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RESEARCH ARTICLE

First records of *Mustha spinosula* and *Perillus bioculatus* (Heteroptera: Pentatomidae) in Romania

Ioan Alexandru Rădac[1, 2], Maximilian Teodorescu[3]

1 *Faculty of Biology and Geology, Babeș-Bolyai University, Clinicilor Str., No. 5–7, 400015 Cluj-Napoca, Romania*
2 *Faculty of Chemistry, Biology, Geography, West University of Timișoara, Pestalozzi Str., No. 16, 300115 Timișoara, Romania*
3 *Fizicienilor Str., No. 15, L2 Block, 077125 Măgurele, Romania*

Corresponding author: *Ioan Alexandru Rădac* (radac.alexandru@yahoo.ro)

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Abstract

This study provides data on the first known established populations of two allochthonous species in Romania, *Mustha spinosula* and *Perillus bioculatus*. Overwintering, host plants and distribution of these two pentatomids (Heteroptera: Pentatomidae) are briefly discussed. We also provide information on color variation in nymphs of *Mustha spinosula* and report a new host plant for this species.

Keywords

Alien species, distribution, Southern Europe, true bugs, two-spotted stink bug.

Introduction

The true bugs are a highly diverse group of insects with over 40000 species worldwide (Schuh and Slater 1995) from which only around 9400 species are occurring in the Palaearctic region (Aukema et al. 2013). In the context of globalization, at least 42 species are considered alien in Europe (Rabitsch 2008) with most of the species recorded in central and western European countries (Rabitsch 2010). Although

Heteroptera is a big and heterogeneous group of insects, data on the alien taxa's presence and occurrence are still scarce in Romania. Only six alien true bugs species were recorded so far in Romania, with three taxa reported in the last 10 years (Grozea et al. 2012; Macavei et al. 2015; Don et al. 2016). This is obviously a low number considering that Romania represents an important economic route point for both terrestrial and marine ecosystems. Many allochthonous species are prone to become invasive species, thus it is critical to quickly detect them in order to correctly assess their potential impact on native fauna and flora. In the present paper, we report for the first time well-established populations of two taxa: *Mustha spinosula* (Lefebvre, 1831) a range expanding species and the alien species *Perillus bioculatus* (Fabricius, 1775), both from the family Pentatomidae.

Material and methods

Data collection was carried out by collecting, photographing or just observing specimens *in situ* as both genera are easy to identify in the field by the unique habitus. In the case of *M. spinosula,* overwintered nymphs were also collected in the spring by handpicking. Rearing the *M. spinosula* nymphs was performed indoors in a transparent mesh cage, at room temperature and sprayed with water regularly. As food source we provided fresh branches of *Prunus cerasifera*, *P. spinosa*, *Crataegus monogyna*, *Quercus robur* and *Acer campestre.* We also provided them apple and banana fruits. A few specimens were dry-mounted on glue boards. Photos of living specimens were taken in the studio using a Canon EOS 6D and Yongnuo 560 studio flashes with a diffuser, the RAW files were processed in Adobe Photoshop CS2. The map was done in ArcGIS. Voucher specimens are stored in the private collection of the last author and in the Grigore Antipa National Museum of Natural History, Bucharest, Romania.

Results

Both taxa were identified in southern Romania. *Mustha spinosula* was found at three sites as overwintered nymphs (Fig. 1). A total of 28 specimens were observed of which 14 were collected. *Perillus bioculatus* was found at three sites, two adults were photographed and several specimens were found dead near a house wall.

