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First record of the non-native *Cnestus mutilatus* (Blandford) and further findings of other Xyleborini (Curculionidae, Scolytinae) recently recorded in Slovenia

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Abstract

Ambrosia beetles of the subfamily Scolytinae (Coleoptera: Curculionidae) have successfully invaded many parts of the world and are increasingly being introduced to new areas, primarily through international trade. The number of non-native species recorded in Slovenia is also increasing. During the national survey of quarantine species in 2024, a new non-native scolytine beetle, *Cnestus mutilatus*, was recorded for the first time in Slovenia. Two specimens were trapped at Radmožanci, a location in northeastern Slovenia. New data on two other species recently recorded in Slovenia, *Anisandrus maiche* and *Xylosandrus compactus*, are also presented.

Key words: ambrosia beetles, biological invasions, forest, invasive alien species, surveys, trapping

Introduction

Ambrosia beetles of the subfamily Scolytinae (Coleoptera: Curculionidae) are often accidentally introduced into new parts of the world (Kirkendall & Faccoli 2010; Hulcr & Skelton 2023). The primary pathway for their introduction to areas outside their natural range of distribution includes international trade in wood products, plants, pallets and wood packaging material (Lantschner *et al*. 2020). Their cryptic lifestyle and small size increase the likelihood of introductions going unnoticed, while other characteristics, such as associations with symbiotic fungi, a broad host range, inbreeding capabilities and adaptability to different climates, facilitate their successful establishment in new areas.

The arrival and establishment of non-native Scolytinae species in new areas is increasing, and this trend is expected to continue (Ruzzier *et al*. 2022). In Slovenia, the number of recorded non-native species has also been growing, with seven new taxa discovered in the last decade (Kavčič 2018; Hauptman *et al*. 2019a; Hauptman *et al*. 2019b; Kavčič *et al*. 2023; Hauptman *et al*. 2024; Jurc *et al*. 2024).

Cnestus mutilatus (Blandford) belongs to the tribe Xyleborini and is easily distinguished from other members of the tribe by its truncate elytra, which are shorter than the pronotum (Fig. 1), and a distinct carina separating the elytral disc and declivity (Gomez *et al*. 2018). A mycangial tuft is also clearly visible at the base of the pronotum. Its natural range extends across southern and eastern Asia (Smith *et al*. 2020). In 1999, it was first recorded in North America, in Mississippi (Schiefer & Bright 2004), and it is now well-established in several southeastern states of the United States (Gomez *et al*. 2018; Olatinwo *et al*. 2014). In Europe, *C. mutilatus* was first found in northeastern Italy

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in 2021, in the Veneto Region (Colombari *et al*. 2022). Since then, several new records have been reported from the same region (Marchioro *et al*. 2022; Colombari & Battisti 2023; Ruzzier *et al*. 2023a), indicating its successful establishment. The species was also recently recorded for the first time in Croatia (Dinka Matošević, personal communication).

During the national survey of quarantine species in 2024, *Cnestus mutilatus* was recorded for the first time in Slovenia. Here we report the first finding of this non-native species, and in addition, we present new data on the polyphagous ambrosia beetles *Anisandrus maiche* (Kurentzov) and *Xylosandrus compactus* (Eichhoff), which were first recorded in Slovenia in 2023.

FIGURE 1. Habitus view of an adult female *Cnestus mutilatus* trapped in Slovenia.

Materials and Methods

Trapping and identification

Traps were set in 2024 in 10 locations throughout Slovenia (Tab. 1) as part of a survey for the early detection of quarantine oak bark beetles (*Pseudopityophthorus* spp.). We used 10 black 5+1 segmented multi-funnel traps (Witasek), one per site, baited with 70% ethanol dispensed by a perforated 50 ml tube. A mixture of propylene glycol and water served as a preservative. The traps were suspended at a height of approximately 1.5 m above the ground in oak-dominated forest stands (*Quercus* spp.). The trapping period in 2024 started in May or June (varying by location) and lasted approximately 8 to 10 weeks. During this period, each trap was emptied three times at intervals of two to three weeks (Tab. 1). Samples were collected in 500 ml plastic containers, transported to the Laboratory for Forest Protection at the Slovenian Forestry Institute and stored at 4°C until analysis. All Scolytinae were separated from the rest of the catch and identified according to specific morphological keys (Grüne 1979; Pfeffer 1995; Smith *et al*. 2020). The specimen selected for additional molecular analysis was individually frozen at -20°C in 70% ethanol.

