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NEW RECORDS OF THE INVASIVE AMPHIPOD *ECHINOGAMMARUS ISCHNUS* (STEBBING, 1899) IN CROATIA

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The Ponto-Caspian alien and invasive amphipod, *Echinogammarus ischnus* (Stebbing, 1899), was found in Croatia at three sites in the lower course of the Drava River and at three sites in the Danube River (Batina, Borovo and Ilok). The most upstream site in the Drava where the species was found is a locality near Donji Miholjac, approximately 82 km upstream from the confluence with the Danube River. At this site as well as at two downstream sites (Osijek and Belišće) in Drava it was found together with the dominant invasive amphipods *Dikerogammarus villosus* and *Chelicorophium curvispinum*, while at the Donji Miholjac site it also co-occurs with two native species, *Gammarus fossarum* and *G. roeseli*.

Key words: Amphipoda, Gammaridae, invasive species, large rivers, Danube, Drava

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Ponto-kaspijski strani i invazivni rakušac *Echinogammarus ischnus* (Stebbing, 1899), pronađen je u Hrvatskoj u donjem toku rijeke Drave i na tri lokaliteta u Dunavu (Batina, Borovo i Ilok). Najuzvodnija postaja na kojoj je nađen u Dravi, kod mjesta Donji Miholjac, udaljena je otprilike 82 km od ušća u Dunav. Na toj, kao i na dvije nizvodne postaje (Osijek i Belišće) u Dravi, zabilježen je zajedno s druge dvije invazivne vrste rakušaca *Dikerogammarus villosus* i *Chelicorophium curvispinum*, dok na postaji kod Donjeg Miholjca uz ove dvije dominantne strane vrste dolazi zajedno s još dvije autohtone vrste *Gammarus fossarum* i *G. roeseli*.

Ključne riječi: Amphipoda, Gammaridae, invazivna vrsta, velike rijeke, Dunav, Drava

INTRODUCTION

Alien and invasive amphipods, mostly originating from the Ponto-Caspian Basin, have been spreading throughout European freshwaters during the last two centuries. This rapid range extension has been facilitated by inter-basin canal connections, river ship traffic and many different means of unintentional introduction as well as by intentional introductions (Jazdzewski, 1980; Holdich & Pöckl, 2007). Since the Danube was connected with the Rhine through the Main-Danube Canal, and especially after the reopening of this canal in 1992, this became the most important migration route for Ponto-Caspian species' range extension to the west, known as the South European corridor (Bij De Vaate *et al*., 2002). Rivers of the Danube catchment drain 62% of Croatian territory, with the largest tributaries the Drava and Sava Rivers, and the Danube at

Fig. 1. a) Male specimen of *Echinogammarus ischnus* from the site Drava-Osijek sampled on 13th February 2015; b) detail of flagellum of antenna with characteristic long, curly and abundant setation on second antenna; c) third uropods (U3) and telson viewed from above.

the easternmost border of Croatia with Serbia. Five alien amphipods, *Dikerogammarus villosus, D. haemobaphes, D. bispinosus, Obesogammarus obesus and Chelicorophium curvispinum* are known to be present in large rivers of Croatia, their distribution being established in a previous study (Žganec *et al*., 2009).

