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Rapid Communication

First record of the northern spruce bark beetle, *Ips duplicatus* (Sahlberg, 1836), in Slovenia

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Abstract

The northern spruce bark beetle, *Ips duplicatus* (Sahlberg), has invaded Central Europe in the past century and continues to spread southwards and westwards. It has caused damage in some parts of Europe in recent decades and poses a serious threat to spruce forests across the continent. From 2018 to 2021, we conducted intensive monitoring to determine the status of *I. duplicatus* in Slovenia. We used different types of traps and pheromone lures that attract *I. duplicatus* beetles and took bark samples from bark beetle-infested trees. Of the 26 traps used during the four years of monitoring, *I. duplicatus* was found in 16 traps in the central part of Slovenia in 2020. Adult beetles were caught in traps containing both *I. typographus* and *I. duplicatus* lures. The identity of this species was confirmed using morphological keys and molecular methods. One to five *I. duplicatus* beetles were found per trap, totaling 25 specimens. However, we did not confirm any *I. duplicatus* in samples taken from trees in 2021. We conclude that *I. duplicatus* is present in Slovenia, but our results suggest that the species is only present locally and that the population is small.

Key words: biological invasions, forest pests, temperate forests, conifers, damage, forest pest monitoring, confirmation

Introduction

Ips duplicatus (Sahlberg, 1836) (Coleoptera: Curculionidae: Scolytinae), also known as the double-spined bark beetle or the northern spruce bark beetle, is a Eurasian species, originally found in north-eastern Europe and northern Asia. In the past century, the bark beetle has been reported for the first time in several European countries (Turčáni and Zúbrik 1999; Holuša et al. 2010; Steyrer and Hoch 2020; Wermelinger et al. 2020; Hölling and Queloz 2021), indicating that the species is expanding its distribution range southwards and westwards in Europe (Lakatos et al. 2007; Wermelinger et al. 2020). The species is mostly found at elevations up to 600 m a.s.l. (Holuša et al. 2013a, b) but can also be found in mountainous areas up to 1,000 m a.s.l. (Grodzki 2003; Duduman et al. 2011; Holuša et al. 2013b) or higher (Grodzki 2020). *Ips duplicatus* has attracted attention in recent decades due to its potential to develop local outbreaks and cause damage in coniferous forests in Central Europe (Knížek and Zahradník 1996;

Olenici et al. 2009; Holuša et al. 2010; Kašák and Foit 2015; Zahradník and Zahradníková 2019; Vakula et al. 2021; Grodzki and Jabłoński 2022; Lubojacký et al. 2022). The first outbreaks of *I. duplicatus* in Central Europe were recorded in southern Poland and the Czech Republic in the 1990s.

Ips duplicatus mainly attacks spruce trees (*Picea* spp.) but can also develop on pines (*Pinus* spp.) and Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco, 1950) (Holuša and Grodzki 2008; Kašák and Foit 2015). The species often occurs together with other spruce bark beetles, mostly *I. typographus* (Linnaeus, 1758) and *Pityogenes chalcographus* (Linnaeus, 1761) (Grodzki 2012; Vakula et al. 2021; Lubojacký et al. 2022). Therefore, its presence in a tree can easily be overlooked. The main differences between *I. typographus* and *I. duplicatus* are that *I. duplicatus* only rarely attacks fallen trees (Lubojacký et al. 2018; Šotola et al. 2021), and its gallery systems are typically found in higher parts of tree trunks and in larger branches. However, in some cases spatial competition with *I. typographus* in the lower parts of spruce trees has been observed (Grodzki 2012). The phenology of *I. duplicatus* roughly matches that of *I. typographus*, with the main peaks of emergence in May and July (Holuša et al. 2012, 2013a; Steyrer and Hoch 2020). In contrast to *I. typographus*, *I. duplicatus* develops at lower optimum temperatures (Davídková and Doležal 2019) and has a shorter overall development time under the same environmental conditions (Duduman et al. 2011; Davídková and Doležal 2019). Therefore, this species can develop up to four generations per season (Davídková and Doležal 2019). *Ips duplicatus* overwinters mainly as adult beetles in the forest litter (Davídková and Doležal 2019; Davídková et al. 2023).

