*Zootaxa* 5415 (2): 339–345
https://www.mapress.com/zt/

**Article**

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

https://doi.org/10.11646/zootaxa.5415.2.8
http://zoobank.org/urn:lsid:zoobank.org:pub:C493F3A1-97DC-4AF3-8186-6464A052B25B

# First record of non-native *Xylosandrus compactus* and *Anisandrus maiche* (Coleoptera: Curculionidae, Scolytinae) in Slovenia

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## Abstract

The introduction of non-native organisms into new areas may pose major threats to natural ecosystems. Therefore, continuous and careful national surveys for the interception of invasive non-native species are necessary. During the national survey of quarantine species in 2023, two new non-native ambrosia beetles, *Anisandrus maiche* (Kurentzov) and *Xylosandrus compactus* (Eichhoff), were recorded in Slovenia. *Anisandrus maiche* was recorded in three locations in the eastern part of Slovenia, with a total of 386 individuals. Three individuals of *Xylosandrus compactus* were collected in one location near the port of Koper. We discuss the possible pathways of introduction and dispersal.

**Key words:** Biological invasions, surveys, trapping, invasive alien species, forest

## Introduction

Invasive insects pose a major threat to ecosystems (Kenis *et al*. 2009), with international trade recognized as a major driver of invasion worldwide (Hulme 2021). Bark and ambrosia beetles (Coleoptera: Curculionidae, Scolytinae) are often unintentionally introduced to new areas through wood products, live plants, and wood packaging material (Lantschner *et al*. 2020). Due to their cryptic lifestyles, associations with symbiotic fungi, inbreeding, broad host ranges, and adaptability to various climates, ambrosia beetles have proven to be especially successful as invasive species in Europe (Kirkendall & Faccoli 2010). In 2023, two new non-native ambrosia beetles, *Anisandrus maiche* (Kurentzov) and *Xylosandrus compactus* (Eichhoff), were recorded for the first time in Slovenia.

*Anisandrus maiche* is native to Northeast Asia (China, Japan, North and South Korea, and the Russian Far East). It was recorded for the first time in North America in 2005 (Rabaglia *et al*. 2009) and has since become widely distributed in the northeastern part of the continent (Gómez *et al*. 2018). In Europe, the species was first recorded in 2007 in Ukraine and European Russia (Terekhova & Skrylnik 2012; Nikulina *et al*. 2015). While *A. maiche* has been observed attacking trees such as *Betula pendula* Roth., *Populus tremula* L., *Quercus robur* L., *Quercus rubra* L., and *Ulmus minor* Mill. in Ukraine, it appears to have no significant economic impact (Terekhova & Skrylnik 2012). More recently, in 2020 and 2022, the species was also detected in Northern Italy (Mola *et al*. 2023) and Southern Switzerland (Ribeiro-Correia *et al*. 2023). The presence of the species was confirmed using various traps, but no tree damage has been recorded thus far.

*Xylosandrus compactus* is believed to be native to East Asia. However, the species is also widespread in tropical and subtropical regions of Africa and South America. It has been introduced to North America, some Pacific islands, and Europe (EPPO 2020). In Europe, *Xylosandrus compactus* was found for the first time in Italy in 2011 (Garonna

*Accepted by S. Smith: 30 Jan. 2024; published: 21 Feb. 2024*

*et al*. 2012) and subsequently in France in 2015 (Barnouin *et al*. 2020), Greece in 2019 (Spanou *et al*. 2019), Spain in 2019 (Leza *et al*. 2020), Malta in 2021 (EPPO 2024), and Turkey in 2021 (Hizal *et al*. 2023). Numerous attacks by this species have been recorded in Europe, particularly in Italy and France, affecting 54 host genera of forest, agricultural, and ornamental plants (Riba-Flinch *et al*. 2021). The species poses a serious threat to several Mediterranean host plants (Gugliuzzo *et al*. 2020).

Here, we report the first findings of these two species in Slovenia and discuss the possible pathways of introduction and dispersal.