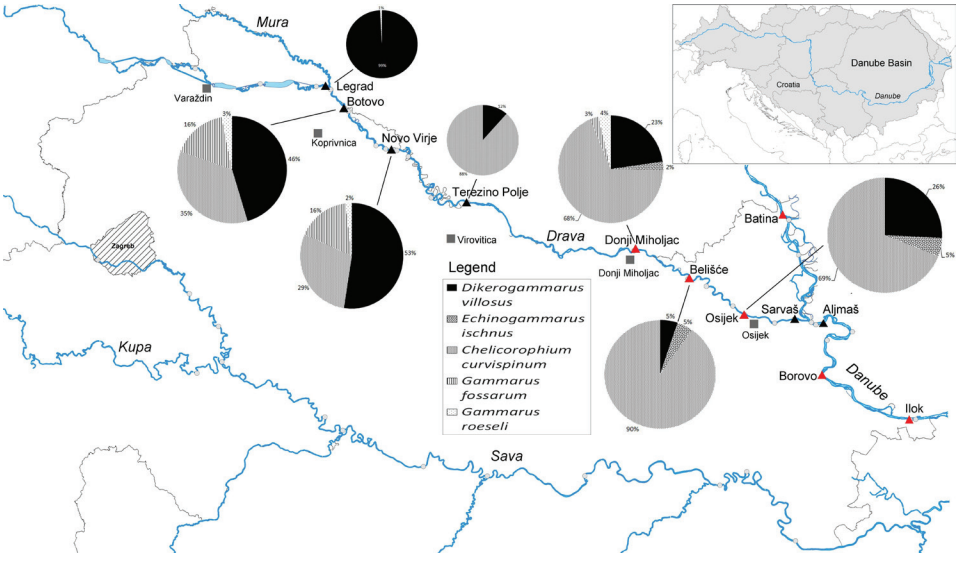

Fig. 2. Map of all study sites sampled in 2015 in Danube and Drava (triangles) and the sites in Sava, Drava and Danube sampled in 2007 and 2008 from Žganec *et al*. (2009) (grey circles). Red triangles represent sites where *Echinogammarus ischnus* was found in 2015.

The species *Echinogammarus ischnus* (Stebbing, 1899) (syn. *Chaetogammarus ischnus*) originates from the rivers of Ponto-Caspian Basin. It has migrated to Western Europe through Central and Southern European corridors (Jazdzewski, 1980; Bij De Vaate *et al*., 2002; Cristescu *et al*., 2004). Morphologically, this species is easily recognizable (Fig. 1) because of the relatively small size (11 mm of total length in males), large eyes (typical for the genus *Echinogammarus*), the abundant curly setation of its second antenna, very short telson lobes, third uropod without seta and only with spines (Fig. 1) and characteristic setation of mandibule palp, a combination which is unique in the genus *Echinogammarus* (Pinkster, 1993).

MATERIALS AND METHODS

Quantitative samples (n=20) were collected at eight sites along the Drava River in February 2015 and at four sites in the Danube in July 2015 (Fig. 2) using hand nets (mesh size 500 μm) over an area of 0.0625 m^2 in a shallow bank area. Samples were collected from all available types of substrate, taking into consideration the relative contribution of each microhabitat type. The number of samples from each substrate/microhabitat type corresponds to the relative contribution of a particular microhabitat to the substrate of the assessed river reach (5% = 1 sample) (Hrn En 16150). Samples were fixed with 96% alcohol. In the laboratory, amphipods were separated from the sediment, organic detritus and other invertebrates and stored in 75% alcohol for later identification. Amphipods were identified using the keys of Cărăuşu *et al*. (1955), Karaman & Pinkster (1977a, b, 1987), Pinkster (1993) and Eggers & Martens (2001). A GPS receiver was used for the geocoding of field observations on the spot, and Gauss-Krüger coordinates were determined for each site. Data were mapped using Diva-Gis program packet.

RESULTS

Echinogammarus ischnus was found at three of the eight sites in the lower course of Drava and at three of the four sites in the Croatian section of the Danube (Fig. 2). In the Danube it was found at Batina (river kilometre-rkm 1424, 8 specimens), Borovo (rkm 1336, 2 specimens) and at Ilok (rkm 1300, 5 specimens). In the Drava it was found upstream of the town of Osijek, approximately 24 km upstream from the confluence with Danube (5 specimens), in Belišće (rkm 56, 8 specimens) and at the most upstream site, Donji Miholjac (rkm 82, 2 specimens). At the sites Osijek and Belišće the focal species was found together with *Dikerogammarus villosus* and *Chelicorophium curvispinum*. *Echinogammarus ischnus* had a relative abundance of 5% at both sites. At the site Donji Miholjac two native species, *Gammarus fossarum and G. roeseli*, were found together with the former three alien species; and *E. ischnus* had a relative abundance of 2% (Fig. 2). Interestingly, amphipods were not found at the most downstream site, Drava-Sarvaš, 12 km downstream of Osijek, i.e. 5 km upstream from the confluence with the Danube River.

