

An overlooked invader: *Cryptorchestia garbinii* (Amphipoda: Talitridae) colonizing Serbian banks of the Danube

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ABSTRACT

The talitrid *Cryptorchestia garbinii* is a terrestrial and riparian European amphipod, distributed throughout the continent and well-known as a recent invader of the shores and banks of many water bodies. In the last decades, the species significantly expanded its range in the Balkans. First records of this species in Serbia are presented here and include descriptions of the habitats. The species was found at several localities alongside the Danube at the Iron Gates. The species was first observed in the Serbian section of the river in 2017. Abundance at this time was very high indicating that colonization almost certainly occurred much earlier. We discuss possible reasons why the species was not detected in Serbia before, as well as probable ways of its arrival and spread. Even though *C. garbinii* is the eighth allochthonous amphipod detected in Serbia, it is the first semiterrestrial representative, and also, the first one from the infraorder Talitrida recorded in the country.

KEYWORDS

Allochthonous species, Crustacea, new record, Serbia, the Iron Gates



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INTRODUCTION

The cosmopolitan amphipod family Talitridae Rafinesque, 1815 includes more than 70 genera that inhabit mainly supralittoral or terrestrial habitats (Horton et al., 2024). The recent classification schemes include four subfamilies: Floresorchestiinae Myers and Lowry, 2020; Platorchestiinae Lowry and Myers, 2022; Pseudorchestoideinae Myers and Lowry, 2020, and Talitrinae Rafinesque, 1815, of which the last one is the richest in genera (Myers and Lowry, 2020; Lowry and Myers, 2022; Horton et al., 2024). Although amphipods in this family are sometimes terrestrial, they present few structural modifications compared to aquatic representatives of other amphipod families. That can be seen in the lack of adaptations to limit water loss, as well as their typical aquatic, branchial method of respiration (Williamson, 1951).

The species *Cryptorchestia garbinii* Ruffo, Tarocco and Latella, 2014 (Fig. 1) is relatively recently described from the shores of Lake Garda, northern Italy (Ruffo et al., 2014). Although recently described, the majority of its populations across Europe have been known for a long time, but specimens were usually incorrectly identified as *Orchestia cavimana* Heller, 1865, but also as *Orchestia gammarellus* (Pallas, 1766), and *Orchestia bottae* H. Milne Edwards, 1840 (Ruffo et al., 2014). Lowry and Fanini (2013) described a new genus, *Cryptorchestia* Lowry and Fanini, 2013, and transferred the species *O. cavimana* to the newly described genus. Ruffo et al. (2014) described the species *C. garbinii*, and concluded that its distribution includes the Mediterranean basin, central Europe, and England, which is a much larger area of occupancy compared to *O. cavimana*, now considered endemic to freshwater habitats in Cyprus only. Davolos et al. (2018a) extended the description of *C. garbinii* based on specimens from the Marmara region, northwestern Turkey. Ruffo et al. (2014) noted that the distribution of *C. garbinii* is discontinuous, and that it included both freshwater and brackish habitats in Italy, France, Croatia, United Kingdom, Germany, and the Netherlands. Davolos et al. (2018b) highlighted the importance of the usage of valid species names in future basic and applied research, given that earlier

studies often use the name *C. cavimana*, but for specimens now considered *C. garbinii*.

Cryptorchestia garbinii (often under the synonym *O. cavimana*) has been used as a model species in laboratory research (e.g., Gerberding and Scholtz, 1999; Scholtz and Wolff, 2002; Ungerer et al., 2011). It has also been shown to be a good model for monitoring of microplastic pollution in the environment (Battistin et al., 2023; Ciotti et al., 2023). Fanini et al. (2019) found that *C. garbinii* is largely nocturnal, active even when air temperatures were 0 °C (but not when the soil is frozen), and is able to survive, and release live offspring in water. It is also strongly associated with rocks on the shores which are used as shelter. *Cryptorchestia garbinii* is also a well-known allochthonous species associated with several European freshwater ecosystems, both rivers and lakes, such as the Oder River (Rewicz et al., 2020), the Vistula River (Konopacka et al., 2009), Ohrid Lake (Karaman, 2013), and Balaton Lake (Juhász et al., 2006). Relatively large numbers of new records were reported by Rewicz et al. (2020) and Latella et al. (2023).