***Mustha spinosula* (Lefebvre, 1831)**

Material examined: 1 nymph, Pruni (44.33149°N, 25.97195°E), 78 m a.s.l., 16.02.2020, under the bark of *Quercus robur*, leg. Teodorescu Maximilian; 1 nymph, Pruni (44.32869°N, 25.97690°E), 73 m a.s.l., 01.03.2020, under the bark of *Fraxinus excelsior*, leg. Teodorescu Maximilian; 2 nymphs, Pruni (44.32988°N, 25.97556°E), 74 m a.s.l., 13, 16.03.2020, on *Fraxinus excelsior*, leg. Teodorescu Maximilian;

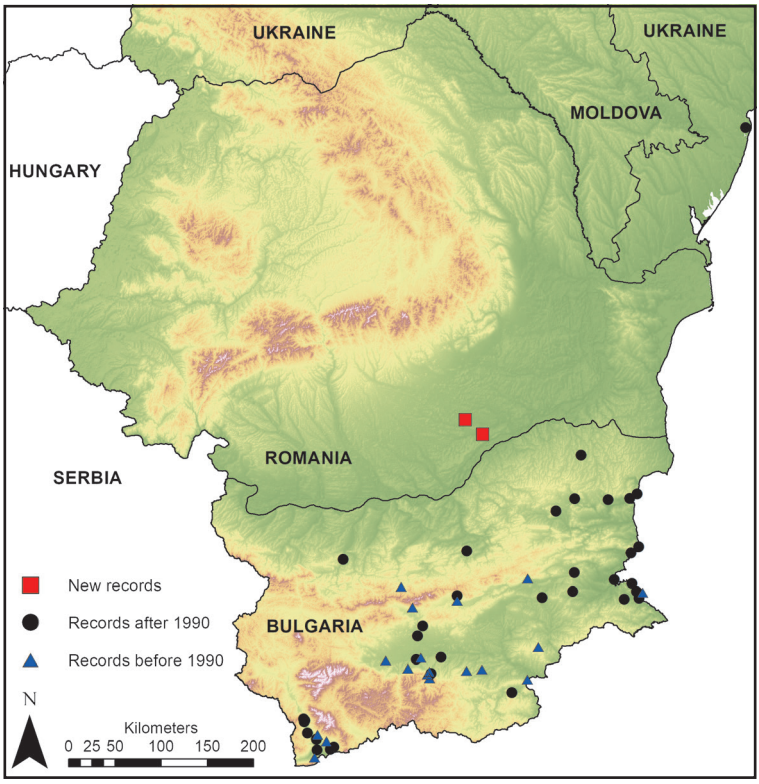

Figure 1. Current distribution of *Mustha spinosula* in Bulgaria, Ukraine and Romania.

1 nymph, Pruni (44.33040°N, 25.97473°E), 74 m a.s.l., 17.03.2020, on *Fraxinus excelsior*, leg. Teodorescu Maximilian; 1 nymph, Pruni (44.32945°N, 25.97731°E), 75 m a.s.l., 18.03.2020, on *Acer campestre*, leg. Teodorescu Maximilian; 1 nymph, Pruni (44.32831°N, 25.97707°E), 75 m a.s.l., 21.03.2020, on *Acer campestre*, leg. Teodorescu Maximilian; 14 nymphs, Pruni (44.32945°N, 25.97731°E), 75 m a.s.l., 28.03–09.04.2020, on *Acer campestre* and *Ulmus* sp., 2 specimens collected, the rest observed by Teodorescu Maximilian; 1♂ nymph, Comana (44.15653°N, 26.16667°E), 76 m a.s.l., 09.05.2020, on *Acer campestre*, leg. Teodorescu Maximilian; 3 nymphs, Comana (44.15872°N, 26.10438°E), 76 m a.s.l., 24.12.2020, under a fallen tree log, leg. Teodorescu Maximilian; 1 nymph Măgurele (44.35011°N, 26.02914°E), 80 m a.s.l., 07.04.2021, specimen with dirt on exoskeleton found on *Populus* sp., leg. Teodorescu Maximilian.

***Perillus bioculatus* (Fabricius, 1775)**

Material examined: 1♂, Deșli-Caira Hill (45.07366°N, 28.80058°E), 14.09.2017, under a rock, leg. Ștefan Vasile; several specimens, Telița (45.13205°N, 28.58267°E), 14.09.2017, dead specimens near a house wall observed by Ștefan Vasile; 1♂, Tulcea,

12.10.2018, observed by Cuzic Mariana; several specimens, Bucharest (44.40906°N, 26.18689°E), 09.08.2020, on *Ambrosia artemisiifolia*, observed by Eugenia Petrescu.