The density of *E. ischnus* in the Danube was very similar at the three sites and ranged from 4.8 to 6.7 $ind.m^{-2}$, with a similar relative abundance of 2.4 to 2.8% in total number of amphipods collected at each site. At the sites Batina and Ilok most samples were collected on embankments made of large stones, while at two other sites they were collected mostly on sand and mud substrate. In the Drava, density decreased in the upstream direction from 24 $ind.m^{-2}$ in Osijek to 1.6 $ind.m^{-2}$ in Donji Miholjac. At three sites where samples were collected in the Drava, the substrate was mostly sand (90–95%) and mud

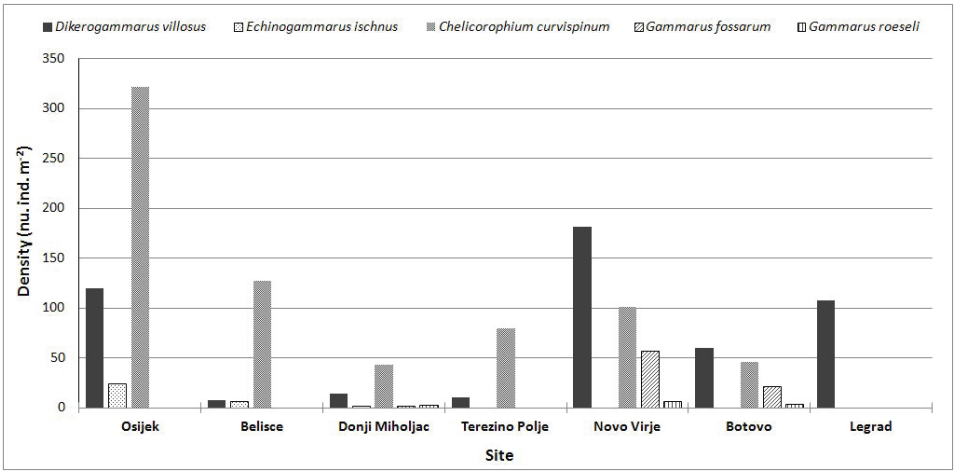

Fig. 3. Densities of all amphipod species at seven sites in Drava in February 2015.

(5%) and most of the replicate samples (18–19) were collected on these types of substrate. *Chelicorophium curvispinum* had the highest density at four downstream sites in Drava and *D. villosus* was the second most abundant amphipod at these sites (Fig. 3). At upstream sites Novo Virje, Botovo and Legrad, *D. villosus* was the most abundant species. The relation between species densities of two alien and two native amphipods was the same at the sites Novo Virje and Botovo. Interestingly, native species, *Gammarus fossarum* and *G. roeseli,* were found at three and four sites, respectively, co-occurring with alien amphipods. Density of *G. fossarum* ranged from 1.6 ind.m^{-2} at Donji Miholjac to 56.6 ind.m^{-2} at Novo Virje, while *G. roeseli* was less abundant (0.8–6.9 ind.m^{-2}) but it was found at four sites (site Legrad: 1% or 0.8 ind.m^{-2} not visible on Fig. 3).