Molecular analyses

Genomic DNA was extracted non-destructively with a DNeasy Blood & Tissue commercial extraction kit (Qiagen, Venlo, Netherlands). The manufacturer's instructions for total DNA extraction from animal tissue were followed, with some modifications. During preparation of the specimen, a hole was punctured in the abdomen using a sterile needle, followed by overnight lysis in a Thermomixer C (Eppendorf, Germany) at 56°C and 300 rpm. After lysis, the specimen was washed with sterile molecular grade water (Sigma-Aldrich, Missouri, USA) and stored frozen in 97%

ethanol. Additionally, the columns were left to dry for 10 minutes before elution, which was performed with 50 µl of elution buffer heated to 56°C. A fragment of COI was amplified using primers LCO1490 and HCO2198 (Folmer 1994), following the conditions published in EPPO Standard PM7/129 (2) (EPPO 2021), with denaturation times and temperature adapted for use with the AmpliTaq Gold 360 Master Mix (ThermoFisher Scientific, Massachusetts, USA), according to the manufacturer's instructions. PCR products were purified using 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 and manually edited using Geneious Prime® v 2025.0.3. The obtained sequence was deposited in the GenBank database (PV257754). Megablast searches against the core_nt collection in the GenBank database were performed on 14.1.2025 and 11.3.2025. For phylogenetic analyses, sequences from Colombari *et al*. (2022) and Cognato *et al*. (2020), along with sequences from GenBank showing 99% identity or higher, were included (Supplementary table 1). The final nucleotide matrix comprised 23 COI sequences, aligned using MAFFT Alignment v7.490 within Geneious Prime with the E-INS-i algorithm. The alignment ends were trimmed to 578 bp, and a maximum likelihood analysis was conducted using 1000 rapid bootstrap inferences under the GTRGAMMA model in RAxML version 8.2.11 within Geneious Prime (Stamatakis 2014).

TABLE 1. Trap locations (WGS84 coordinates) and sampling periods in 2024.

	Location	Latitude	Longitude	Trapping period
1	Srmin*	45.5617	13.7744	22.5.2024–31.7.2024
2	Sežana*	45.6987	13.8509	6.6.2024–7.8.2024
3	Breg ob Savi*	46.2039	14.3689	10.5.2024–17.7.2024
4	Volčji potok	46.1862	14.6146	14.5.2024–18.7.2024
5	Črnomelj	45.5830	15.1814	15.5.2024–16.7.2024
6	Malo Mraševo*	45.8715	15.4249	15.5.2024–24.7.2024
7	Gotovlje	46.2760	15.1613	14.5.2024–18.7.2024
8	Celje	46.2280	15.2791	14.5.2024–18.7.2024
9	Marjeta na Dravskem polju	46.4575	15.7313	14.5.2024–11.7.2024
10	Radmožanci	46.6191	16.3769	5.6.2024–13.8.2024

* Locations where the survey was also conducted in 2023

Results and Discussion

During our survey, we trapped 18 different Scolytinae species (Tab. 2). Among these, five were non-native, with *Cnestus mutilatus* representing a new record for the fauna of Slovenia. Species identification was confirmed through both morphological and genetic analyses.

A total of two *Cnestus mutilatus* specimens were captured, both at Radmožanci, a location in northeastern Slovenia (Fig. 2). The first specimen was found on July 3, 2024, and the second on August 13, 2024. The COI sequence from the Slovenian specimen (PV257754) clusters with *C. mutilatus* sequences and further subgroups with sequences from specimens in the USA and Croatia (Fig. 3). Although the species is already established in northern Italy, we assume that it did not spread to Slovenia naturally. The first finding in Slovenia was located on the opposite side of the country, with no successful detections between this location and the Italian border. Given Slovenia's role as a transit country for road transport from the Adriatic Sea into mainland Europe, particularly towards Hungary, this pathway of spread cannot be ruled out and may explain the lack of detection closer to the established population in Italy. Moreover, the Italian populations of *C. mutilatus* are genetically different from the specimen found in Slovenia, indicating that Italian and Slovenian populations might also be the result of separate introductions.

Cnestus mutilatus is a polyphagous species, with 20 host families recorded worldwide (Ruzzier *et al*., 2023b). Its host range includes many deciduous genera of economic relevance, such as *Acer* (Sapindaceae), *Carpinus* (Betulaceae), *Castanea* (Fagaceae), *Fagus* (Fagaceae), *Juglans* (Juglandaceae), *Prunus* (Rosaceae) and *Ulmus* (Ulmaceae). The species is a major pest of young *Castanea mollissima* Blume trees in China but is not treated as such in other areas of its distribution, as it prefers thin branches (1–5 cm) of stressed, weakened, or recently dead trees. Unusual damage to plastic gasoline containers caused by females has been recorded in North America (Carlton & Bayless 2011). In Europe, its host plants are not yet known (Ruzzier *et al*. 2023a).