## Materials and methods

### Trapping and identification

Traps were set as part of a survey of quarantine species in Slovenia, specifically for the early detection of the oak bark beetle, *Pseudopityophthorus minutissimus* (Zimmermann). In this survey, we used 10 black multi-funnel traps (Chemtica, USA), each baited with 70% ethanol dispensed by a perforated 50 ml tube. Propylene glycol served as preservative. The traps were suspended at heights ranging from 1 to 1.5 m above the ground in forest stands with a large share of oaks (*Quercus* spp.). The trapping period occurred in spring 2023 and lasted approximately 6 to 7 weeks, varying by site. During this period, each trap was emptied three times (Table 1). Samples were stored 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 bark and ambrosia beetles were separated from the rest of the catch and identified according to specific morphological keys (Grüne 1979; Pfeffer 1995). As some specimens differed in their morphological characteristics from known native and non-native species present in Slovenia, additional keys for non-European species were used (Rabaglia *et al*. 2009; Gallego *et al*. 2017; Smith *et al*. 2020). Specimens selected for additional molecular analysis were individually frozen at -20°C in 70% ethanol.

**TABLE 1:** Trap locations (WGS84 coordinates) and sampling dates.

| | Location | Latitude | Longitude | Trap setting | Sample 1 | Sample 2 | Sample 3 |
|---|---|---|---|---|---|---|---|
| 1 | Srmin | 45.5617 | 13.7744 | 14.4.2023 | 04.5.2023 | 17.5.2023 | 31.5.2023 |
| 2 | Sežana | 45.6987 | 13.8509 | 18.4.2023 | 04.5.2023 | 18.5.2023 | 01.6.2023 |
| 3 | Breg ob Savi | 46.2039 | 14.3689 | 04.5.2023 | 18.5.2023 | 02.6.2023 | 15.6.2023 |
| 4 | Kranj | 46.2356 | 14.3725 | 04.5.2023 | 18.5.2023 | 02.6.2023 | 15.6.2023 |
| 5 | Pondor | 46.2389 | 14.9903 | 24.4.2023 | 10.5.2023 | 26.5.2023 | 08.6.2023 |
| 6 | Zalog pri Šempetru | 46.2744 | 15.1251 | 24.4.2023 | 10.5.2023 | 26.5.2023 | 08.6.2023 |
| 7 | Ragovo | 45.8078 | 15.1823 | 25.4.2023 | 10.5.2023 | 24.5.2023 | 09.6.2023 |
| 8 | Malo Mraševo | 45.8715 | 15.4249 | 25.4.2023 | 10.5.2023 | 24.5.2023 | 09.6.2023 |
| 9 | Apače | 46.394 | 15.8021 | 05.5.2023 | 19.5.2023 | 05.6.2023 | 19.6.2023 |
| 10 | Murska šuma | 46.5006 | 16.5134 | 05.5.2023 | 19.5.2023 | 05.6.2023 | 19.6.2023 |

### Molecular analyses

For DNA extraction, only the head, thorax, and legs of individual beetles (four *A. maiche* and one *X. compactus*) were used. Genomic DNA was extracted with a DNeasy Blood & Tissue commercial extraction kit (Qiagen, Venlo, Netherlands) following the manufacturer's instructions for total DNA extraction from animal tissue, with some modifications. The samples were lysed overnight in a Thermomixer C (Eppendorf, Germany) at 56°C and 300 rpm. 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 from 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® v2023.1.2. (Biomatters Ltd., Auckland, New Zealand). The obtained sequences were deposited in the GenBank database. Megablast searches against the nr/nt collection in the GenBank database were performed on 22.11.2023. In addition, species identification was conducted via the identification engine in BOLD, Barcode of Life Data System (https://www.boldsystems.org/, accessed on 22.11.2023).

## Results and discussion

In total, 15 different bark and ambrosia beetle species were found in the traps during our survey (Tab. 2). Six of these species were non-native, and among them, two species (*A. maiche* and *X. compactus*) were new records for the fauna of Slovenia. The species identification was consistent both morphologically and genetically.

*Anisandrus maiche* was trapped in three locations in the eastern part of Slovenia (Fig. 1) and ranked as the third most numerous species (386 individuals) according to the total trap catches (Tab. 2). This taxon was detected at Murska šuma and Ragovo during the second and third samplings rounds, while it was found only in the third catch at Malo Mraševo. Out of the 386 *A. maiche* beetles captured, the majority was caught at Murska šuma (357 individuals), with a smaller number at Ragovo (17) and Malo Mraševo (12). *A. maiche* was the most numerous species (285 individuals) in the captures of the third sampling at the Murska šuma location.