Discussion

***Mustha spinosula* (Lefebvre, 1831)**

It is a Ponto-East-Mediterranean phytophagous species (Figs 2A, B) recorded in Albania, Armenia, Azerbaijan, Bulgaria, Cyprus, Egypt, Georgia, Greece, Iran, Northern Macedonia, Montenegro, Russia, Turkey, Turkmenistan, Ukraine, Syria and Iraq (Protić 2001; Derjanschi and Péricart 2005; Markina et al. 2018). Regarding its Pontic areal, until 1990 all the records of the species were situated up to the Balkans Mountains (Joakimov 1912; Josifov 1954, 1963, 1964, 1969, 1981; Göllner-Scheiding and Arnold 1988). After 1990, the records of the species in the southern areal continued (Josifov 1999; Simov 2001; Josifov and Simov 2004) but also a gradual expansion to the north started (Markina et al. 2018; GBIF 2020; Nikolay Simov personal communication) and is presented in Fig. 1. This taxon develops on *Quercus*, *Prunus* and *Crataegus* but it was also collected on *Acacia*, *Cupressus* and *Olea* (Derjanschi and Péricart 2005). We collected it on *Fraxinus excelsior*, *Quercus*

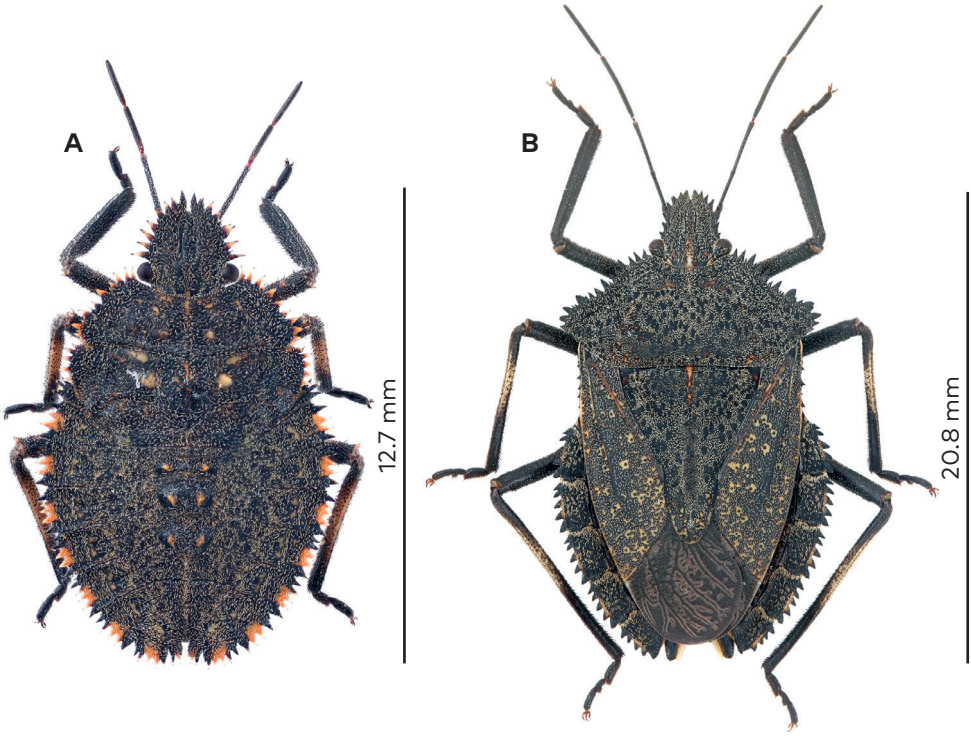

Figure 2. *Mustha spinosula*: **A** third instar nymph; **B** adult male.