Ips duplicatus and *I. typographus* are similar in morphology, type and size of gallery system, and genetics (Grüne 1979; Stauffer et al. 2003; Faccoli 2015). However, *I. duplicatus* is generally smaller than *I. typographus* (about 25% smaller; body length 2.8–4.0 mm vs. 4.2–5.5 mm, respectively) and has darker colouring. The species can be distinguished mainly by the surface of the elytral declivity and the arrangement of teeth on the declivity. In *I. duplicatus*, the surface of the declivity is shiny and strongly punctured, and the second and third tooth/spine on each side of the elytral declivity arise from a common base and are slightly above the level of other teeth/spines (Grüne 1979; Freude et al. 1981; Pfeffer 1995). Furthermore, *I. duplicatus* can be distinguished from *I. typographus* by the aspect of the antennal sutures (see Figure 1 in Olenici et al. 2009). The gallery system of *I. duplicatus* is generally shorter than that of *I. typographus* (John et al. 2019). Recently, Becker et al. (2021) developed a molecular protocol for the rapid detection of *I. duplicatus* and *I. typographus*, making the differentiation of these species easier and more straightforward.

Recent reports of outbreaks and damage caused by *I. duplicatus* in several European countries, as well as its spread across Europe, motivated us to conduct a monitoring programme to determine the status of this species in Slovenia. Here, we report the first finding of *I. duplicatus* in Slovenia.

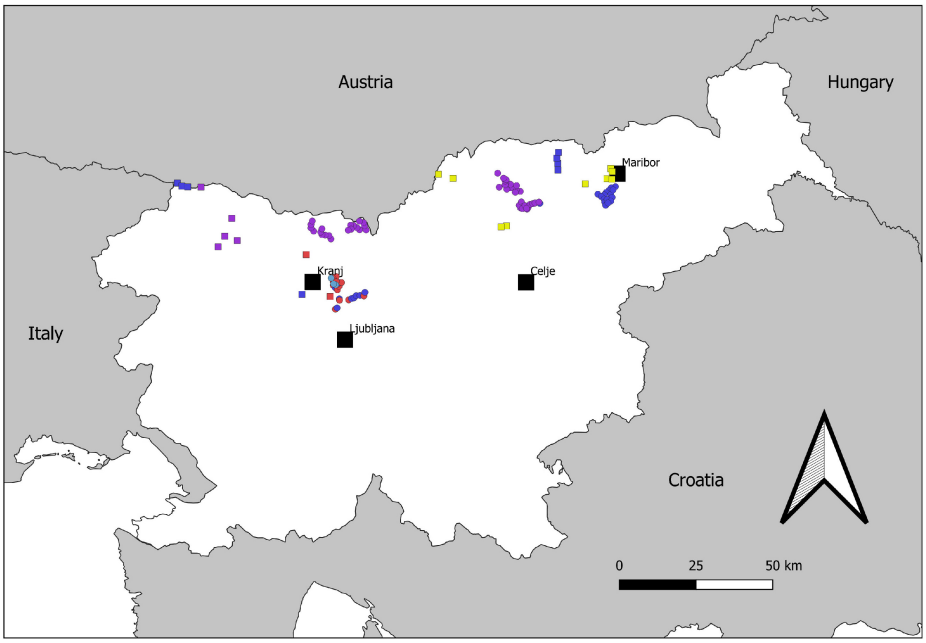

Figure 1. Locations of sampling sites for *Ips duplicatus* in the period 2018–2021. Squares denote traps in the *I. duplicatus* survey in different years (yellow: 2018, purple: 2019, dark blue: 2020). Red squares denote traps where *I. duplicatus* was found in 2020. Circles denote traps in the *I. typographus* survey in different years (purple: 2019, dark blue: 2020). Red circles denote traps where *I. duplicatus* was found in 2020. Light blue circles denote locations where trees were sampled in 2021 (for details see Supplementary material Table S1)

Materials and methods

Three survey methods were used to detect *I. duplicatus*. We used 1) traps with species-specific lures for *I. duplicatus* and 2) checked for *I. duplicatus* as a non-target organism in traps with species-specific lures for *I. typographus*. Pheromone traps are known to be efficient at detecting small populations of invasive alien insect species (e.g. bark beetles) in the early stage of their invasion. In addition, (3) direct observation on spruce trees was used after the presence of the species in Slovenia had been confirmed to check whether *Ips duplicatus* was also present in natural habitats, i.e. on spruce trees in forests.