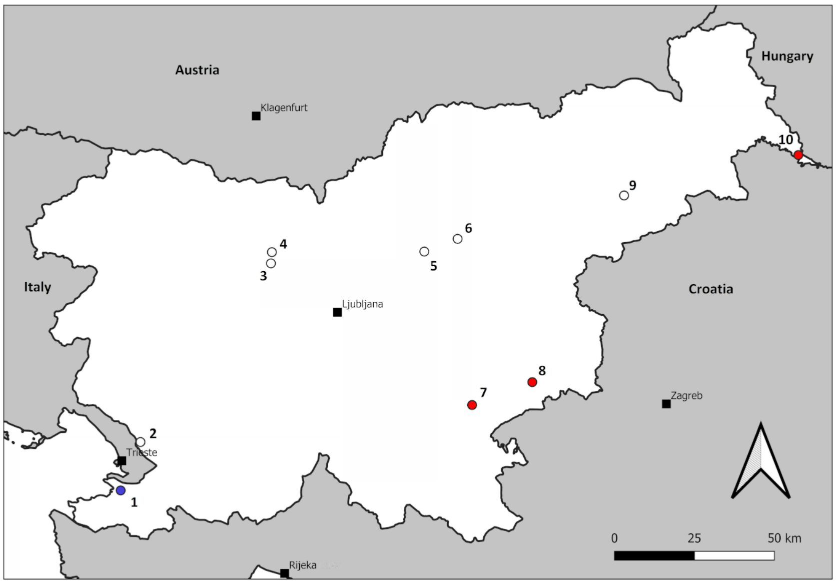

**FIGURE 1:** Trapping locations. Red dots represent locations where *A. maiche* was caught, and the blue dot represents the finding of *X. compactus*. White dots represent traps set during the survey, but where none of the two species were found.

At first glance, *A. maiche* resembles the congeneric and native *Anisandrus dispar* (Fabricius), except that *A. maiche* is smaller and has a slightly bisulcate appearing declivity (Fig. 2, Fig. 3) (Rabaglia *et al*. 2009; Smith *et al*. 2020; Colombari *et al*. 2022).

According to Ribeiro-Correia *et al*. (2023), the flight period of *A. maiche* in Ticino (Switzerland) is between early May and late August. Since our survey concluded in mid-June, it is likely that the catch of *A. maiche* would have been even higher if the survey continued. Despite its recent discovery, the species is widespread in Northern Italy, with a distribution area covering approximately 9,900 square kilometers in 2022 (Ruzzier *et al*. 2022), and even more in 2023 (Ruzzier *et al*. 2023). The sudden occurrence of *A. maiche* in such a large area might indicate its ability to spread rapidly following a single or possibly multiple introductions. However, Ribeiro-Correia *et al*. (2023) have also highlighted the possibility that the species has been present in Northern Italy and Southern Switzerland for many years, and its resemblance to the common and widespread *X. germanus* (Blandford) and *A. dispar* may have hindered its earlier detection. The relatively large captures at Murska šuma indicate that *A. maiche* has most likely also been present in Slovenia for several years. However, no tree damage has been recorded so far.

**FIGURE 2:** Dorsal (left) and lateral (right) habitus views of an adult female *Anisandrus maiche*.

**FIGURE 3:** Lateral habitus views of adult female *Anisandrus dispar* (up) and *Anisandrus maiche* (down).

In Slovenia, only one COI haplotype of *A. maiche* was found in the three specimens analyzed (OR544795–OR544797). This haplotype differs in 38 bp out of 658 bp from the haplotype reported by Colombari *et al*. (2022) in Italy (OL451219). However, sequences from Slovenian specimens showed a 100% identity match with a private accession in BOLD, designated as *A. maiche* from Switzerland (closest matching BIN BOLD: ADU0788). Despite the genetic similarity, we speculate that our *A. maiche* population is most likely not the result of the natural spread of the species from Switzerland or/and Italy, as no *A. maiche* specimens were found in traps set in the western part of Slovenia along the Slovenian-Italian border (not even in some additional ethanol baited traps used for monitoring other bark beetles). Since *A. maiche* is not yet recorded in the eastern neighboring countries, the Slovenian populations are probably not the result of the natural spread of the species from Ukraine but instead an introduction via infested wood or plants. This introduction pathway is similar to that suggested by Hölling & Brockerhoff (2023) for Swiss populations.

*Xylosandrus compactus* (Fig. 4), the other new non-native species found in Slovenia, was much less abundant, with only three individuals found on 31.05.2023 at Srmin in the western Slovenia (Fig. 1). The sequence obtained from one specimen (OR544798) showed a 100% identity match to sequences of *X. compactus* from Italy (MW532748) and Spain (MW961427). The species identification was also confirmed via the BOLD identification engine (closest matching BIN BOLD: AEM9552). *Xylosandrus compactus* is widely distributed in central and southern Italy (EPPO 2024), but it is not yet present in the northern Italian regions along the border with Slovenia. Therefore, it most likely did not spread to Slovenia naturally. The trap in which we found *X. compactus* was located less than two kilometers from the port of Koper, which represents the most possible point of entry. Since the climatic conditions suit the species (Urvois *et al*. 2021), *X. compactus* will most likely spread throughout the area. As of now, no tree damage has been recorded in Slovenia due to *X. compactus*.

