

Rapid Communication**First records of seed beetles *Megabruchidius dorsalis* (Fähræus, 1839) and *M. tonkineus* (Pic, 1904) from three Balkan countries**

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Citation: Šipek M, Horvat E, Sajna N (2022) First records of seed beetles *Megabruchidius dorsalis* (Fähræus, 1839) and *M. tonkineus* (Pic, 1904) from three Balkan countries. *BioInvasions Records* 11(1): 101–109, <https://doi.org/10.3391/bir.2022.11.1.11>

Received: 3 September 2021

Accepted: 15 December 2021

Published: 10 January 2022

Handling editor: Alejandro Zaldivar-Riverón

Thematic editor: Angeliki Martinou

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OPEN ACCESS**Abstract**

Asian seed beetles of the genus *Megabruchidius* have been recorded recently in many European countries. Our contribution serves to formally report the first records of *M. dorsalis* in Bosnia and Herzegovina, Montenegro and Albania and the first record of *M. tonkineus* in Montenegro, all found in 2021. Both species develop inside the hard seeds of the ornamental tree *Gleditsia triacanthos*. Mature *G. triacanthos* pods were collected from the ground in urban parks of Nikšić and Podgorica (Montenegro) and Tirana (Albania) in July 2021, and Banja Luka (Bosnia and Herzegovina) in August 2021. Fully developed beetles were recorded after two weeks. *Megabruchidius dorsalis* emerged from the pods collected in all locations, while *M. tonkineus* was reared only from the pods collected in Podgorica. Because in Podgorica both species were found in the same pods, this also represents confirmation that in some locations these species do co-exist. Overall, *M. dorsalis* was more widespread and abundant.

Key words: alien species, invasive species, Bruchinae, Chrysomelidae, Coleoptera, range expansion, Europe, *Gleditsia triacanthos*

Introduction

Seed beetles of the subfamily Bruchinae (Coleoptera: Chrysomelidae) include about 1,700 species (Johnson et al. 2004), and among them some are economically significant pests of stored legume seeds intended for human consumption (Delobel and Tran 2003). Generally, bruchine larvae feed and develop inside seeds and are specialized feeders of Fabaceae (Southgate 1979; Johnson 1981). Therefore, the infestations can happen almost unnoticeably, and species can spread inadvertently across geographic boundaries through transport of infested seeds (Njoroge et al. 2017). This can pose a threat of alien species introduction. A review by Yus-Ramos et al. (2014) showed that dozens of bruchine species in Europe are alien, originating from the Western hemisphere and from Asia and Africa; however, their introduction happened largely through the importation of infested host plants, which are used for ornamental, agriculture or forestry purposes. Such alien species are often perceived as being low in importance, and their impact and spread are expected to be very limited (Seidel et al.

2021). However, recent studies suggest that encounters of host plants and their seed predators can lead to intriguing interactions, which go beyond straightforward predator – host interactions, particularly if host plant switching happens (Horvat and Sajna 2021a).

The species of the genus *Megabruchidius* Borowiec, 1984 were initially reported to be monophagous or strictly oligophagous, specialized on *Gleditsia* L. species as hosts (Zacher 1952). In Europe, two *Megabruchidius* species, originating from East Asia, were introduced: *M. dorsalis* (Fähræus, 1839), first recorded in 1989 in Italy (Migliaccio and Zampeti 1989); and *M. tonkineus* (Pic, 1904), first recorded in 1980 in Germany (Wendt 1980). *Megabruchidius dorsalis* develops in seeds of *Gleditsia fera* (Lour.) Merr., *Gleditsia japonica* Miq., *G. sinensis* Lam. and *G. triacanthos* L. (Tuda and Morimoto 2004), and *Gymnocladus dioicus* (L.) K. Koch (Callot and Wagner 2016; Temreshev and Valiyeva 2016; György and Tuda 2019). *Megabruchidius tonkineus* develops in *G. triacanthos* seeds (Tuda and Morimoto 2004).

Here we report for the first time the presence of *M. dorsalis* and *M. tonkineus* in Montenegro and *M. dorsalis* in Albania and Bosnia and Herzegovina, where they were reared from seeds of *G. triacanthos*. We also report the co-occurrence of *M. dorsalis* and *M. tonkineus* in the same pods in one location, something that has been previously evidenced by Yus Ramos (2009) in Hungary and Fritzsche and Delobel (2012) in France.

