



***Faxonius limosus* (Rafinesque, 1817) (Decapoda: Cambaridae), a New Invasive Alien Species of European Union Concern in Bulgaria**

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Abstract: A representative of the family Cambaridae (Decapoda) was recorded for the first time in Bulgaria. This is the North American spiny-cheek crayfish *Faxonius limosus*, considered as an invasive alien species of European Union concern. A total of 92 specimens (42 males and 50 females) were found in the Danube tributaries, the rivers Topolovets, Voinishka and Archar in northwestern Bulgaria, in 2015–2016. The morphometric characters of all specimens were studied and no significant differences were found between the three populations and between the males and females. Furthermore, the morphometric features distinguishing *F. limosus* from the native crayfish species of the genera *Astacus*, *Pontastacus* and *Austropotamobius* in Bulgaria were presented. We assume that *F. limosus* was introduced in Bulgaria through the Danube River, coming downstream from the Serbian and Romanian sectors, where this species have been recorded earlier. Our results also indicate an early stage of establishment of the species in the Bulgarian sector of the Danube River. The further spread of *F. limosus* upstream of the Danube tributaries and in the inland waters will threaten the native crayfish populations in Bulgaria. Therefore, urgent measures in accordance with the Regulation (EU) No 1143/2014 need to be taken at national and regional level in order to prevent the range extension and the negative impact of this invasive alien species in Bulgaria.

Key words: Invasive alien species, spiny-cheek crayfish, morphometry, introduction, establishment, Bulgarian fauna

Introduction

The spiny-cheek crayfish *Faxonius limosus* (Rafinesque, 1817) (also known as *Orconectes limosus* in the literature) belongs to the largest crayfish family Cambaridae, which comprises 14 genera and 441 species (440 extant and one known only from fossil material), and is native to Northern and Central America (CRANDALL & DE GRAVE 2017). It was the first alien crayfish species introduced to Europe. The first and the only known successful introduction of *F. limosus* was in 1890, when 90

individuals of a batch sent by the US Commission of Fish and Fisheries were released to a fishpond near Barno'wko (Berneuchen) in Pomerania, currently in western Poland. Another recorded introduction from New York to France in 1895 failed (KULMATYCKI 1935, KOSSAKOWSKI 1966, FILIPOVÁ et al. 2011). Subsequently, *F. limosus* has spread from the initial place of its successful introduction to neighbouring countries through natural dispersal and human-assisted secondary introductions. Currently, the species has been reported from river systems, canals and lakes of 23 European territories,

including the island of Corsica, and is not found only in the Scandinavian Peninsula, as well as in many of the Balkan and Eastern European countries (HOLDICH 2002, HOLDICH et al. 2006, 2009, KOUBA et al. 2014, KOZÁK et al. 2015, GOVEDIČ 2017).

The invasive *F. limosus* is eurybiotic and can be found in various types of both standing and running waters, but prefers warm, slow-flowing and standing waters, with rich aquatic vegetation and silty sediments (HAMR 2002, HOLDICH et al. 2006, PETRUSEK et al. 2006, PÖCKL et al. 2006, KOZÁK et al. 2015). In the Atlas Mountains in Morocco, *F. limosus* has established population at altitudes from 1400 to 2078 m a.s.l. (HOLDICH et al. 2006). The species is very tolerant to a variety of environmental conditions, including highly eutrophic waters, decreased oxygen concentrations, low and elevated water temperatures, brackish water, and even drying of its habitats for several weeks (LAURENT 1988, FÜREDER et al. 2006, HOLDICH et al. 2006, PÖCKL et al. 2006, KOZÁK et al. 2015). It is highly resistant to both organic and inorganic pollution (SCHILDERMAN et al. 1999, FÜREDER et al. 2006). The species has been observed to leave the water and can colonise new habitats by migrating overland (PUKY 2014, HERRMANN et al. 2018). The lifespan of *F. limosus* is short, usually 3–4, exceptionally up to five years. Its fecundity is mostly varying from 30 to 500 eggs, depending on female size (HOLDICH et al. 2006, KOZÁK et al. 2006, 2015), with a maximum number of 1,156 ovarian eggs (PÂRVULESCU et al. 2015). This species has also highly plastic reproductive biology as it is the only one decapod capable of facultative parthenogenesis which is presumably important at occasions when population densities are low (invasion front, secondary introductions which are frequent, with low number of specimens (BUŘIČ et al. 2011, 2013).