Specific monitoring of I. duplicatus

The survey for *I. duplicatus* started in 2018 and continued in 2019, 2020 and 2021 (Figure 1, Table S1). In 2018, 2019 and 2020, we used nine, seven and ten traps, respectively. One trap was set per location, with a distance of 1,000 m or more between adjacent traps. To attract *I. duplicatus*, we used single black slit traps (Theysohn type, Witasek, Austria) and commercial pheromone lures, namely Dupliwit (Witasek, Austria), ID Ecolure (Fytofarm Ltd., Slovak Republic) and Atradup (Raluca Ripan' Chemical Institute, Cluj Napoca, Romania (https://feromoni.iccrr.institute.ubbcluj.ro/gandacul-de-scoarta-2/)). One type of lure was used per trap. Traps were fixed on wooden or metal frames approximately 1.5 m above the ground and 10 to 25 m from spruce stands. Lures were replaced according to manufacturer's instructions (approximately every 6 to 8 weeks).

In 2018, the traps were set in the northern part of Slovenia (500–600 m a.s.l.) near the border with Austria, using Dupliwit as the lure. In 2019, traps were set in NW Slovenia at higher altitudes (900–1,400 m a.s.l.), using ID Ecolure in all seven traps. In 2020, traps were set in three parts of Slovenia at different altitudes. Three traps were set in NW Slovenia (1,500 m a.s.l.), three in central Slovenia (400 m a.s.l.) and four in NE Slovenia (750–900 m a.s.l.). We used Atradup in the traps in central Slovenia and ID Ecolure in the traps in NW and NE Slovenia.

We surveyed for the presence of *I. duplicatus* in mature forest stands at a range of altitudes from 500 to 1,500 m above sea level, and the percentage of spruce in wood stock was higher than 50% at the selected locations. At most locations, spruce did not grow naturally but was planted in the past. Wind damage and *I. typographus* foci were reported at some of the locations.

In 2018, the survey to detect *I. duplicatus* lasted for one month, from the end of June to the end of July. In 2019, the survey took place from July to October. In 2020, the traps to detect *I. duplicatus* were used from April to July in NW Slovenia, from April to September in central Slovenia and from July to October in NE Slovenia. In all cases, samples were collected from the traps at 7 to 14 day intervals.

In June 2021, we sampled spruce trees infested by *Ips* bark beetles in the spring of the same year. The trees were cut as a part of sanitary felling and immediately checked to see if *Ips duplicatus* was one of the bark beetle species present in the trees. Sampling was carried out on June 20th near Kranj in the vicinity of locations where *I. duplicatus* beetles had been found in traps the previous year. Bark samples were randomly taken from freshly felled trees of different sizes and from different parts of the tree trunk. A total of 15 samples were taken from three locations.

Non-target catch during monitoring of I. typographus

In 2019 and 2020, research was carried out to test lures from different manufacturers and different types of traps for the monitoring of the European spruce bark beetle, *I. typographus,* in Slovenia (Šramel et al. 2021, 2022). The traps were set in 2019 in two areas near each other in the northern part of Slovenia and in two areas near each other in the north-eastern part of Slovenia. Similarly, in 2020, traps were set in two areas near each other in the central part of Slovenia and in two areas near each other in the north-eastern part of Slovenia (Figure 1). The locations of traps for the monitoring of *I. typographus* in one area in the north-eastern part of Slovenia were the same in 2019 and 2020. One of the variables analysed was the amount and diversity of non-target organisms (specimens other than *I. typographus*) in the traps. Therefore, we also used this experiment to check for *I. duplicatus* beetles. Fifty traps were set in spruce forests in each year, as shown in Figure 1, with one type of trap set per location and the distance between the neighbouring traps being 1,000 m or more.