Materials and methods

Several mature *G. triacanthos* pods were collected in July or August 2021 from the ground and from the lowest branches (if present): on July 17th from a park in Nikšić (42.768599; 18.954820), on July 19th from a park in Podgorica (42.445176; 19.266206; both Montenegro), on July 26th in Tirana (41.323056; 19.833323; Albania), and on August 18th in Banja Luka (44.770975; 17.218756; Bosnia and Herzegovina). Pods had been produced during the previous year. Collected material was kept in a car trunk outdoors until it was transported to the ecology laboratory at the Faculty of Natural Sciences and Mathematics in Maribor, Slovenia, and stored in dark cloth bags until examination. We examined the pods from Montenegro and Albania for reared beetles twice: on the 5th and the 10th of August 2021. On the latter date, we counted the number of characteristic adult seed beetle emergence holes on each collected *G. triacanthos* pod. The pods from Bosnia were examined and emergence holes counted on September 1st, 2021. Because the collected pods were stored in a car trunk for some time, we recorded both dead and live beetles.

In Europe, both *Megabruchidius* species can be distinguished from other seeds beetles found by their specialization for larval hosts of the non-native ornamental *Gleditsia* genus and by their pygidium with present a pair of conspicuous oval and blackish depressions (larger in females). *Megabruchidius*

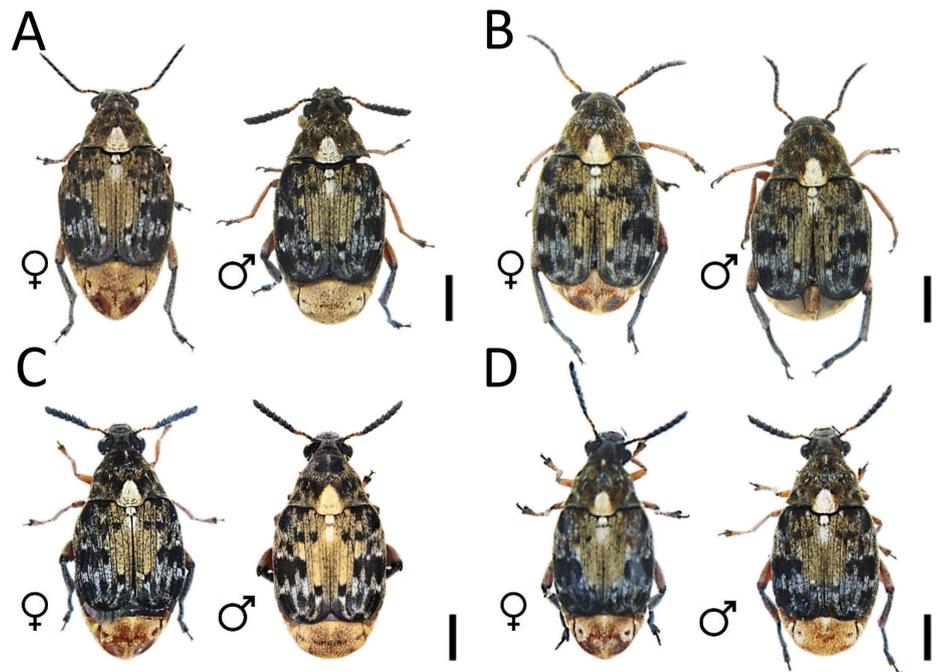


Figure 1. Adult *Megabruchidius dorsalis* beetles emerged from pods collected in A) Nikšić (Montenegro), B) Podgorica (Montenegro), C) Tirana (Albania) and D) Banja Luka (Bosnia and Herzegovina). Dorsal views of female (♀) and male (♂) are shown. Scale bars represent 1 mm. Photographs by M. Šipek and E. Horvat.