In the last decades, the number of aquatic allochthonous and invasive invertebrates in Serbian waters has increased drastically (Zorić et al., 2020). The Danube (including the Iron Gates) is well-known as a corridor in the spread of allochthonous and invasive species (Bij de Vaate et al., 2002). There are numerous examples among different invertebrate groups from Serbia, such as the snail *Clathrocasapia knipowitschii* (Makarov, 1938) (Szekeres et al., 2022), the polychaete *Manayunkia caspica* (Annenkova, 1928) (Jakovčev-Todorović et al., 2006), and the freshwater crab *Eriocheir sinensis* (H. Milne Edwards, 1853) (Paunovic et al., 2004; Živić and Vesović, 2019).

The aim of this study is to present the first records of the allochthonous amphipod species, *C. garbinii* at several localities in Serbia, in the Iron Gates area.

MATERIAL AND METHODS

Surveys were conducted from 2017 to 2024, and the study areas include several localities along the Danube River in Serbia. The complete list of surveyed localities is provided in Tab. 1. The initially surveyed



Figure 1. The female of *Cryptorchestia garbinii* Ruffo, Tarocco and Latella, 2014, collected in the village of Brnjica, the Iron Gates, northeastern Serbia (Photo: N. Vesović).

Table 1. The list of all surveyed localities along Serbian section of the Danube River (IT: Ivan Tot; MV: Mihailo Vujić; NV: Nikola Vesović; VG: Vukašin Gojšina).

Administrative district	Wider locality	Precise locality	Coordinates	Year of survey	Substrate type	Surveyor
Bor	Donji Milanovac	Mosna (Poreč Bay)	44.453915, 22.170183	2017	Sand	NV
South Banat	Banatska Palanka	Labudovo Okno	44.831960, 21.310357	2022	Sand, debris, mud	NV, MV, VG
South Banat	Kovin	Kovinski Dunavac	44.725892, 20.978134	2022	Mud, debris	NV, VG, MV
Bor	Donji Milanovac	Kostol	44.614475, 22.661832	2022	Sand, gravel	NV
Braničevo	Golubac	Golubac - Marina	44.654779, 21.622812	2023	Concrete, debris	NV, MV, VG
Braničevo	Golubac	Brnjica	44.656525, 21.765964	2023	Sand, gravel	NV, MV, VG
Braničevo	Golubac	Brnjica (Šumica)	44.654398, 21.752051	2023	Sand, gravel	NV, VG
Braničevo	Golubac	Dobra	44.635003, 21.939756	2023	Sand, gravel	NV, MV, VG
Braničevo	Golubac	Beg Bunar	44.656018, 21.706682	2023	Pebbles	NV, VG
Braničevo	Veliko Gradište	VG Quay	44.764821, 21.503462	2023	Pebbles	NV, MV, VG
Braničevo	Veliko Gradište	Ostrovo - Silver Lake	44.767453, 21.478717	2023	Sand	NV, VG, MV
Braničevo	Veliko Gradište	Zatonje	44.798812, 21.380821	2023	Sand	MV
Braničevo	Ram	Ram Marina, Quay	44.814935, 21.331257	2023	Pebbles, debris	MV
Belgrade	Belgrade	Grocka	44.674705, 20.721880	2023	Pebbles	MV

Table 1. Cont.

