., 1-2 cores per tree). Among these 37 trees, 27 were killed by the beetle and 10 were live trees (with green crown). The cores were dried, mounted on wooden support, and polished on sandpaper with progressively fined grain (to 1000 ISO 6344).

The polished cores were scanned with a resolution of 2400 dpi and ring width was measured in CooRecorder software (Cybis, Sweden). The obtained time series was cross-dated in CooRecorder software (Cybis, Sweden). Before cross-dating, the series was detrended by first-order differences. Cross-dating was performed by leave-one-out method; the Pearson correlation coefficient (r) 0.4 indicated the suc-

cess for the dated series. In total, 28 out of 37 trees (i.e., 18 dead trees and 10 live trees) were dated successfully, and 9 dead trees failed to be dated. The series with obviously inconsistent radial growth changes were excluded. The tree-ring statistics for the series chosen in the previous step (including living trees) after prewhitening (Cook and Kairiukstis 1990) were calculated in the dplR package (Bunn 2008). The first of the key statistics is effective correlation coefficient, i.e., measure of overall (joined within-tree and between-tree) radial growth consistency. The second statistic, EPS (expressed population signal), is an indicator of the representativeness of our sample. The conventional lower limit of EPS is 0.85 (Cook and Kairiukstis 1990). Prewhitening allowed us to remove the trend and low-frequency oscillations but retain the high-frequency ones. At this step, Pearson's coefficient *r* for the series was calculated using the leave-one-out method after prewhitening, which is a well-elaborated method of detrending (Cook 1985).

Results

Search for *P. proximus* in the Natural Park in 2020 and 2023

In mid-July 2023, fir trees with notable resin flow on the trunks, and the beetle's galleries under the bark were discovered in the forest stand next to the village of Bazhukovo (i.e., in the administrative center of the park) (Fig. 2). A more detailed examination performed in mid-August 2023 revealed the trees with additional signs of colonization by the beetle, i.e., redness of tree crowns, numerous exit holes on the bark, maternal galleries, and pupal chambers in sapwood.

In 2023, the number of fir trees with red needles and exit holes was relatively small. Overall, 40 out of 200 trees (i.e., 20% of the trees examined) had notable resin flow and numerous entrance holes in the bark, corresponding to the tree state categories III-IV (i.e., severely weakened and attacked or already colonized by the beetle), according to the classification given in Krivets et al. (2015). Furthermore, the number of fir trees with red crowns and numerous exit holes, which corresponded to the category V (recently dead standing trees), was relatively small, about 7% of all trees examined. The density of the exit holes in the trees with red needles varied from 3-4 to 10-15 holes per dm^2. And finally, around 6% of the trees examined were assigned to the category VI (old dead standing trees without needles), suggesting that *P. proximus* was already present on the territory of the Natural Park for some years. The density of exit holes on the trees with red needles ranged from 3-4 to 10-15 holes per dm^2. Some beetles were unable to vacate the galleries, and at the time of examination, they were found dead under the bark.

Identification and dating the invasion

The beetle specimens were identified to the species level by V.I. Ponomarev and B.V. Krasutsky (Institute Botanic Garden Ural Branch Russian Academy of Sciences, Ekaterinburg) based on the morphological characteristics (Krivets et al. 2015; Kirichenko et al. 2023). The species was confirmed by Yu.N. Baranchikov and A.A. Efremenko (Sukachev Institute of Forest Siberian Branch of Russian Academy of Sciences, Krasnoyarsk). At about the same time, the species was also reported by the Sverdlovsk Department of the Federal Center of Animal Health (FGBI "ARRIAH") in the phytosanitary document (No 006602-558-23).

The results of the dendrochronological analysis of the cores sampled from the fir trees that died due to *P. proximus* provided more clarity about the invasion of the pest. The values of the effective correlation coefficient and the expressed population signal (EPS) indicated that the cross-dating results were reliable and the samples studied were representative (Table 1).