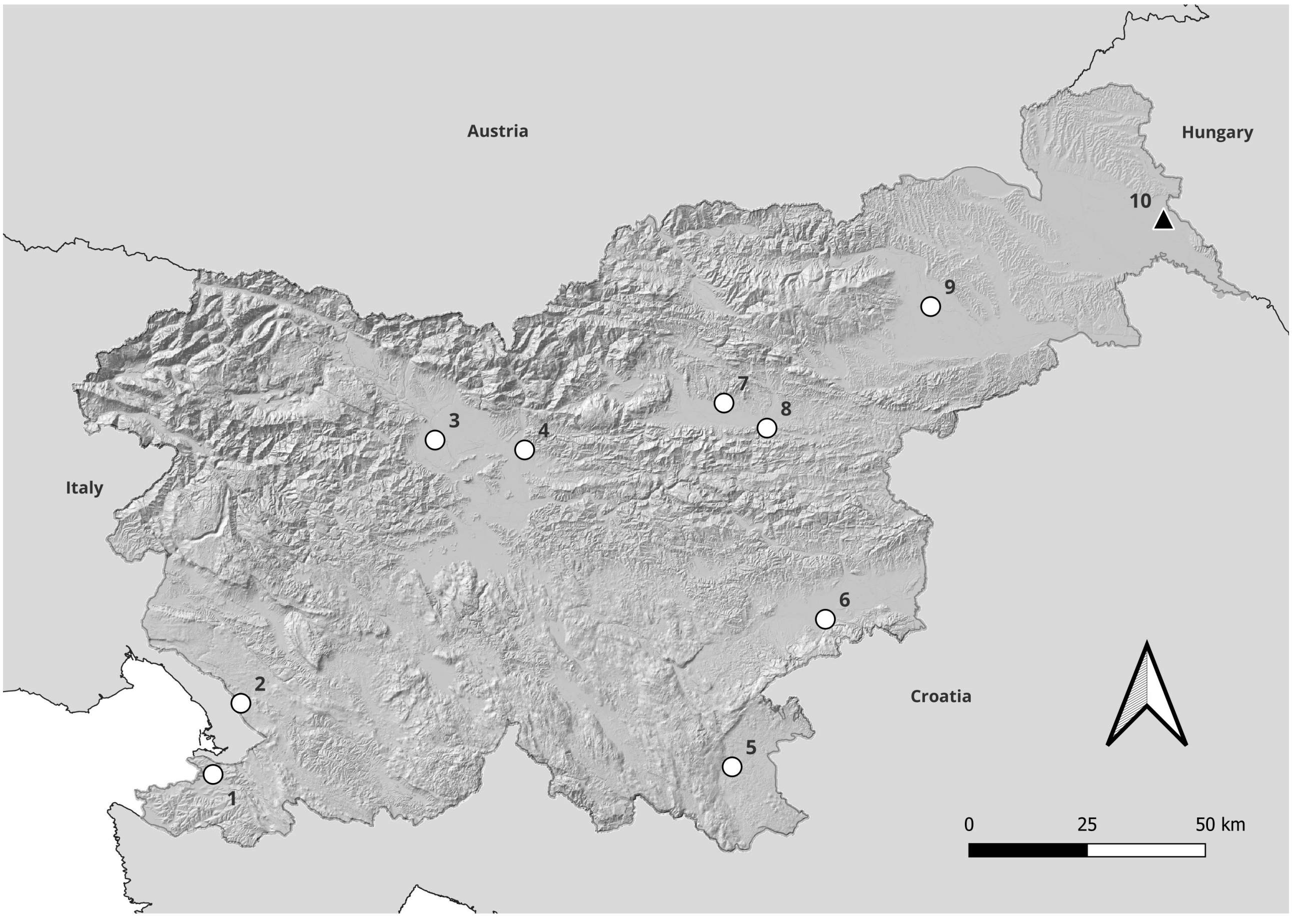

FIGURE 2. Trapping locations in 2024. The black triangle (site 10) represents the location where *Cnestus mutilatus* was found, while white circles represent the other trapping locations. The location numbers correspond to the locations in Table 1.