Administrative district	Wider locality	Precise locality	Coordinates	Year of survey	Substrate type	Surveyor
Belgrade	Belgrade	New Belgrade	44.824461, 20.425919	2023	Mud, debris	MV, NV, VG
West Bačka	Apatin	Kupusina	45.723908, 18.903430	2023	Sand, mud	IT
West Bačka	Bezdan	Baračka	45.854485, 18.858919	2023	Concrete, pebbles	IT
South Bačka	Bač	Plavna	45.345059, 19.067793	2023	Sand, debris	IT
South Bačka	Bač	Bačko Novo Selo	45.283941, 19.142744	2023	Sand, mud, debris	IT
South Bačka	Bačka Palanka	Mladenovo	45.283828, 19.219735	2023	Mud, debris	IT
South Bačka	Bačka Palanka	Karadorđevo	45.245724, 19.259903	2023	Sand, gravel	IT
South Bačka	Bačka Palanka	Jama	45.238555, 19.305438	2023	Pebbles, sand	IT
South Bačka	Bačka Palanka	Tikvara	45.236203, 19.325060	2023	Sand, mud	IT
South Bačka	Bačka Palanka	Mali Liman	45.233516, 19.368111	2023	Pebbles	IT
South Bačka	Bačka Palanka	Bager	45.239518, 19.410389	2023	Gravel	IT
South Bačka	Bačka Palanka	Poloj	45.240676, 19.479734	2023	Sand, pebbles	IT
South Bačka	Bačka Palanka	Čelarevo	45.255290, 19.539645	2023	Sand, pebbles	IT
South Bačka	Bački Petrovac	Gložan	45.245382, 19.556725	2023	Pebbles, debris	IT
South Bačka	Novi Sad	Begeč	45.222997, 19.623697	2023	Sand, gravel	IT
South Bačka	Novi Sad	Futog	45.234875, 19.710655	2023	Gravel	IT
South Bačka	Novi Sad	Sremska Kamenica	45.230234, 19.848427	2023	Pebbles, mud	IT
South Bačka	Novi Sad	Šodroš	45.229946, 19.816674	2024	Mud, sand	IT
South Bačka	Novi Sad	Quay of the Raid Victims	45.252593, 19.855917	2024	Concrete, pebbles	IT
South Bačka	Novi Sad	Petrovaradin	45.261774, 19.865650	2024	Sand	IT

habitats were selected accidentally, when the first specimens of *C. garbinii* have been recorded. After that, the selection of surveyed habitats was based on the accessibility of the riverbanks, the presence of adequate substrate and objects such as logs, rocks, and litter. The studied localities along the Serbian section of the river are arranged to cover most of the river section. The surveyed localities are presented in Fig. 2. Specimens were collected manually, but also using pitfall traps. The traps were filled with 9% alcoholic vinegar and poured into a 0.2-litre plastic cup (95 mm high and 70 mm in diameter) as an attractant

intended for collecting riparian carabid fauna. They were subsequently fixed in 70% ethanol and stored in the collection of the Institute of Zoology, University of Belgrade – Faculty of Biology. Specimens were photographed with a Zeiss SteREO Discovery.V12 stereomicroscope equipped with a Leica Flexacam C3 camera. Photographs of whole specimens were taken using a Nikon D5300 digital camera equipped with a Tamron SP Di AF 90 mm F/2.8 macro lens and a Sigma EM-140 DG ring flash with a DIY light diffuser. In order to obtain full focused images, singular frames were stacked using the Zerene Stacker 1.04.



Figure 2. The distribution map of *Cryptorchestia garbinii* Ruffo, Tarocco and Latella, 2014 in southeastern Europe and Asia Minor. Yellow triangles - literature data; red squares - new records (Serbia); green squares – localities where the species was not found (Serbia).

RESULTS

Cryptorchestia garbinii (Figs. 1, 3) was recorded at seven localities on the right bank of the Danube, in Bor and Braničevo Districts, exclusively in Eastern Serbia. The list of localities where *C. garbinii* was recorded is presented in Tab. 2. The species was not recorded at 27 surveyed localities, including all localities in South Banat, South Bačka, West Bačka, and Belgrade Districts, as well as some localities in Braničevo Districts. At four localities [Mosna (Poreč Bay), Kostol, Brnjica, and Brnjica (Šumica)] the species was relatively abundant, indicating the presence of large populations. At other three localities (Golubac-Marina, Dobra, and Beg Bunar) only a smaller number of specimens was found.