Figure 2. Trees damaged by *Polygraphus proximus* in the Natural Park "Olenyi Ruchyi": a) resin flaw on recently attacked tree trunk; b) dead tree with beetle galleries under the bark. Photos: V.I. Ponomarev.

Table 1. Tree-ring series statistics of fir trees analyzed in the "Olenyi Ruchyi" Natural Park

No of series	No of trees	Average length of series, years	Average tree diameter, cm	Average ring width, mm	Effective correlation	EPS
34	28	62	23.5	1.5	0.604	0.977

Dendrochronological analysis showed that the majority of trees attacked by *P. proximus*, i.e., 13 out of 18 trees (72% of all successfully dated dead trees), died in the "Olenyi Ruchyi" in 2021-2022 (Fig. 3).

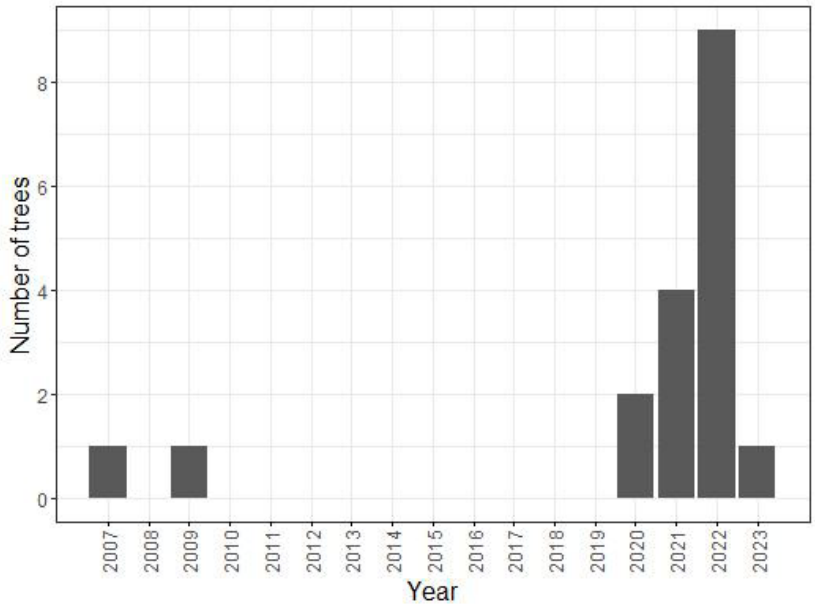

Figure 3. The mortality dynamic of fir trees colonized by *P. proximus* in the "Olenyi Ruchyi" Natural Park based on the dendrochronological analysis.

The first tree death associated with *P. proximus* occurred in 2007 (tree no. 2; the core series OlR02a in our study) and 2009 (tree no. 21; the core series OlR21b). The correlation coefficients with the master chronology calculated using the leave-one-out method were equal to 0.55 and 0.35, respectively (Fig. 4). The diameter of attacked trees was relatively small, and these trees were probably oppressed by the trees from the canopy. The attacked trees had smaller ring width over the average ring width across all studied samples (0.74 mm for OlR02a and 0.61 mm for OlR21b) (Table 1).

Identification of phytopathogenic fungi associated with *P. proximus*

The late timing of the sample collection did not allow us to identify the whole complex of ophiostomatoid fungi associated with the bark beetle in the Middle Urals. However, in all parts of the beetle galleries (i.e., mating chambers, maternal, and larval galleries), large perithecia of varying degree of development were found. In all the bark samples and the beetle galleries, large perithecia were documented. The isolation of the fungus, which formed these perithecia, into a pure culture allowed to unmistakably identify the species, *Grosmannia aoshimae* (Ohtaka, Masuya & Yamaoka) Masuya & Yamaoka.

In addition to *G. aoshimae*, the bark samples studied in the beetle galleries contained the conidiophores of other unidentified fungi from the genus *Geosmithia* Pitt, which are usual members of the mycocomplex associated with *P. proximus* in its primary and secondary ranges (Pashenova et al. 2017).