In 2019, we used pheromone lures for *I. typographus* from five different manufacturers: Pheroprax (BASF, Germany), IT Ecolure Extra (Fytofarm Ltd., Slovak Republic), Ipsowit (Witasek, Austria), Ipstyp (AlphaScents Inc., USA) and Typosan (Sintagro AG, Switzerland). In 2020, we used IT Ecolure Extra and IT Ecolure Tubus (Fytofarm Ltd., Slovak Republic) in five different trap types: single-, double- and triple-slit traps (Theysohn type), multifunnel traps and cross-vane traps (all Witasek, Austria). The traps were fixed on wooden or metal frames approximately 1.5 m above the ground at a distance of 10 to 25 m from spruce stands. The lures were replaced according to the manufacturer's instructions (approximately every 8 weeks). Monitoring lasted from mid-April to the end of September each year. We collected samples from the traps at 7 to 14 day intervals.

Laboratory analyses

All samples were analysed in the Laboratory for Forest Protection at the Slovenian Forestry Institute. *Ips duplicatus* bark beetles were identified using morphological keys (Grüne 1979; Freude et al. 1981; Pfeffer 1995) and reference materials (adult beetles of *I. duplicatus*, kindly provided by Gernot Hoch, Federal Research and Training Centre for Forests, Natural Hazards, and Landscape (BFW), Vienna) and later deposited in the entomological collection of the Laboratory for Forest Protection at the Slovenian Forestry Institute.

To confirm the morphological identification of *I. duplicatus*, a species-specific PCR-based approach developed by Becker et al. (2021) was used. Total DNA was extracted from three previously morphologically identified *I. duplicatus* adults using a Nucleospin DNA Insect kit (Macherey Nagel, Düren, Germany) according to the manufacturer's instructions. *Ips duplicatus* specific primers (dupl_F_spec and Ips_R_cons) were used for PCR amplification of a part of the arthropod COI according to Becker et al. (2021). The PCR master mix was prepared using an Amplitaq Gold 360 MM (ThermoFisher Scientific, Massachusetts, USA): 25 µl of master mix, 1 µl of GC Enhancer, 1 µl of each primer and 4 µl DNA (standardized to a concentration of 10 ng/µl) per 50 µl reaction. PCR products were separated on a 1% agarose gel and, in the case of the successful amplification of a product of expected size, purified with a Wizard SV Gel and PCR Clean-Up System (Promega, Wisconsin, USA) and sequenced at a sequencing facility (Eurofins, Köln, Germany) in both forward and reverse directions using the same primers as for the PCR. Sequences were visualized, manually edited using Geneious Prime® v.2021.0.1. (Biomatters Ltd., Auckland, New Zealand) and deposited at GenBank.

Results

In 2020, *I. duplicatus* was recorded for the first time in Slovenia. In total, we found 25 specimens of *I. duplicatus* in 16 traps in the central part of Slovenia in the vicinity of Kranj. In 2018, 2019 and 2021, no *I. duplicatus*