Description of localities, habitats, and remarks

All the habitats where the specimens of *C. garbinii* were found are riparian, on the right bank of the Danube. They were found on the banks, with the main substrate consisting of sand, pebbles, gravel, concrete, and debris, as well as various large objects such as logs, large rocks, and litter. Given that all the habitats are riparian, all of them are relatively wet, but not soaked in water, and usually distant from direct contact with the water course. The species was found co-occurring with other allochthonous/invasive species, such as zebra mussel, *Dreissena polymorpha* (Pallas, 1771), Asian gold clam, *Corbicula fluminea* (O.F. Müller, 1774), and spinycheek crayfish, *Faxonius limosus* (Rafinesque, 1817).

The locality at Mosna (Poreč Bay) is a mainly natural riverbank, consisting of gravel, mud, rocks, and riparian vegetation. The locality where the specimens were found at Kostol is a river bend consisting of large rocks, with a gravel belt between the bend and river course. The locality at Golubac - Marina is a town quay, with a concrete riverbank with abundant deposits of litter and debris. Localities Brnjica, Brnjica (Šumica), and Beg Bunar are all semi-natural river banks, with substrate consisting of sand and gravel, as well as various larger objects, such as driftwood logs and trunks, large rocks, and deposits of snail and bivalve shells. All localities are also in partial shade of willow and poplar trees. The locality at Dobra is quite similar to previously mentioned ones, except for the presence of some larger pebbles and absence of trees.

Description of the specimens collected in Serbia

Living specimens, dark grayish in color with a silver hue (Figs. 1, 3). This pigmentation is lost after preservation when the animal becomes uniformly pale with a reddish dorsal reticulated pattern; eyes large and dark (Fig. 1); antenna I much shorter than antenna II, consisting of 9 articles (including the flagellum and the pedunculus as well); antenna II long, consisting of 21 articles and measuring nearly half the body length (Fig. 3: 1, 2, 16, 17); gnathopod I and II in male and female pronounced, sexually dimorphic; meropodite of gnathopod I in males with a small lobe which is absent in females, propodite with a slightly sinuous palm (absent in females), dactylopodite relatively long in comparison to the margin of the propodite; gnathopod II in males with widened and almost oval propodite (absent in females), dactylopodite much slender (Fig. 3: 3, 4, 18, 19); mandibles strong; maxillae I and II very delicate (Fig. 3: 5, 20); maxillipeds partly equipped with densely arranged setae which are not present basally; coxal plates wide and irregularly setiferous with smaller and larger setae (Fig. 3: 6, 21); pereopods III and IV showing almost the same morphology except for the longer meropodite in pereopod III than pereopod IV (Fig. 3: 8, 9, 22, 23); pereopod V with considerably wider basis than pereopod III and IV but similar to those of pereopods VI and VII (Fig. 3: 9, 24); pereopod VI shorter than pereopod VII but showing almost the same morphology (Fig. 3: 10, 11, 25, 26);

all pleopods biramous and densely setiferous, inner ramus very slightly shorter than the outer (Fig 3: 12–14, 27–29); uropods also biramous, rami of equal length and setiferous (Fig 3: 15, 30). The morphology of specimens agrees with that already described by Ruffo et al. (2014) and Davolos et al. (2018a).

Behavior

Individuals express strong negative phototactic behavior, both *in situ* and in the laboratory, when exposed to natural/artificial light, by constant, unpredictable jumping, until they find a suitable new shelter (under rock or in substrate).

DISCUSSION

The newly recorded allochthonous amphipod *C. garbinii* is the first species from the family Talitridae recorded in Serbia, as well as the first terrestrial amphipod in the country. It is also the first recorded species from infraorder Talitrida in the country. Paunovic et al., (2007) mentioned the presence of the family at one locality, on the bank of the Danube, but without precise identification, or detailed explanation of the record. All the localities where *C. garbinii* was found in Serbia were within the Iron Gates section of the Danube, where the original river status was significantly altered due to the existence of the hydroaccumulation.