Already during the first week of laboratory observations, the formation of fruiting bodies of *G. aoshimae* with varying degrees of abundance was observed in the bark samples (Fig. 5). This indicates that at the end of the growing season, the inner bark of tree trunks inhabited by the bark beetle was largely colonized by the fungus mycelium.

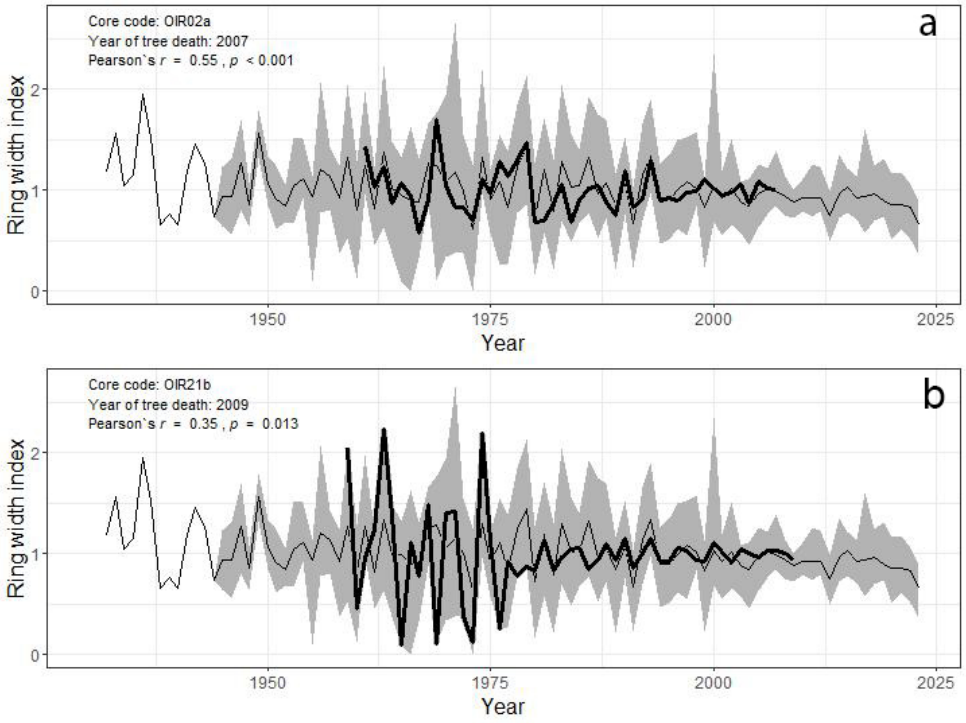

Figure 4. The verification of the dating quality of trees colonized by *P. proximus* vs. the earliest tree death recorded in 2007 (a) and 2009 (b) in the "Olenyi Ruchyi" Natural Park. The master chronology is indicated by thin dark-gray line; the range between min and max values of growing indices in series is shown by the band filled with gray color; the probe series are shown by bold line.

The signs of *G. aoshimae* were absent in the bark samples of the trees that were attacked for the first time and/or actively resisted the attack of *P. proximus*: the necrotic spots of the fir phloem after unsuccessful attacks varied from 5 to 10 mm in diameter. However, in the samples collected from the weakened trees, which were slower to implement a protective response, fruiting bodies of *G. aoshimae* were already observed. In such trees, phloem necrosis around the beetle entrance holes has

reached 25-35 mm in diameter. This suggests that *G. aoshimae* can begin to weaken the host plant even before the intense colonization of the tree trunk by *P. proximus*.

Discussion

We performed a first survey of fir trees in the Natural Park "Olenyi Ruchyi" in 2020, but were unable to discover the pest signs in the trap trees exposed in the park's forested area. It could suggest the absence of *P. proximus* in the examined stands in 2020 or its presence in small number. The latter seems to be more likely, given the beetle abundance in the same forest stand in 2023, as per our current observations.