DISCUSSION AND CONCLUSIONS

Echinogammarus ischnus (syn. *Chaetogammarus ischnus*) originates from the rivers of the Ponto-Caspian Basin, the lower Volga and the Danube River Delta (Carausu *et al*., 1955; Birštejn & Romanova, 1968), and the Dnieper River Delta (Jazdzewski, 1980). It migrated to Western Europe from the Dnieper River via the Pripet-Bug canal system, which connects to the Vistula River in Poland (Konopacka & Jasionowska, 1995), which is known as Central European corridor (Bij De Vaate *et al*., 2002). Through this corridor it advanced all the way to the North Sea and the Baltic Sea (Jazdzewski, 1980). In the Rhine (The Netherlands) it was discovered in the early 1990s (Schöll, 1990; Van Den Brink *et al*., 1993). The species also managed to cross the Atlantic by ship ballast water and it was discovered in North America in the Great Lakes basin in 1995 (Witt *et al*., 1997). The rapid range extension of this invasive species along natural and artificial watercourses is well documented in both Europe (Jazdzewski, 1980) and North America (Kang, 2003).

In the Danube it had dispersed up to the Slovakian section, more than 1900 km upstream from the mouth, by the second half of 20th century (Jazdzewski, 1980). Karaman (1953) reports its findings in the Serbian section of Danube (Smederevo, downstream from Belgrade). In the samples collected in 1961 and 1962 at ten sites along the Serbian

section of the Danube (rkm 1092–1179), Pljakić (1965) established *E. ischnus* and *Obesogammarus obesus* to be the most frequent and dominant species among six recorded amphipod species. However, some more recent studies of the macroinvertebrate communities of Croatian and Serbian section of Danube failed to detect its presence in the Danube (Paunović *et al*. 2007), while other studies report its presence in the Serbian part of Danube (Paunović *et al*., 2010). However, in the most recent study Borza *et al.* (2015) reported findings of the species in the Croatian section of the Danube. Based on these and our data we assume that *E. ischnus* was more abundant in the Danube in the past, while now has low abundance and it is therefore difficult to detect. It is possible that *Dikerogammarus villosus* and *Chelicorophium curvispinum*, which are now the dominant amphipods in the Danube (Žganec *et al*., 2009; Borza *et al.*, 2015), have caused the decline of *E. ischnus*. The decline of *E. ischnus* observed in the lower Rhine was probably facilitated by the mass abundance of *C. curvispinum*, which probably reduced the colonization success of *E. ischnus*, a lithophilic dweller, by covering the solid substrates in the river with mud tubes (Bij De Vaate *et al*., 2002). Although Jazdzewski (1980) assumed that *E. ischnus* entered the lower reaches of the Sava and Drava, it was not found in Croatian sections of these rivers before (Žganec *et al*., 2009). A previous study failed to detect *E. ischnus* in the lower course of the Drava probably because it was still rare and sampling at this section of the river was not intensive enough to detect rare species.

In its native range *E. ischnus* was found in fresh and slightly brackish waters of large tributaries of Caspian and Black Sea, but also in the littoral of the open Caspian and Black Sea. However, the taxonomic confusion about freshwater and marine forms is still not resolved (Jazdzewski, 1980; Pinkster, 1993). The freshwater form, *E. ischnus* (s.s.) is euryhaline, primarily riverine and prefers large, slow-flowing waters with stony substrates. It was found mainly on stone or gravel substrates along the shallow margins of lakes and rivers, and the greatest densities were found in *Dreissena* clumps (Jazdzewski, 1980; Kohn & Waterstraat, 1990; Dermott *et al*., 1998; Kang, 2003). At three sites along the Drava, samples were collected mostly from sand (psammal) microhabitats. Therefore, we assume that the relatively low densities of *E. ischnus* established in lower Drava are the consequence of the prevalence of sand and mud substrates int this section of the river. In the Danube it was only not found at the Aljmaš site, where the dominant substrate from which samples were collected was a mix of sand and mud. At two sites in Danube (Batina and Borovo) most samples were collected on artificial substrates (technolithal), while in Ilok, where sand and mud dominated, one sample was collected on small stones (microlithal). In the lower (Serbian) section of the Sava with muddy sediments, *E. ischnus* was found only on the large stones of embankments (Žganec *et al*., unpublished). Those findings are in accordance with the well-known preference of this litophilic gammarid for stony substrates (Kang, 2003). It is very likely that much the higher densities of *E. ischnus* in Drava could be found on the technolithal substrate of embankments and groynes, where future sampling should be conducted. Also, due to the *E. ischnus* preference for *Dreissena* clumps, which are abundant in the middle Drava and especially in Drava reservoirs (Lajtner *et al*., 2004), if it spreads further upstream it could easily became much more abundant than it is now in the lower Drava. Due to the mentioned problems with the detection of *E. ischnus* in the lower Drava in previous studies, it is not possible to say when exactly this species colonized Drava. We estimate that this happened probably 10–20 years ago since it has already colonized 58 km of the lower Drava between Osijek and Donji Miholjac. Further, we cannot say much about the speed of its upstream spreading. Therefore, further studies should check its distribution in the least studied section of the Drava between Donji Miholjac and Tere-