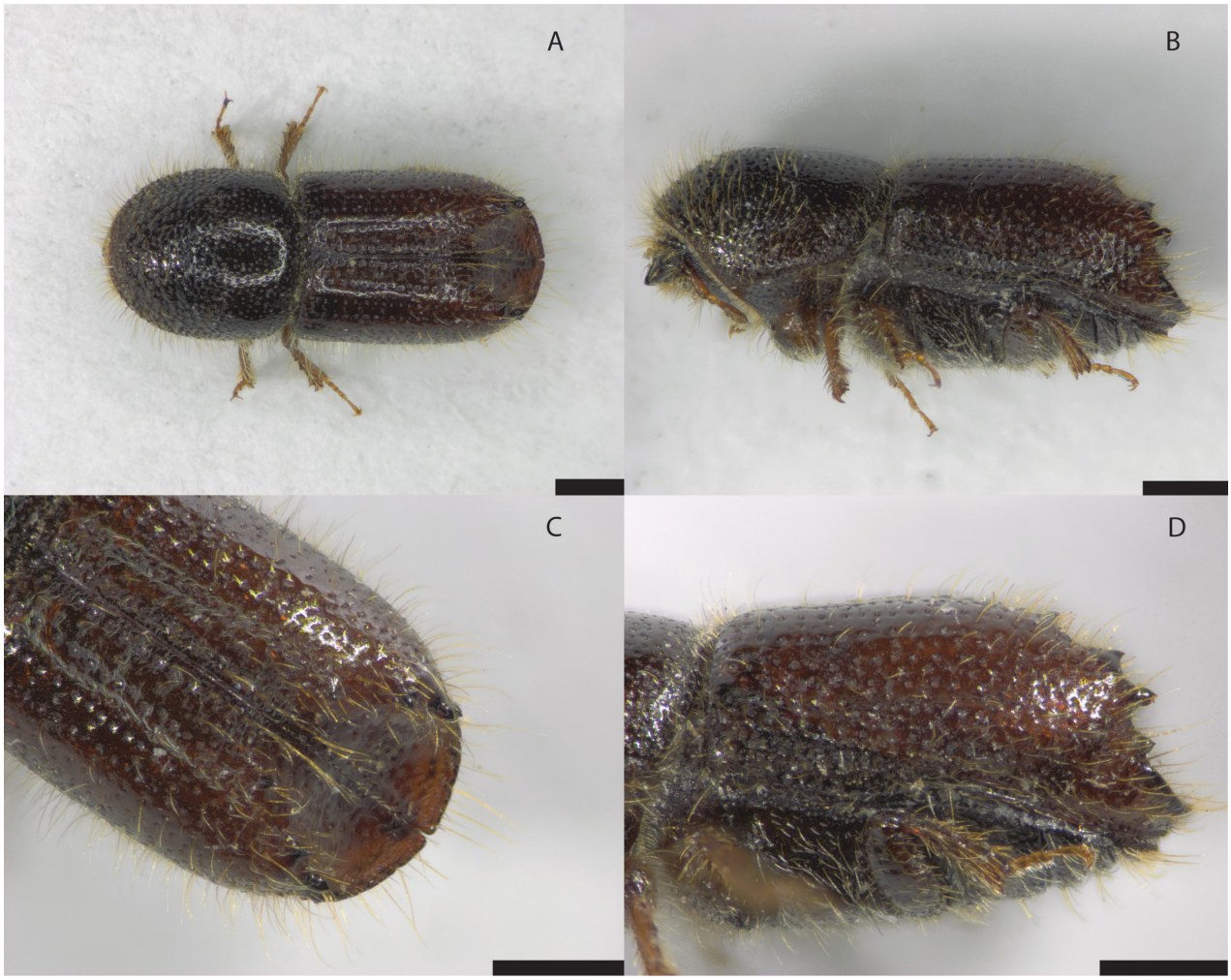

Figure 2. *Ips duplicatus* A) adult: dorsal view, B) adult: lateral view, C) abdomen: dorso-lateral view, surface of elytral declivity, D) abdomen: lateral view, teeth/spines on elytral declivity. Scale bar – 1 mm (Photo: Andreja Kavčič, Slovenian Forestry Institute).

beetles were recorded either in samples taken during the survey to detect *I. duplicatus* using pheromone traps or in samples taken from bark beetle infested trees.

Monitoring of I. typographus

During the monitoring of *I. typographus* in 2020, two *Ips* bark beetles that were not typical for *I. typographus* were found in a trap in the central part of Slovenia near Vodice (Lat: 46.195, Lon: 14.5243). The specimens were found in a multifunnel trap baited with IT Ecolure Extra in a sample from April 24th. The specimens had a shiny surface on the elytral declivity, and the teeth on each side of the declivity were not evenly spaced as in *I. typographus*. Also, the second and third tooth emerged from a common base and rose above the level of teeth one and four (Figure 2). We identified the beetles as *I. duplicatus*. The identity was confirmed by VULHM (Forestry and Game Management Research Institute, Prague, Czech Republic – Miloš Knížek) and BFW (Austrian Research Centre for Forests, Vienna, Austria – Gernot Hoch and Gottfried Steyrer) on the basis of photographic material (Olympus cellSens imaging software). This was the first record of *I. duplicatus* in

Slovenia. *Ips duplicatus* beetles were found again in the same trap in samples from June 29th (one specimen), and in three other traps for the monitoring of *I. typographus* and baited with IT Ecolure Extra in the same area in the central part of Slovenia (Lat: 46.1557, Lon: 14.4045; Lat: 46.1819, Lon: 14.4212; Lat: 46.1833, Lon: 14.4602), namely in samples from May 12th (one trap – two *I. duplicatus* beetles) and July 1st (two traps – one *I. duplicatus* beetle per trap).