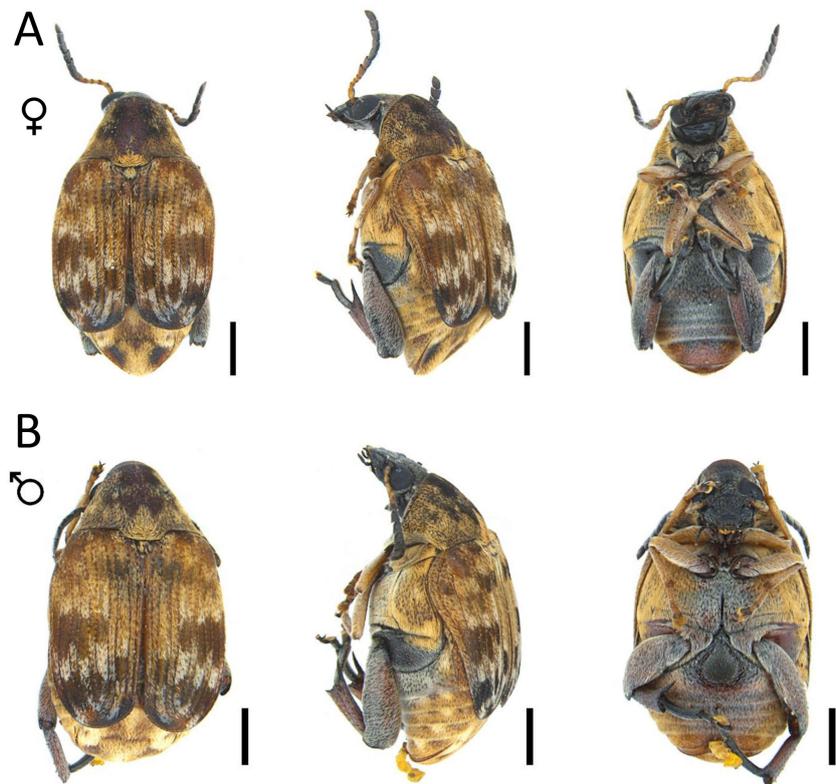


Figure 2. Adult *Megabruchidius tonkineus* emerged from collected *Gleditsia triacanthos* pods in Podgorica – (A) female and (B) male dorsal, lateral and ventral views. Scale bars are 1 mm. Photographs by M. Šipek and E. Horvat.

dorsalis (Figure 1) and *M. tonkineus* (Figure 2) are similar in size; both species can reach up to 6 mm, however, *M. dorsalis* is generally larger than

Table 1. List and description of species and specimens found in pods in Montenegro (Nikšić, Podgorica), Albania (Tirana) (collected in July 2021, checked on August 10th, 2021), and Bosnia and Herzegovina (Banja Luka) (collected on August 18th and checked on September 1st, 2021).

Location	No. of pods checked, species, no. of specimens found	No. of males (♂), females (♀), organism live (a)/dead (d)
Nikšić	34 pods, <i>M. dorsalis</i> 129 specimens	45♂a; 15♂d 54♀a; 15♀d
Podgorica	47 pods, <i>M. dorsalis</i> 17 specimens, <i>M. tonkineus</i> 14 specimens	6♂a; 1♂d 9♀a; 1♀d 2♂a; 5♂d 4♀a; 3♀d
Tirana	72 pods, <i>M. dorsalis</i> 30 specimens	17♂a; 0♂d 13♀a; 0♀d
Banja Luka	54 pods, <i>M. dorsalis</i> 15 specimens	8♂a; 3♂d 3♀a; 1♀d

M. tonkineus (Tuda and Morimoto 2004). The main morphological difference between them is the distinctly shorter “tooth” (spiniform extension) on the apex of the hind tibia in *M. dorsalis* (Korotyaev 2015) compared to *M. tonkineus*, which exhibits a large “tooth” exceeding the first tarsal segment (visible in lateral views in Figure 2). Additionally, in *M. dorsalis* the pronotum is campaniform and elytra are dark grey, while in *M. tonkineus* the pronotum is subconical and the elytra are reddish yellow to reddish brown with blackish apex (Tuda and Morimoto 2004; Pintilioaie 2018).

In both species, differences in the dark-pigmented oval depressions on the female pygidium (visible in Figure 1 and Figure 2A), and the respective length/width relation of the pygidium (longer and narrower in females, shorter and broader in males) allow for successful separation between males and females (Tuda and Morimoto 2004; Sajna 2019).

Results

Altogether, 207 pods were collected: 34 in Nikšić, 47 in Podgorica, 72 in Tirana, and 54 in Banja Luka. At the time of inspection (September 1st for samples from Bosnia and August 10th for all other samples), 79% of all pods showed characteristic adult beetle emergence holes. Those pods with emergence holes exhibited from 1 to 14 holes in Nikšić (on average 7), up to 12 holes in Podgorica (on average 4), up to 11 holes in Tirana (on average 4), and up to 20 in Banja Luka (on average 8). From pods from all locations *M. dorsalis* emerged (Figure 1), while only in the pods collected in Podgorica, did we also find *M. tonkineus* (Figure 2).

After the incubation period of 19 days for pods collected in Nikšić, 112 specimens of *M. dorsalis* were counted, 54 males (28% dead) and 58 females (26% dead). After an additional 5 days (on August 10th), 6 males and 11 females emerged (Table 1), amounting to 60 males and 69 females in total.

After incubation for 16 days, the pods collected in Podgorica yielded 14 specimens of *M. dorsalis* and 12 specimens of *M. tonkineus*. Among the

M. dorsalis beetles, we found 6 males (17% dead) and 8 females (13% dead), while for *M. tonkineus* we recorded 7 males (71% dead) and 5 females (40% dead). After an additional 5 days, 1 male and 2 female *M. dorsalis* (7 males and 10 females altogether) and 2 female *M. tonkineus* emerged (7 males and 7 females altogether).