Anisandrus maiche was the most abundant species in the catch (Tab. 2), although it was first found in Slovenia during a similar survey only the previous year (Hauptman *et al*. 2024). The distribution of traps across Slovenia was approximately the same in 2023 and 2024. In 2023, *A. maiche* was confirmed at three locations, with a total of 386 specimens caught (Hauptman *et al*. 2024). In contrast, in this study, we caught a total of 5603 individuals at six locations. However, it should be noted that the 2024 survey was conducted later in the vegetation period, thus capturing the emergence peak of *A. maiche*, which, according to our results, occurs in June. So far, *A. maiche* is only present in the eastern part of the country (Fig. 4), but it appears to be becoming one of the most abundant ambrosia beetles in Slovenia, similar to what has already occurred in parts of North America (Ranger *et al*. 2019).

Xylosandrus compactus, the other non-native Xyleborini found in Slovenia, is not as abundant or widely distributed. In 2024, we found it at the same location as in 2023 (Hauptman *et al*. 2024), and in both years, only three individuals were caught. However, during other official monitoring activities in 2024, the species was also captured in a bark beetle slit trap (Witasek) equipped with 70% ethanol. It was located approximately 2.5 km (WGS84, Lat: 45.5852, Lon: 13.7730) from the location of the first finding in Slovenia. In this case, two individuals were caught (T. Hauptman, personal observation). This suggests that the *X. compactus* population in Slovenia is currently small and restricted to the coastal area (Fig. 4).

As of now, no tree damage in Slovenia has been attributed to recently discovered non-native Xyleborini beetles. All findings of *A. maiche*, *C. mutilatus* and *X. compactus* in Slovenia have been linked exclusively to ethanol-baited traps. However, repeated captures over two consecutive years suggest the probable establishment of these species. In 2023 and 2024, we analyzed over 30 plant samples showing signs of ambrosia beetle attack in our laboratory. The only confirmed damage-causing agents were the indigenous species *Anisandrus dispar* (Fabricius) and the non-native *Xylosandrus germanus* (Blandford). The latter was also relatively abundant in our survey (Tab. 2). However, *X. germanus* has been present in Slovenia since 2000 and is now widely distributed throughout the country (Hauptman *et al*. 2019a). Apart from occasional attacks on weakened hosts or freshly felled trees, no major economic damage has been recorded so far in Slovenia. The last non-native species trapped in this survey was *Gnathotrichus materiarius* (Fitch), first recorded in Slovenia in 2003. While much less abundant than *X. germanus*, it is now widespread in coniferous stands across most of the country (Hauptman *et al*. 2019a; Jurc *et al*. 2024), although it has not yet been found on a host plant in Slovenia.

New non-native scolytine beetles in Slovenia have often been detected as bycatch during national surveys for quarantine species (Kavčič 2018; Hauptman *et al*. 2024; this study), highlighting the effectiveness and necessity of such activities. The three non-native ambrosia beetles recently discovered in Slovenia likely do not meet the criteria for inclusion on the list of potential EU quarantine pests (EFSA 2024). Nevertheless, establishing a monitoring system to track their spread and potential impact on forest ecosystems is crucial. Continued efforts to identify entry points and pathways could provide valuable insights into the spread of non-native ambrosia beetles. Enhanced collaboration with neighboring countries may also improve surveillance efforts and offer a more comprehensive understanding of their distribution and impact.