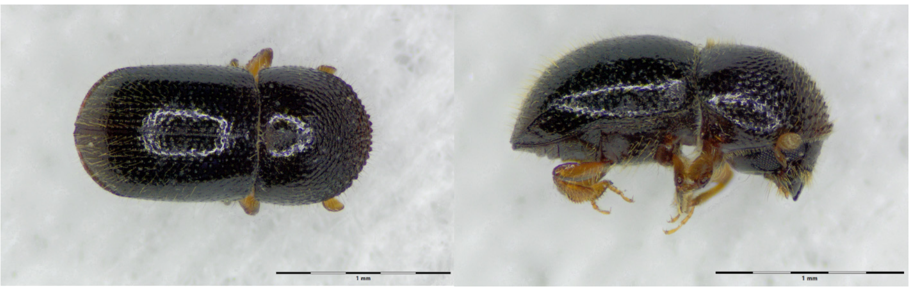

**FIGURE 4:** Dorsal (left) and lateral (right) habitus views of adult female *Xylosandrus compactus*.

Following the discovery of *X. germanus* in 2000 (Jurc *et al*. 2010) and *X. crassiusculus* in 2017 (Kavčič 2018), *X. compactus* is the third non-native *Xylosandrus* species found in Slovenia. *Xylosandrus germanus* is now widely distributed throughout Slovenia (Hauptman *et al*. 2019a), and it was also found in all monitoring sites during this survey (Tab. 2). Conversely, *X. crassiusculus* was not detected in the traps during this survey, although it has already spread to a large part of southwestern Slovenia with a sub-Mediterranean climate (T. Hauptman, personal observation).

In the last decade, seven new non-native species have been detected for the first time in Slovenia (Kavčič 2018; Hauptman *et al*. 2019a; Hauptman *et al*. 2019b; Kavčič *et al*. 2023; this study). Together with neighboring Italy, which is considered one of the EU countries with the largest number of intercepted and established non-native bark and ambrosia beetles (Ruzzier *et al*. 2022), Slovenia sits at an important crossroads of international trade routes and an area with diverse ecosystems, both of which has a significant effect on introduction and successful establishment of non-native species. All these findings underscore the necessity of continuous and careful national surveys for the interception of invasive non-native species. Furthermore, a monitoring system should be established in Slovenian forests for early detection and to track the spread and potential impact of all newly introduced species.

**TABLE 2:** Bark beetle species caught in 10 ethanol-baited multi-funnel traps. The total number of specimens and sites where particular species were caught are shown. Non-native species are highlighted in bold.

| Species[1] | Presence at locations | Total N of individuals |
|---|---|---|
| *Xyleborinus saxesenii* (Ratzeburg) | 1–10 | 1907 |
| ***Xylosandrus germanus* (Blandford)** | **1–10** | **1581** |
| ***Anisandrus maiche* (Kurentzov)** | **7, 8, 10** | **386** |
| *Anisandrus dispar* (Fabricius) | 2, 3, 5–8, 10 | 164 |
| *Dryocoetes villosus* (Fabricius) | 3–5, 7, 8 | 57 |
| *Xyleborus dryographus* (Ratzeburg) | 3, 4 | 12 |
| *Trypodendron signatum* (Fabricius) | 3, 8, 10 | 3 |
| *Xyleborus monographus* (Fabricius) | 1, 3, 10 | 3 |
| *Hylesinus toranio* (D'Anthoine) | 2, 10 | 3 |
| *Hylesinus fraxini* (Panzer) | 2 | 3 |
| ***Xylosandrus compactus* (Eichhoff)** | **1** | **3** |
| ***Gnathotrichus materiarius* (Fitch)** | **5** | **2** |
| ***Hypothenemus eruditus* Westwood** | **8** | **1** |
| *Taphrorychus bicolor* (Herbst) | 8 | 1 |
| ***Xyleborinus attenuatus* (Blandford)** | **8** | **1** |

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

## Acknowledgements

This study was supported by the Slovenian Research and Innovation Agency (Research Programme P4-0107) and by the Administration of the Republic of Slovenia for Food Safety, Veterinary Sector and Plant Protection.

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