The negative impact of the spiny-cheek crayfish *F. limosus* on the native crayfish populations in Europe is expressed in competition for habitats, in which the invader is more adaptive, as well as transmission of the crayfish plague pathogen, the oomycete *Aphanomyces astaci* Schikora, 1906, which is lethal to the native species, and to which *F. limosus* is resistant. Thus, after the appearance of *F. limosus* in Europe, the native species have dramatically decreased in abundance (HOLDICH et al. 2009, PÂRVULESCU et al. 2012, 2015, SCHRIMPF et al. 2012). Economically, *F. limosus* is not equivalent to the native species from the genera *Astacus* and *Pontastacus* due to its small size. It is rarely used in pet trade and by the fishery managers as a food supplement (CHUCHOLL 2013). Because

of its invasive potential and negative impact on native crayfish species, since 2016, *F. limosus* has been listed as invasive alien species of European Union concern according to the Regulation (EU) No 1143/2014 of the European Parliament and of the Council of 22 October 2014 on the prevention and management of the introduction and spread of invasive alien species (EU 2014).

The aim of the present article is to report on the first finding of the invasive alien crayfish species *F. limosus* and to present up-to date information about its distribution and the status of its populations in the river systems of Bulgaria.

Materials and Methods

The materials for this study were collected in the period June 2015 – November 2016. Eight surveys were conducted in the Danube River, the adjacent water bodies and the lower reaches of 21 Danube tributaries. A total of 91 sites were sampled, of which 33 in the main Danube River channel, 38 in the tributaries and 20 in the adjacent standing water bodies (reservoirs and canals).

The crayfish were caught by hands or using a dip net with a diameter of 60 cm. In some cases (rich submerged vegetation), a triangular hand dredge was also used. In each locality, transects with a length of 100 m and a width of 1 m were sampled. The total number of specimens in each transect was counted. All captured specimens were identified, sexed and measured in the field. The main morphometric features of the collected specimens were studied, and the total length, carapace length and carapace width of each specimen were measured to the nearest mm. After that, the native species were carefully returned in the place of their capture, while the alien species were fixed in 95% ethanol to avoid possible spreading of the crayfish plague pathogen *Aphanomyces astaci* in the wild. The materials have been stored in the collections of the Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences.

Results

The spiny-cheek crayfish *Faxonius limosus* was recorded for the first time in Bulgaria in June 2015. Fourteen specimens (6 male and 8 female) were caught in the Topolovets River, a tributary of the Danube River, near the town of Vidin, on 17.06.2015 (Fig. 1, Table 1). During the subsequent more intensive studies of the main Danube River channel and the lower reaches of its tributaries



Fig. 1. Habitats of *Faxonius limosus* in Bulgaria. (A) Locality of the first finding of the species in the Toplovets River near Vidin Town; (B) Voinishka River at Dunavtsi Village; (C) Archar River downstream of Archar Village; (D) Archar River – a weir formed by the destroyed old bridge, separating populations of *F. limosus* (downstream of the bridge) and *Pontastacus leptodactylus* (upstream of the bridge).

Table 1. Localities of the spiny-cheek crayfish *Faxonius limosus* in Bulgaria, with geographic names and coordinates, altitude, UTM codes, dates of finding, and number of individuals collected in the period 2015–2016.

№	River	Locality (Distance to the confluence with the Danube River, km)	Altitude (m a.s.l.)	Geographic coordinates	UTM Code	Date of finding	Number of individuals at 100 m transect
1	Toplovets	At the confluence with the Danube River (0.2 km)	33	N 43.937583 E 22.847194	FP 46	19.08.2015	2
2	Toplovets	At the bridge on the road Sofia City – Vidin Town (1.2 km)	35	N 43.941306 E 22.838611	FP 46	17.06.2015	14
						25.09.2016	3
						30.09.2016	5
3	Toplovets	At the bridge on the road Vidin Town – Slana Bara Village (4.7 km)	35	N 43.969417 E 22.825750	FP 47	19.08.2015	13
4	Toplovets	At the bridge on the road Vidin Town – Ruptsi Village (8.0 km)	38	N 43.997056 E 22.826611	FP 47	19.08.2015	2
5	Toplovets	At the bridge on the offroad Gradets Village – Druzhba Village (18 km)	57	N 44.019833 E 22.719250	FP 48	27.09.2016	1
6	Voinishka	At Dunavtsi Village, the bridge on the road Sofia City – Vidin Town (2.0 km)	34	N 43.920500 E 22.825472	FP 46	20.08.2015	6
						30.09.2016	4
						08.11.2016	1
7	Archar	Downstream of the Archar Village (1.0 km)	32	N 43.813306 E 22.921806	FP 55	22.08.2015	34
						30.09.2016	6
						08.11.2016	1