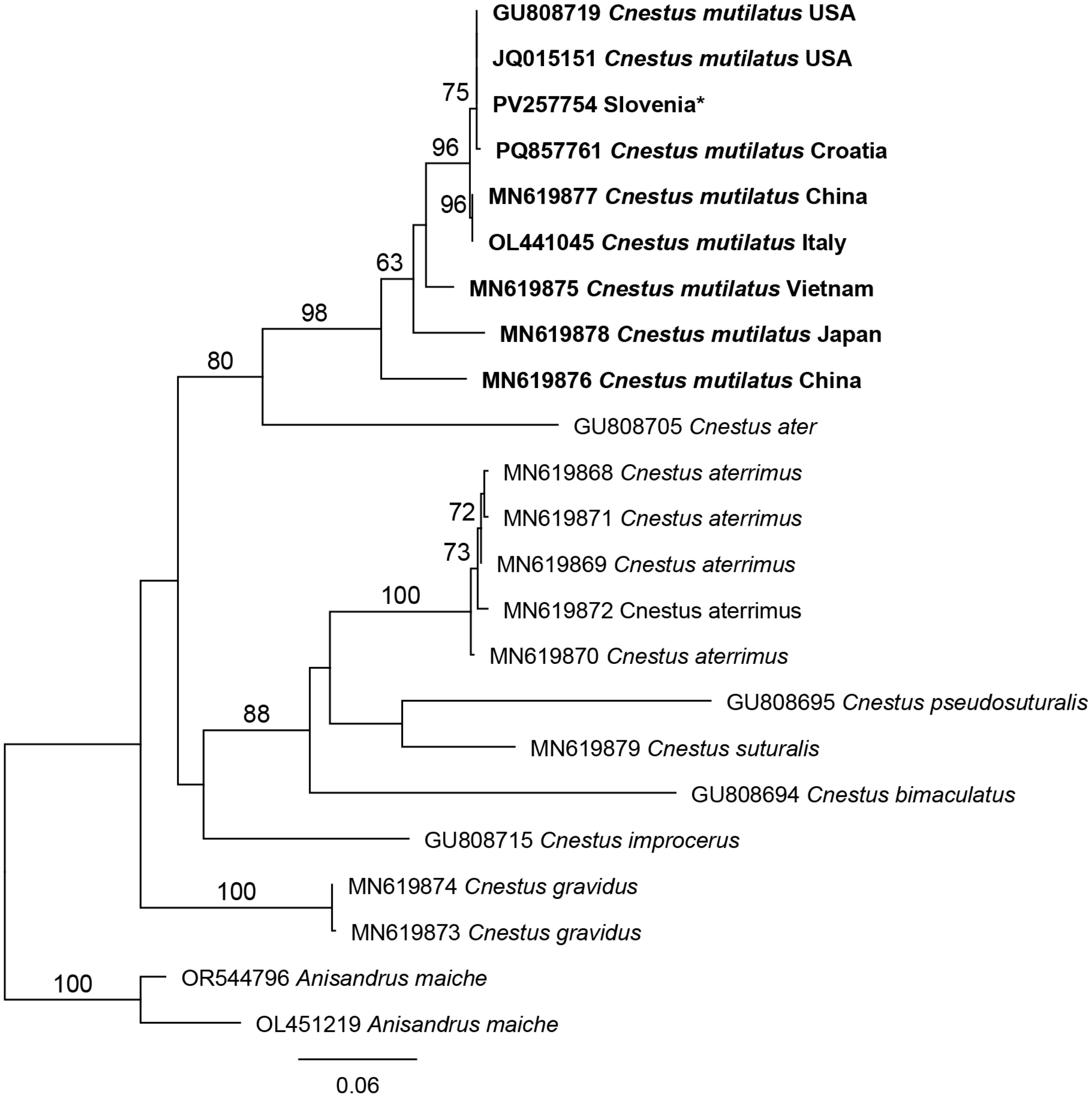

FIGURE 3. Maximum likelihood tree based on the COI dataset of selected species from the genus *Cnestus*. Tree topology was obtained after 1000 runs and was rooted against *Anisandrus maiche*. Bootstrap values (>60%) are indicated at the nodes. The scale bar indicates nucleotide substitutions per site. The sequence of the *C. mutilatus* specimen from Slovenia is marked with *.

TABLE 2. Bark and ambrosia beetle species caught in 10 ethanol-baited multi-funnel traps. The total number of specimens and sites (as listed in Tab. 1) where each species was caught are shown. Non-native species are highlighted in bold.

Species[1]	Presence at locations	Total N of individuals
***Anisandrus maiche* (Kurentzov)**	**4–8, 10**	**5603**
Xyleborinus saxesenii (Ratzeburg)	1–10	2318
Anisandrus dispar (Fabricius)	2–10	1449
***Xylosandrus germanus* (Blandford)**	**1, 3–10**	**1198**
Trypodendron signatum (Fabricius)	5–8	11
Taphrorychus bicolor (Herbst)	7, 8	8
Xyleborus dryographus (Ratzeburg)	4, 7, 9	5
Xyleborus monographus (Fabricius)	6, 7, 9	4
***Xylosandrus compactus* (Eichhoff)**	**1**	**3**
Polygraphus poligraphus (Linnaeus)	7	3
Dryocoetes autographus (Fabricius)	7, 8	2
***Cnestus mutilatus* (Blandford)**	**10**	**2**
***Gnathotrichus materiarius* (Fitch)**	**7**	**1**
Hylurgops palliatus (Gyllenhal)	7	1
Hylesinus toranio (D'Anthoine)	2	1
Scolytus intricatus (Ratzeburg)	2	1
Pityogenes chalcographus (Linnaeus)	7	1
Pityophthorus pubescens (Marsham)	9	1

[1] Species are listed according to the total number of individuals found in the traps.