Most likely, the beetle arrived to the "Olenyi Ruchyi" about 20 years ago by the Trans-Siberian Railway, which runs approximately 10 km from the northern border of the park. This is a common route of *P. proximus* dispersal from the Far East to Siberia (Krivets et al. 2015). Another possibility is that the beetle arrived to the "Olenyi Ruchyi" from Chelyabinsk Oblast, where it had appeared erlier and killed firs on a large territory of the Natural Park "Taganay" (near the Zlatoust town) in 2023 (Four-eyed fir bark beetle… 2023). The distance from the "Taganay" to Bazhukovo village (the administrative center of "Olenyi Ruchyi") is about 100 km in a straight line.

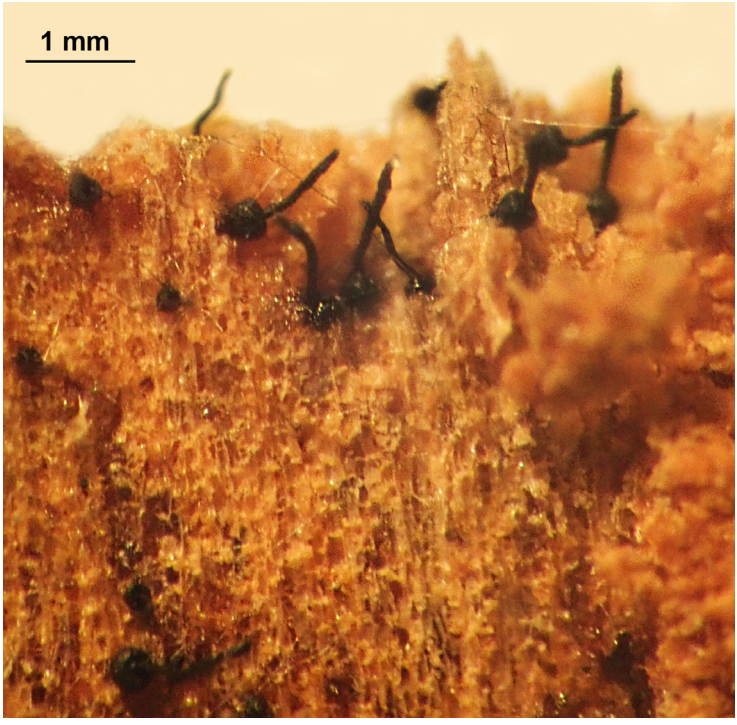

Figure 5. Perithecia of the phytopathogenic fungus *Grosmannia aoshimae* developed in the bark of a fir tree next to the gallery of *P. proximus*. Photo: N.V. Pashenova.

The gap between the first cases of tree death (i.e., 2007 and 2009) in the "Olenyi Ruchyi" and the subsequently observed decline of tree stand in 2021-2023 is probably related to the active decomposition of wind fallen trees and difficulty to date the weakened trees correctly, because of their differences in radial growth with the general pattern of the examined fir stand. For example, nine dead fir trees were excluded from the dendrochronological analysis due to this problem.

Usually, when *P. proximus* arrives to the new territory, it first colonizes weak trees and, under favorable conditions, it starts to increase the population density (Krivets et al. 2015). At the next step, increasing attacks on healthy fir trees usually occur, which is an evidence of the pest foci development. In this situation, *P. proximus* is able to weaken and colonize trees of different age, including young trees with a stem diameter of 6 cm (Krivets et al. 2015). We believe that in the "Olenyi Ruchyi", the bark beetle did not show a notable aggressiveness to the fir trees in the park until recently, and it first attacked weakened trees with small stem diameter. After the beetle population grew, it started colonizing healthy trees with larger diameters. In the galleries of beetles, we detected the presence of the ophiostomatoid fungi *G. aoshimae*, the Far Eastern associate vectored by the beetle, which adds to the tree decline attacked by *P. proximus*.