robur, *Acer campestre* and *Ulmus* sp. and observed feeding in laboratory conditions on *Crataegus monogyna*. We also observed *in situ* feeding on *Acer campestre,* a new host plant for this species. Although we provided them fresh branches of the known host species, rearing of the nymphs was mainly unsuccessful. However, 5 nymphs that were developed enough molted in laboratory conditions including one individual that molted from the last instar to the adult stage. To our knowledge, there is no documented rearing of any *Mustha* species but our results show that providing fresh branches to the nymphs can maintain them alive for a period of time and even help them molt. The species overwinters as nymphs; we found this species in February under the bark of *Fraxinus* and *Quercus*, and in March, a sunbathing nymph covered with dirt and sand particles, suggesting that the species also overwinter in leaf litter. Furthermore, during the winter, nymphs were also found under a fallen tree which also indicates that the species uses a wide range of shelters to overwinter. Collecting nymphs proved to be more efficient than adults, due to undeveloped vegetation in the spring which makes the nymphs easier to spot. Therefore, collecting the last nymphal stage and providing them fresh branches can be a useful approach in obtaining adults as the identification to species level of the nymphs can be problematic. Keys for nymph identification of the Palearctic species do not exist; only a short description of the fifth instar of *Mustha spinosula* is provided by Derjanschi and Péricart (2005). Based on the observed specimens we noted a color variation of the spines on the head and thorax, varying from almost entirely yellow (Fig. 2A) to completely dark brown (Fig. 3B). The second nymphal stage has a lighter color, the spines are whitish with a yellow base and the abdomen of cream color with many dark spots (Fig. 3A). All specimens were covered with whitish waxy secretions which were more abundant on the third and fourth nymphal stages. In laboratory conditions, some individuals lose a big part of the waxy secretions. The same thing usually occurs on dead specimens stored in collections (Petr Kment, personal communication).

Although the records from the north of Danube are close to important commercial routes (Bucharest, the capital of Romania and Odessa, the largest Ukrainian seaport), the analysis of the distribution data suggests that the new records are most likely due to natural range expansion.

***Perillus bioculatus* (Fabricius, 1775)**

The two-spotted stink bug (Fig. 3D) is native to North America and it was repeatedly introduced into several European countries like Belgium, France, Germany, Italy, Russia, Slovakia, Ukraine or the former Yugoslavia, in order to control the populations of *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae) (Rabitsch 2008). After the first attempts of introduction, studies have shown that only a few individuals manage to overwinter (7.3–15.7%) and the introduction projects were abandoned in many countries (Derjanschi and Elisoveţcaia 2014). In the lack of further records, the introduction of the species was considered in many places unsuccessful (Rabitsch 2008; Derjanschi and Elisoveţcaia 2014). Till recently, the only viable populations in Europe were known in Greece and European Turkey (Rabitsch

Figure 3. *Mustha spinosula*: **A** second instar nymph; **B** fourth instar nymph; **C** fifth instar nymph; *Perillus bioculatus*; **D** adult male

2008). In these areas, the taxon is known since 1992 (Rabitsch 2008). However, more recent studies revealed other populations in Serbia (1996, Prokletije Mt.), Bulgaria (2002, Stara Planina and Maleshevska Planina Mt.), Russia (2008, Krasnodar region), Ukraine (2013, Donetsk region) and Moldova (2013, Chișinău) (Protić and Živić 2012; Simov et al. 2012; Derjanschi and Elisoveţcaia 2014; Levchenko and Martynov 2018; Tarla and Tarla 2018). In Romania, there are no mentions of releases of *P. bioculatus* (Kurzeluk 2018) and as far as we know there were no recent releases done in agriculture. The first individuals were found in 2017 and 2018 in the same area, suggesting that a stable population was present in the region. *Perillus bioculatus* probably arrived in Romania naturally, from the neighboring Republic of Moldova, as first records were close to this country (around 40 km), or from Bulgaria. Although the current distribution is hard to explain due to the multiple introductions in Europe, a scenario is emerging: a southern population of *P. bioculatus* was adapting to the new areal and started to spread north-west (Bulgaria and Serbia) and north-east along the Black Sea (East Turkey, Russia, Ukraine, Moldova and Romania), probably helped by humans through hitchhiking along commercial routes. However, spreading from multiple locations cannot be excluded, as deliberate introductions are still made (Elisoveţcaia et al. 2016) and records such as those from North India (Prasad and Pal 2015) cannot be explained without further human introduction.

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