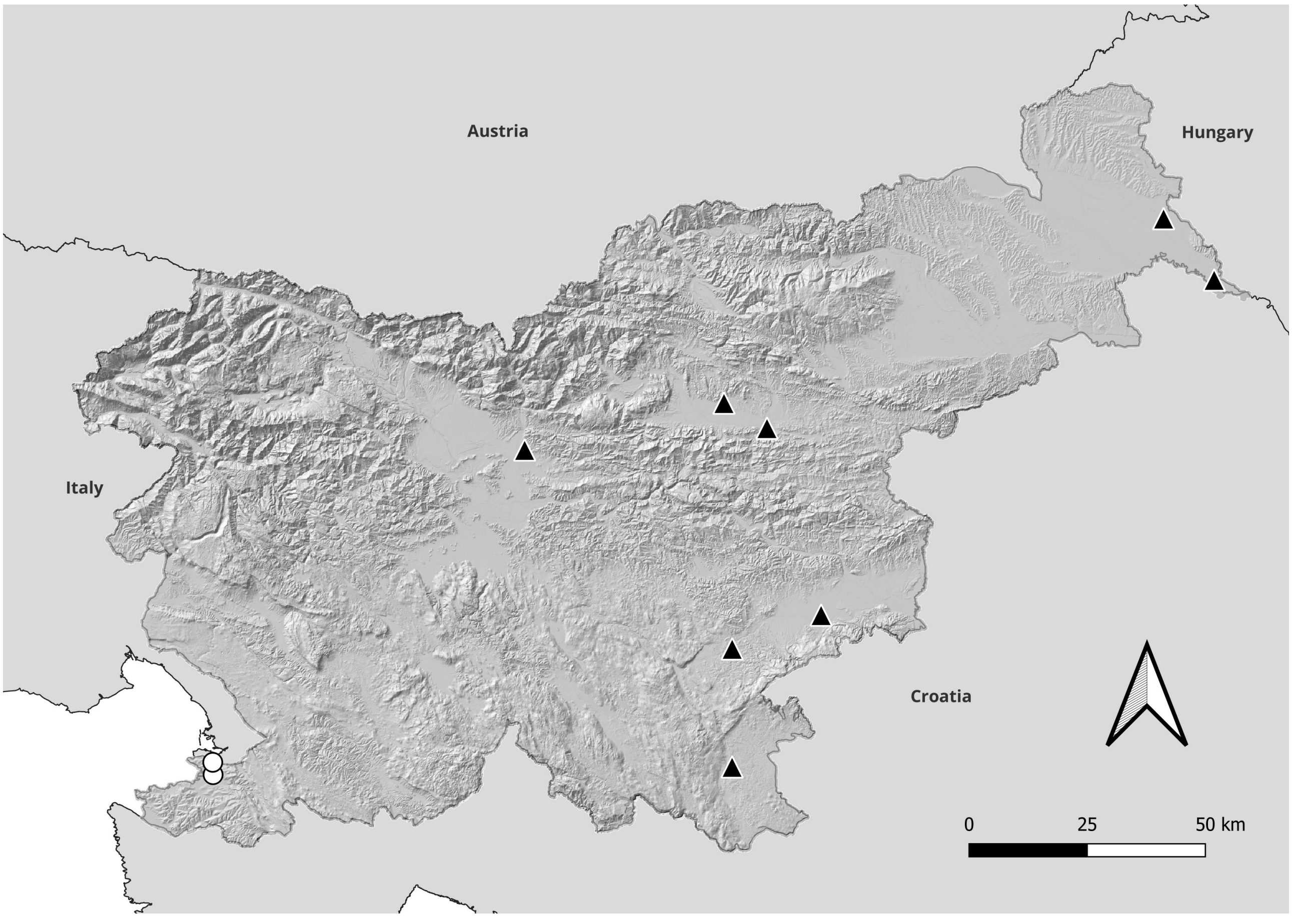

FIGURE 4. Locations where *Anisandrus maiche* (black triangle) and *Xylosandrus compactus* (white circles) were detected in Slovenia in 2023 and 2024.

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Supplementary Material. The following supporting information can be downloaded at the DOI landing page of this paper:

Supplementary table 1: Sequences used for phylogenetic analyses.