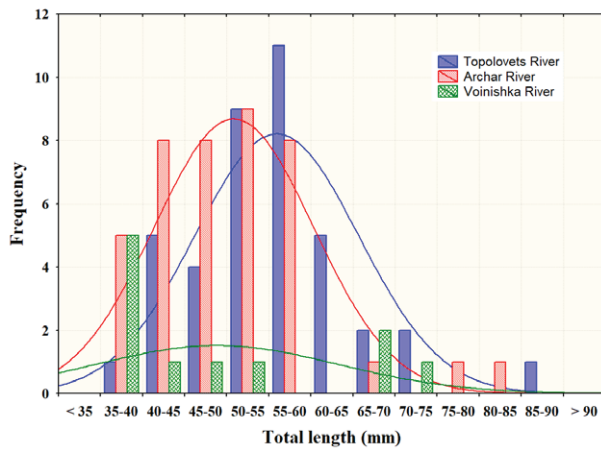


Fig. 2. Histograms showing the size frequency distribution of the total length in the three studied populations of *Faxonius limosus* in Bulgaria.

Table 2. Morphometric characters of *Faxonius limosus* from Bulgaria. Min – minimum value; Max – maximum value; SD – standard deviation; n – number of specimens examined. The measurements are given in mm.

Characters / sex		Mean	Min	Max	SD	n
Total length (TL)	total	52.7	36	86	10.51	92
	males	52.9	36	78	9.35	42
	females	52.5	36	86	11.48	50
Carapace length (CL)	total	25.6	17	40	5.02	92
	males	25.9	18	40	4.66	42
	females	25.4	17	40	5.35	50
Carapace width (CW)	total	12.1	8	20	2.65	92
	males	12.3	8	20	2.48	42
	females	12.0	8	20	2.80	50
CL/TL ratio	total	0.49	0.42	0.53	0.02	92
	males	0.49	0.45	0.53	0.02	42
	females	0.48	0.42	0.53	0.02	50
CW/TL ratio	total	0.23	0.20	0.26	0.01	92
	males	0.23	0.20	0.26	0.01	42
	females	0.23	0.20	0.25	0.01	50
CW/CL ratio	total	0.47	0.38	0.54	0.03	92
	males	0.47	0.41	0.53	0.02	42
	females	0.47	0.38	0.54	0.03	50

in northwestern Bulgaria, *F. limosus* was found at other six localities in the rivers Topolovets, Voinishka and Archar, while it was not found in the Danube River main channel (Table 1). As a result of all surveys conducted in 2015–2016, a total of 92 specimens (42 males and 50 females) were recorded at seven sites: 40 specimens at five sites in the Topolovets River, 11 specimens at one site in the Voinishka River, and 41 specimens at one site in the Archar River. The highest population abundance was registered in the Archar River, downstream of

Archar Village. During two surveys at the same site in 2016, a significantly lower abundance was registered. At different localities in the Topolovets River the abundance ranged from low to moderate, while in the Voinishka River it was low (Table 1).

The analysis of size frequency distribution of the total length in the three populations of *F. limosus* indicated that they had very similar characteristics (Fig. 2). Specimens of small to medium sizes (between 35 and 60 mm) predominated in all studied populations. There was only one larger specimen (12%), which did not exceed 86 mm.

The morphometric characters of *F. limosus* from all known populations in Bulgaria are given in Table 2. A strong positive correlation between the crayfish total length, length of carapace and the width of carapace ($r = 0.96–0.98$) was found. The overall sex ratio from all populations was 0.84 (42 males and 50 females). There were no significant differences in the measured sizes and ratios between the males and females (Table 2). The average total length of males was 52.9 ± 9.35 (n=42), ranging from 36 to 78 mm, while that of females was 52.5 ± 11.48 (n=50), ranging from 36 to 81 mm.

Discussion

In Bulgaria, there are three native crayfish species: the noble crayfish *Astacus astacus* (Linnaeus, 1758), narrow-clawed crayfish *Pontastacus leptodactylus* (Eschscholtz, 1823), and the stone crayfish *Austropotamobius torrentium* von Paula Schrank, 1803 (TODOROV et al. 2013, TRICHKOVA et al. 2013). All of them are protected by the Biological Diversity Act (2002) and their capture is prohibited or regulated by the Law on Fisheries and Aquaculture (2001) in Bulgaria. The native crayfish species for the Bulgarian fauna can be easily distinguished from the invasive alien species *F. limosus* by some morphometric characters presented in Table 3 and Fig. 3.

The first introduction of *F. limosus* in the Danube River basin was in 1959, when several thousand individuals purchased from Berlin (Germany) were released into three localities in the vicinity of Budapest (THURÁNSZKY 1960, THURÁNSZKY & FORRÓ 1987). Since then up to 1985, free-living populations of *F. limosus* in the Danube system were not reported. The presence of the species in the Danube River was recorded in 1985 in a large secondary branch of the Hungarian Danube River, called Téli kikötő, near Újpest District in Budapest (1654 rkm) (THURÁNSZKY & FORRÓ 1987), and in the Bavarian Danube River near Ingolstadt Town

Table 3. Comparison of morphometric characters of the native crayfish species of the genera *Astacus*, *Pontastacus* and *Austropotamobius* in Bulgaria and the invasive alien crayfish species *Faxonius limosus*.