What threats does this invasive bark beetle pose to the "Olenyi Ruchyi"? In the Natural Park, the forest covers 86.4% of the territory, where coniferous trees, i.e., Siberian fir (*A. sibirica* Ledeb.), Siberian spruce (*Picea abies* (L.) H. Karst), and Scots pine (*Pinus sylvestris* L.), and deciduous trees, such as silver birch (*Betula pendula* Roth), aspen (*Populus tremula* L.) and gray alder (*Alnus incana* (L.) Moench) are largely present (Taxation description 2016a, 2016b, 2016c). The forests predominated by Siberian fir are uneven throughout the park, with fir wood stock of 10-13% (exceptionally 16%) of the total stock of forest stands of the park. Fir trees are either present singly or by groups/blocks. In forest stands, fir trees are present in the canopy (53% of all forested areas in the park) and as undergrowth without current presence in the canopy (16%) (Table 2). In particular, in the zone of high recreation activity, where the main tourist routes are developed (i.e. 1701.7 ha), fir trees are present in the canopy in an area of 542 ha (i.e. about 32%) and solely in undergrowth on an area of 56 ha (3%) (Table 2).

Based on observations in the secondary range of *P. proximus* in West Siberia, individual fir trees die rapidly, 2-4 years after they are colonized by this invasive species (Krivets et al. 2015). Furthermore, once the pest provides an outbreak, it is capable of killing the entire fir stand in about a decade (Baranchikov et al. 2014b). Dramatic about this pest is that it colonizes fir trees of any age including fir undergrowth (with a diameter of 6 cm and more) (Krivets et al. 2015, 2018; Debkov 2019), resulting in the decline of entire fir stands (Krivets and Bisirova 2012; Krivets et al. 2018, Debkov et al. 2019).

Fir trees are present in the large area of the "Olenyi Ruchyi" Natural Park, i.e. on 6755 hectares (69% of the whole forested area). Bearing in mind that in this park, fir trees started notably declining in early 2020s (as per our dendrochronological

dating analysis), widespread death of firs due to this invasive bark beetle and its associate *G. aoshimae* can be expected up to 2030, if not earlier, in case no prompt and effective measures to eradicate the pest are undertaken.

Table 2. Forested districts of the Natural Park with fir in the canopy and undergrowth*

District, (area in hectares)	Forested area, hectares	Fir in forest canopy		Fir in undergrowth		TOTAL[1]	
		Area, hecteres	Proportion, %	Area, hectares	Proportion, %	Area, hectares	Proportion, %
Mikhailovsky (6340 hectares) [incl. recreation activity zone]	5769	3403	59	795	14	4198	73
	[1702]	[542]	[32]	[56]	[3]	[598]	[16]
Buysky (2571 hectares)	1893	730	39	406	21	1136	60
Bardymsky (888 hectares)	807	379	47	141	18	521	65
Total[2]	8469	4513	53	1342	16	5854	69

Note: * – data provided according to forest management materials [Taxation description, 2016a, 2016b, 2016c]; [1] – calculated as a sum of the columns 3 and 6; [2] – calculated as a sum of tree lines corresponded to the listed districts (Mikhailovsky, Buysky, and Bardymsky), where the data from the recreation zone are included in the indicators of Mikhailovsky district.

Conclusion

The early decline of the Siberian fir stands due to the invasive bark beetle *Polygraphus proximus*, which we recorded in the Natural Park "Olenyi Ruchyi" in 2023, suggests that the pest can spread over the large area of the park already in the coming years, with massive decline of fir trees affecting ecological, cultural, and recreational values of the park. In such case, there will be an obvious risk of fires in dead tree stands, increasing threats to health and life of visitors and the park staff. To prevent the pest outbreak and, thus, mitigate the following dramatic consequences, it is alarming to take all necessary measures to control this invasive tree killer.

Acknowledgments

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Institute of Forest Krasnoyarsk Science Center, Siberian Branch of RAS (project no. FWES-2024-0029; mycological analysis and dendrochronological dating).

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