On August 5th the incubation period for pods collected in Tirana had been 10 days when we found 2 live male *M. dorsalis*. After 5 days, 15 males and 13 females emerged, amounting to 30 beetles reared.

The samples from Banja Luka were incubated for the shortest time – for 14 days. We found 11 male (27% dead) and 4 female (25% dead) *M. dorsalis*. The entomological material is deposited in the collection of the Chair of Ecology, Department of Biology, Faculty of Natural Sciences and Mathematics at the University of Maribor, Slovenia.

Discussion

Characteristics of populations

Our results confirm established populations of *M. dorsalis* and *M. tonkineus* in Montenegro and of *M. dorsalis* in Albania and Bosnia and Herzegovina (Supplementary material Table S1). In each set of collected material, both male and female beetles were present. There is marked sexual dimorphism in both species, exhibited by females possessing two large, dark-pigmented oval patches on the pygidium (Booksmythe et al. 2017; Figures 1 and 2). In both species, the sexual dimorphism is assumed to be the result of distinct natural and/or sexual selection processes on males and females because the natural populations are female-biased (Fritzsche et al. 2016). In our samples, the number of reared females was not much greater than the number of males. The male: female ratio for *M. dorsalis* was 1:1.15 for Nikšić, 1:1.4 for Podgorica, 1:0.8 for Tirana, and 1:0.36 in Banja Luka. The largest population was recorded in Nikšić. The male: female ratio for *M. tonkineus* was 1:1.

Current distribution of species

Since 2000, there have been increasing numbers of *Megabruchidius* species recorded in Europe. Currently, *M. dorsalis* is recorded in 17 European countries, while *M. tonkineus* is present in 12 European countries (Horvat and Sajna 2021b and our results; Figure 3). Occurrence of both species is now recorded for 9 countries (Figure 3). Our data confirms that *M. dorsalis* is more common and abundant compared to *M. tonkineus*.

Even though the timeline of the published records does not indicate how the actual range expansion happened (Horvat and Sajna 2021b), it is still very informative that among the first country records 50% of records for *M. tonkineus* and more than 80% of records for *M. dorsalis* were recorded since 2010. This means that it is likely the species were or still are overlooked in some European countries. Our findings of numerous beetles also indicate