Considering only the amphipod fauna, *C. garbinii* is the eighth allochthonous species recorded in the country, after two corophiids: *Chelicorophium curvispinum* (G.O. Sars, 1895) and *C. robustum* (G.O. Sars, 1895), four gammarids: *Dikerogammarus bispinosus* Martynov, 1925, *D. haemobaphes* (Eichwald, 1841), *D. haemobaphes villosus* (Sowinsky, 1894), *Echinogammarus ischnus* (Stebbing, 1899), and one pontogammarid: *Obesogammarus obesus* (G.O. Sars, 1894) (Zorić et al., 2020). The native range of all previously mentioned allochthonous amphipods include the Ponto-Caspian region (Zorić et al., 2020), while the native range of *C. garbinii* remains unclear. Ruffo et al. (2014) recently described the species from the shores of Lake Garda (northern Italy) and concluded that the actual distribution includes the Mediterranean basin, central Europe, and England. On the other hand, *C. garbinii* is a well-

known allochthonous invader in many parts of Europe, such as the areas around the Baltic Sea (Kotta, 2000; Herkül et al., 2006; Konopacka et al., 2009; Rewicz et al., 2020). Despite intensive hydrobiological research of almost all sections of the Danube in the past, *C. garbinii* has not been detected, so we suggest that the species is a relatively recent invader.

The impact of this species on the native species and habitats remains unknown. Our first records of *C. garbinii* from Serbia originate from 2017, when extremely high abundance was noted in Poreč Bay (the village of Mosna), near the town of Donji Milanovac. Given that the abundance of specimens was very high in 2017, our assumption is that the species was present in Serbia for at least several years before the first observations. For now, it is not even possible to tentatively assume the exact time when the colonization of the Serbian section of the Danube took place. In 2023, numerous specimens were recorded at several localities at the Iron Gates, indicating the presence of large and well-established populations. The most upstream locality where specimens were recorded was the town of Golubac.

The lack of earlier records of *C. garbinii* in Serbia can possibly be explained by its riparian lifestyle. This specific habitat is understudied, so the species was virtually invisible both for the standard methods for collecting water and terrestrial invertebrates. These facts probably play a key role in the lack of records of *C. garbinii* in Serbia, but also in other parts of Europe. We assume that the actual distribution in Serbia, but also in the Balkans, must be much wider than currently known (Fig. 2), despite the fact that we failed to find specimens in additional surveyed localities. Along the Danube, the published records of this species were known only from the Danube Delta (Linetskii et al., 2020; Rewicz et al., 2020). The nearest previously published records of *C. garbinii* from those in Serbia are in the vicinity of the city of Bucharest, Romania.

The time of arrival and cause of the spread of the species in Serbia remains unclear. We hypothesize that completely natural spread, not facilitated or enabled by human activities is less probable, due to the existence of natural and artificial barriers between the nearest populations. As the Danube represents one of the most important water corridors for transportation in this part of Europe, the arrival of *C. garbinii* by

ships is the most probable scenario. The transport of large quantities of suitable goods that are able to retain moisture for a long period of time (e.g., gravel), probably represents the most likely means of arrival. Although dispersion by zoochory is also possible, the chances of this are very slim.

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ADDITIONAL INFORMATION AND DECLARATIONS

Author Contributions

Conceptualization and Design: MV, VG, NV; Performed research: MV, VG, NV; Analysis and interpretation of data: MV, VG, VM, NV; Preparation of figures/tables/maps: NV, VG; Writing - original draft: MV, VG; Writing - critical review and editing: MV, VG, VM, NV.

Consent for publication

All authors declare that they have reviewed the content of the manuscript and gave their consent to submit the document.

Competing interests

The authors declare no competing interest.

Data availability

All study data are included in the article.

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Study permits

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