Ips duplicatus beetles were also found on July 7th, again in traps for the monitoring of *I. typographus* in the central part of Slovenia. However, these traps were set in an area 5–10 km south of the area where the above-mentioned traps were set. Here, *I. duplicatus* was found in 8 traps baited with IT Ecolure Extra (Lat: 46.2515,Lon: 14.4069; Lat: 46.2471, Lon: 14.3929; Lat: 46.2362, Lon: 14.3938; Lat: 46.2262, Lon: 14.394; Lat: 46.2125, Lon: 14.4122; Lat: 46.2233, Lon: 14.4189; Lat: 46.2272, Lon: 14.41; Lat: 46.2339, Lon: 14.4292) and in one trap baited with IT Ecolure Tubus (Lat: 46.2361, Lon: 14.4072). The beetles were found in traps of different types (single-, double- and triple-slit traps (Theysohn type), multifunnel traps and cross-vane traps). The total number of *I. duplicatus* beetles found in traps on July 7th ranged from one to five per trap.

Monitoring of I. duplicatus

During the monitoring of *I. duplicatus* in 2020, two *I. duplicatus* specimens were found in traps containing Atradup in central Slovenia. One beetle was found in a trap located about 5 km south of Kranj (Meja, Lat: 46.1927, Lon: 14.3824) on August 12th, and the other in a trap located about 10 km NW of Kranj (Podbrezje, Lat: 46.3153, Lon: 14.2794) on September 29th. In 2021, no *I. duplicatus* beetles were found in samples taken from spruce trees (Figure 3, Table S2).

The identity of three *I. duplicatus* beetles was confirmed by PCR with *Ips duplicatus* specific primers, which produced an amplification product of approximately 1000 bp visible on the agarose gel. PCR products were sequenced and deposited at GenBank under accession numbers OQ376554-6.

Discussion

Using pheromone traps in the period 2018–2021, we confirmed the presence of *I. duplicatus* in Slovenia. In 2020, we found the first specimens of *I. duplicatus* in pheromone traps in the central part of Slovenia. This finding was expected, as the species has been present in several Central European countries for decades and has been spreading southwards and westwards in Europe (Lakatos et al. 2007; Wermelinger et al. 2020). Moreover, the beetle is already present in Austria and Hungary, Slovenia's neighbouring countries (Wermelinger et al. 2020).

Ips duplicatus originates from north-eastern Europe and northern Asia and has been present in our neighbouring country Austria for thirty years