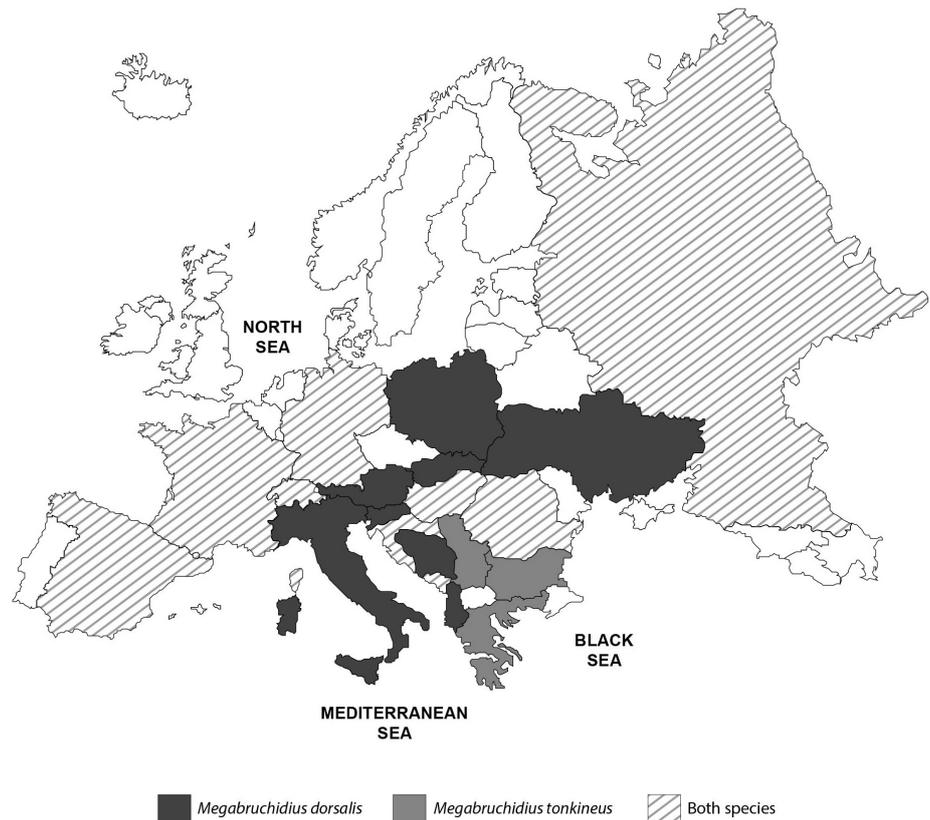


Figure 3. Map of European countries with presence of *Megabruchidius dorsalis* and *M. tonkineus*. Data was obtained from Delobel and Delobel 2008; Fritzsche and Delobel 2012; Gavrilović and Savić 2013; György and Germann 2012; Horvat and Sajna 2021b; Jermy et al. 2002; Korotyayev 2011, 2015; Kurtek et al. 2017; Martynov and Nikulina 2014; Migliaccio and Zampetti 1989; Pintilioaie et al. 2018; Rabitsch 2016; Rheinheimer 2014; Říha and Bezděk 2015; Ruta et al. 2017; Stojanova 2007; Sajna 2019; Wendt 1980; Yus-Ramos and Carles-Tolrá 2017; Yus-Ramos 2009 and our results.

that the recorded populations are well established in all the locations reported here. The infestation rate, indicated by the presence of adult beetle emergence holes, was greatest in Banja Luka, while the most beetles reared were from Nikšić. There might be several additional reasons why we found the most beetles there, such as these having emerged from the earliest collected pods.

Very interesting is the confirmed record of the coexistence of both *Megabruchidius* species in the same pods. As previously mentioned, both species were recorded in 9 European countries; however, up to now only two studies (Yus Ramos 2009; Fritzsche and Delobel 2012) did evidence the coexistence of both species in the same pods. For example, in Croatia, *M. dorsalis* was recorded in Zagreb (Horvat and Sajna 2021b), while *M. tonkineus* was recorded in Osijek and the eastern part of Croatia (Kurtek et al. 2017), and there was no record of the two species coexisting. Generally, the coexistence of *M. dorsalis* and *M. tonkineus* was expected by Bodor (2012) to happen occasionally, because both species are monophagous on the same host, *G. triacanthos* in the introduced range. It was also expected that if both species coexisted, they would be in competition

(Rheinheimer 2014; Korotyayev 2016). This seems to be the case in our sample from Podgorica as well, because in this location the number of reared beetles was much lower for *M. dorsalis* coexisting with *M. tonkineus*, compared to locations where only *M. dorsalis* was present. Interestingly, a similar pattern was recovered by Yus Ramos (2009) and Fritzsche and Delobel (2012).

Conclusions

In cases when an introduced species is a food specialist on a host that is not economically important, the introduced species is frequently perceived as an enrichment of local biodiversity – not a nuisance. Furthermore, for such a species, a general European effort to record it is usually not undertaken (Seidel et al. 2021). This is particularly true if broader scientific consensus exists that the introduced species cannot become established in the new range. However, variability in climate, along with novel habitats in urban areas and beyond, can increase the chances of spontaneous establishment. What is more, the increasing number of invasive species represents a greater probability that new and intriguing interactions could also develop among invasive species (Kueffer et al. 2013). These interactions could become particularly problematic if they represent mutualistic interactions like facilitation, enabling invasion meltdown (Simberloff and von Holle 1999; Relva et al. 2010; Sheppard et al. 2018). Such potentially mutualistic interaction was recently found for *M. dorsalis*, enabling faster and more sporadic germination of its host *G. triacanthos* under certain conditions (Horvat and Sajna 2021a). We believe that it is generally worth the effort of surveying a non-native species even though the species is not immediately problematic; for example, it is monophagous on an introduced ornamental host, because in the new range selection favors rapid evolution. For example, *M. dorsalis* is already feeding on *Gy. dioicus* in the introduced range (Callot and Wagner 2016; Temreshev and Valiyeva 2016; György and Tuda 2019).

Acknowledgements

We are grateful to M. Dabić for collecting the pods in Bosnia and transporting them to Maribor. We are grateful to two anonymous reviewers for valuable suggestions and for pointing out important literature to improve the manuscript.

Funding declaration

The authors acknowledge financial support within the research programs P1-0403 and J1-2457, funded by the Slovenian Research Agency. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Authors' contribution

MŠ performed material collection, MŠ and NS determined and collected data, MŠ and EH prepared figures and all authors wrote the paper.

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Supplementary material

The following supplementary material is available for this article:

Table S1. Geo-referenced species record data.

This material is available as part of online article from:

http://www.reabic.net/journals/bir/2022/Supplements/BIR_2022_Sipek_etal_SupplementaryMaterial.xlsx