zino Polje and try to establish its invasion front for future monitoring of its upstream spreading in Drava.

The species has different life histories in its native and in the invaded range (overview in Konopacka & Jesionowska, 1995). In its native range, the reproduction of *E. ischnus* takes place from March/April until October while in Polish populations it occurs from February until October. Populations in tributaries of the Don River, with a limited annual temperature range, breed throughout the year. Ovigerous females in the native range produce more eggs (Ukraine population had an average 33 and a maximum 55 eggs) than those in the invaded range (Poland: avg-10 and max-27; Germany: <20) (Konopacka & Jesionowska, 1995). Populations from a fluvial lake at the confluence of the St. Lawrence and Ottawa rivers in Canada were found to have maximum brood size of only 19 eggs probably due to the low conductivity of these waters (Kestrup & Ricciardi, 2010). Therefore, fecundity of *E. ischnus* in the invading range is relatively low, which could be expected in Drava as well. However, in spite of this, due to its repeated reproduction and small size this species co-occurs with much larger and more fecund (*D. villosus*) as well as with the more abundant (*C. curvispinum*) dominant invasive amphipods in lower Drava.

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SAŽETAK

Nalazi invazivnog rakušca *Echinogammarus ischnus* (Stebbing, 1899) u Hrvatskoj

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Ponto-kaspijski strani i invazivni rakušac, *Echinogammarus ischnus* (Stebbing, 1899), pronađen je prvi puta u Hrvatskoj u Dunavu na tri postaje (Batina, Borovo i Ilok) i u donjem toku rijeke Drave. Na tri postaje u Dunavu imao je relativno malu gustoću od 4.8–6.7 jed.m^{-2}

i mali udio od 2.4–2.8% u ukupnom broju rakušaca. Najuzvodnija postaja na kojoj je nađen u Dravi, kod mjesta Donji Miholjac, udaljena je otprilike 82 km od ušća u Dunav. Dvije nizvodne postaje na kojoj je nađen udaljene su 56 km (Belišće) i 24 km (Osijek) uzvodno od ušća rijeke Drave. Na postajama Osijek i Belišće zabilježen je zajedno s druge dvije invazivne vrste rakušaca *Dikerogammarus villosus* i *Chelicorophium curvispinum*, a njegov udio bio je 5% ukupnog broja rakušaca na obje postaje. Na postaji kod Donjeg Miholjca uz ove dvije strane vrste zabilježen je zajedno s još dvije autohtone vrste *Gammarus fossarum* i *G. roeseli* s relativnim udjelom od 2%. Gustoća vrste na pretežno pješčanom sedimentu u Dravi smanjivala se u uzvodnom smjeru od 24 jed.m^{-2} u Osijeku do 1.6 jed.m^{-2} u Donjem Miholjcu.