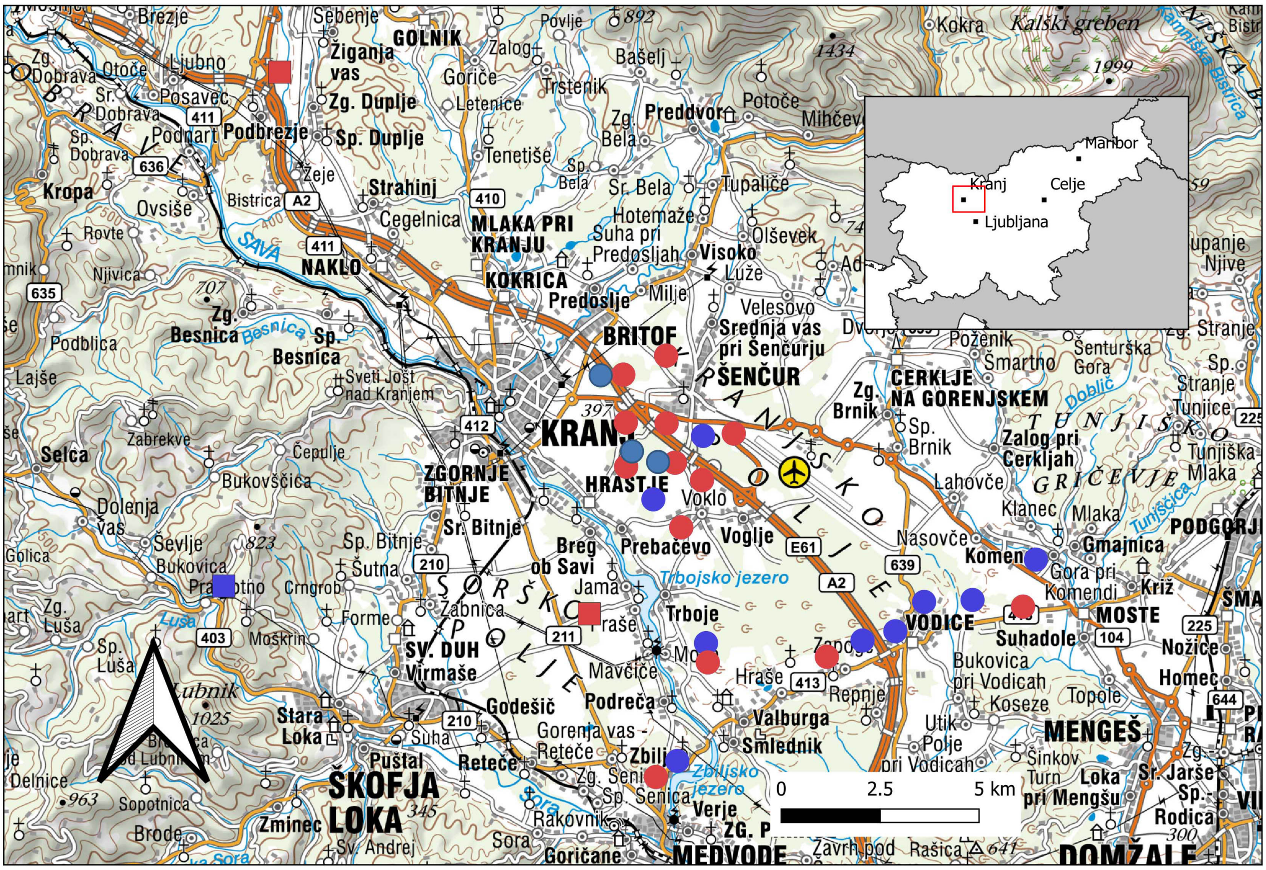

Figure 3. Locations of sampling sites for *Ips duplicatus* in central Slovenia in 2020 and 2021. Dark blue squares denote traps in the *I. duplicatus* survey in 2020 – red squares denote traps where *I. duplicatus* was found. Dark blue circles denote traps in the *I. typographus* survey in 2020 – red circles denote traps where *I. duplicatus* was found. Light blue circles denote locations where trees were sampled in 2021 (for details see Supplementary material Table S2).

(Wermelinger et al. 2020). In Austria, the species is widespread, and its presence has been confirmed at locations close to the Slovenian border (Steyrer and Hoch 2020). Therefore, we focused our survey efforts at higher altitudes and locations near the Slovenian-Austrian border. We expected the beetle to spread to Slovenia over the lower passes of the Karawanks or via Styria/Steiermark. Here, the spread would be possible by natural dispersal, and also by trade in infested timber, as there are the major transportation routes connecting Slovenia and Austria. Contrary to our expectations, we only recorded *I. duplicatus* in traps in the lowlands of the central part of Slovenia at about 400 m a.s.l. This is in line with previous research (Holuša et al. 2013a, b) and field observations (Olenici et al. 2022), which have shown that *I. duplicatus* prefers elevations up to 600 m a.s.l.

It is possible that this is why we did not find *I. duplicatus* in locations near the Slovenian-Austrian border, where the beetles were expected to spread naturally from Austria, as these locations were mainly at higher elevations. However, it is also worth noting that the beetle can be found at high altitudes (Duduman et al. 2011; Grodzki 2003, 2020; Holuša et al. 2013b). In 2018, we also set traps at low altitudes (500–600 m a.s.l.). It is possible that the population density of beetles at these locations was too low to be detected. Alternatively, *I. duplicatus* may not be present at these

locations. The reason for this could be that the extensive mountain ranges in the northern part of Slovenia, which border Austria, may be preventing the natural dispersal of *Ips duplicatus* from Austria into Slovenia. Additionally, wood from Austria is transported via trains and several companies are importing wood with bark. These might be the pathways that *I. duplicatus* arrived in Slovenia.

The total number of beetles caught (25) and the number of specimens per sample (1–5) were low. This may be because the population of *I. duplicatus* is very small in Slovenia. We used black slit traps placed 1.5 m above the ground and 10–25 m from spruce stands, which has been shown to be optimal for trapping *I. duplicatus* (Chen et al. 2009). Moreover, we found *I. duplicatus* beetles in all five types of traps that we used, and we also used lures that have been used for trapping *I. duplicatus* in other studies (Duduman et al. 2011, 2022; Holuša et al. 2010, 2012; Steyrer and Hoch 2020). However, it is possible that the low density of trapped *I. duplicatus* beetles could be a consequence of the type of traps and/or lures used.

It is not surprising that *Ips duplicatus* beetles were caught in traps set up to monitor *I. typographus*. It has been shown that *I. duplicatus* adults are one of many non-target Coleoptera attracted to *I. typographus* pheromones (Grodzki 1997; Valkama et al. 1997). Similarly, *I. typographus* is attracted to *I. duplicatus* pheromones (Duduman et al. 2022), which is why *I. typographus* is often found in traps baited with *I. duplicatus* pheromone lures (Duduman and Olenici 2015).

Ips duplicatus is relatively similar to other *Ips* species, and therefore it is advisable to verify new findings with other experts or use other diagnostic methods. The combination of two different approaches for the identification of trapped beetles proved to be useful for further identification challenges, especially with specimens of lower quality or when morphological identification is not robust.

Ips duplicatus has a high damage potential for Norway spruce, as has already been shown for Poland and the Czech Republic (Grodzki 1997; Knížek and Zahradník 1996). However, early detection and control of the species is more challenging than with *I. typographus*. *Ips duplicatus* is a species that colonizes the upper part of the trunk and larger branches (Grodzki 1997), which makes detection of the symptoms more difficult. Additionally, it only attacks standing trees and not fallen trees or cut logs (Grodzki 1997). Traps with pheromones are also used for early detection (Chen et al. 2009), but the system of control of this species is not well developed yet. More research is needed to improve early detection methods and control strategies for *I. duplicatus*.

It is not clear how *I. duplicatus* was introduced to Slovenia. The species is present in neighbouring countries, Austria and Hungary, and could have spread to Slovenia through several pathways, including natural dispersal (Holuša et al. 2010; Duduman et al. 2011) and trade in infested timber

(Duduman et al. 2011; Wermelinger et al. 2020). However, it seems the natural spread of *Ips duplicatus* from Austria to Slovenia is less likely due to the extensive mountainous area in the northern part of Slovenia bordering Austria, which represents a significant geographical barrier for beetle migration. A more likely pathway for the spread of *I. duplicatus* to Slovenia is the trade/import of infested timber from other European countries where the species is present. Slovenia imports significant amounts of coniferous wood from Austria and other Central European countries (Ščap 2021), and coniferous wood with bark present is a possible source of the species.

Conclusion

This study reports the first finding of *Ips duplicatus* in Slovenia. Our results suggest that this bark beetle is present locally, but at very low densities. In the future, a monitoring system should be established in Slovenian forests to track the spread and potential damage of this species, as has been observed in areas where the species has already been present for a longer period of time.

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Authors' contribution

AK, MdG and BP contributed to research conceptualization; AK and MdG contributed to sample design and methodology; AK, MdG and EG contributed to investigation and data collection; AK, MdG, EG, BP and ZD contributed to data analysis and interpretation; AK, MdG, EG, BP and ZD contributed to preparation of the manuscript.

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Supplementary material

The following supplementary material is available for this article:

Table S1. Locations of sampling sites for *Ips duplicatus* in the period 2018–2021. The coordinate system used is EPSG 4765.

Table S2. Locations of sampling sites for *Ips duplicatus* in central Slovenia in 2020 and 2021. The coordinate system used is EPSG 4765.

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