Native crayfish	<i>Faxonius limosus</i>
Rostrum with median carina and convex medially	Rostrum without median carina
One or two pairs with short postorbital ridges	One pair with very long postorbital ridges
Without noticeable spines on sides of anterior carapace, in front of the cervical groove	With noticeable spines on sides of anterior carapace, in front of the cervical groove
Without prominent spurs on inferior margin of cheliped carpus	With prominent spurs on inferior margin of cheliped carpus
Chelae are large, massive, equal in length or longer than cephalothorax	Chelae are small and narrow, less massive and shorter than the cephalothorax
Total length of adults exceed 12 cm	Total length of adults generally does not exceed 9–10 cm, very rarely reaches 12 cm
The colour of abdominal segments and pleura is homogeneous without visible bands	With characteristic transverse brown-red bands across abdominal segments and on pleura
Tips of fingers (propodus and dactylus) usually have homogeneous colour, sometimes yellowish in the young specimens	Tips of fingers (propodus and dactylus) are orange with contrasting dark blue to black band below

(NESEMANN 1987, NESEMANN et al. 1995). In 1991, the first population of *F. limosus* in the Austrian part of the Danube River, in the Ölhafen Port near Vienna (1918 rkm) was reported (NESEMANN et al. 1995). Later, the species has spread quickly through the Danube River, and has been reported from Austria (PÖCKL 1999), Croatia (MAGUIRE & KLOBUČAR 2003, MAGUIRE et al. 2011), Serbia (KARAMAN & MACHINO 2004, PAVLOVIĆ et al. 2006), Slovakia (JANSKÝ & KAUTMAN 2007, PUKY 2009, LIPTÁK 2013), Romania (PÂRVULESCU et al. 2009, 2012), and Slovenia (GOVEDIČ 2017). *Faxonius limosus* has been recorded not only in the main Danube River channel, but also in the Danube tributaries and the adjacent river basins, such as the Morava River (NESEMANN et al. 1995, PÖCKL & PEKNY 2002), the Tisza River and its tributaries (SALLAI & PUKY 2008, GYÖRE et al. 2013), the Drava River (HUDINA et al. 2009), the Tamiš River (LIPTÁK et al. 2013), and the Sava River (LIPTÁK & VITÁZKOVÁ 2014). Therefore, the appearance of the species in the Bulgarian sector of the Danube River was expected. Although, it was not found at any of the 33 sampling sites in the Danube River main channel, but only in the lower reaches of the smaller tributaries in the northwestern Bulgaria, these findings confirmed the expectations that the species was introduced through the Danube River, coming downstream from the Serbian and Romanian sectors, where *F. limosus* have been recorded much earlier (KARAMAN & MACHINO 2004, PAVLOVIĆ et al. 2006, PÂRVULESCU et al. 2009, 2012).

In the Bulgarian sector of the Danube River, *F. limosus* was characterised with comparatively small sizes of the specimens recorded and relatively low population abundance. Furthermore, the species inhabited only the lower reaches of the tributaries,

the most distant site being 18 km upstream of the Danube River confluence (Table 1). All these facts showed an early stage of establishment of the species in the studied area.

The rivers Topolovets, Voinishka and Archar, where *F. limosus* was found, differ significantly in their hydrological, physical and chemical characteristics. The Topolovets River is slowly flowing, turbid, and muddy, with dense overgrown macrophytic vegetation, while the rivers Voinishka and Archar have clear waters, with faster flows and sandy-gravel bottoms (Fig. 1). As *F. limosus* is tolerant to various environmental conditions (HAMR 2002, HOLDICH et al. 2006, PETRUSEK et al. 2006, PÖCKL et al. 2006, KOZÁK et al. 2015), it can find suitable habitats and has potential for successful and rapid expansion of its range upstream of almost all Danube tributaries in Bulgaria. Currently, there are still some obstacles for the species upstream movement, which we observed during our study. For example, in the Archar River, this species was found with relatively high abundance downstream of the bridge in Archar Village, while there were no records of *F. limosus* upstream of the bridge, where a stable population of *P. leptodactylus* was registered. The spread of the invader in the upper reaches of the Archar River presently is limited by a small weir formed by the destroyed old bridge at the road Vidin – Lom (Fig. 1D). However, as *F. limosus* can colonise new habitats by migrating overland (PUKY 2014, HERRMANN et al. 2018), we can expect that it may easily overcome such obstacles in the near future.

Faxonius limosus has been reported to decrease the native crayfish populations in Europe through competition for resources and by acting as a vector for the crayfish plague (FÜREDER et al.

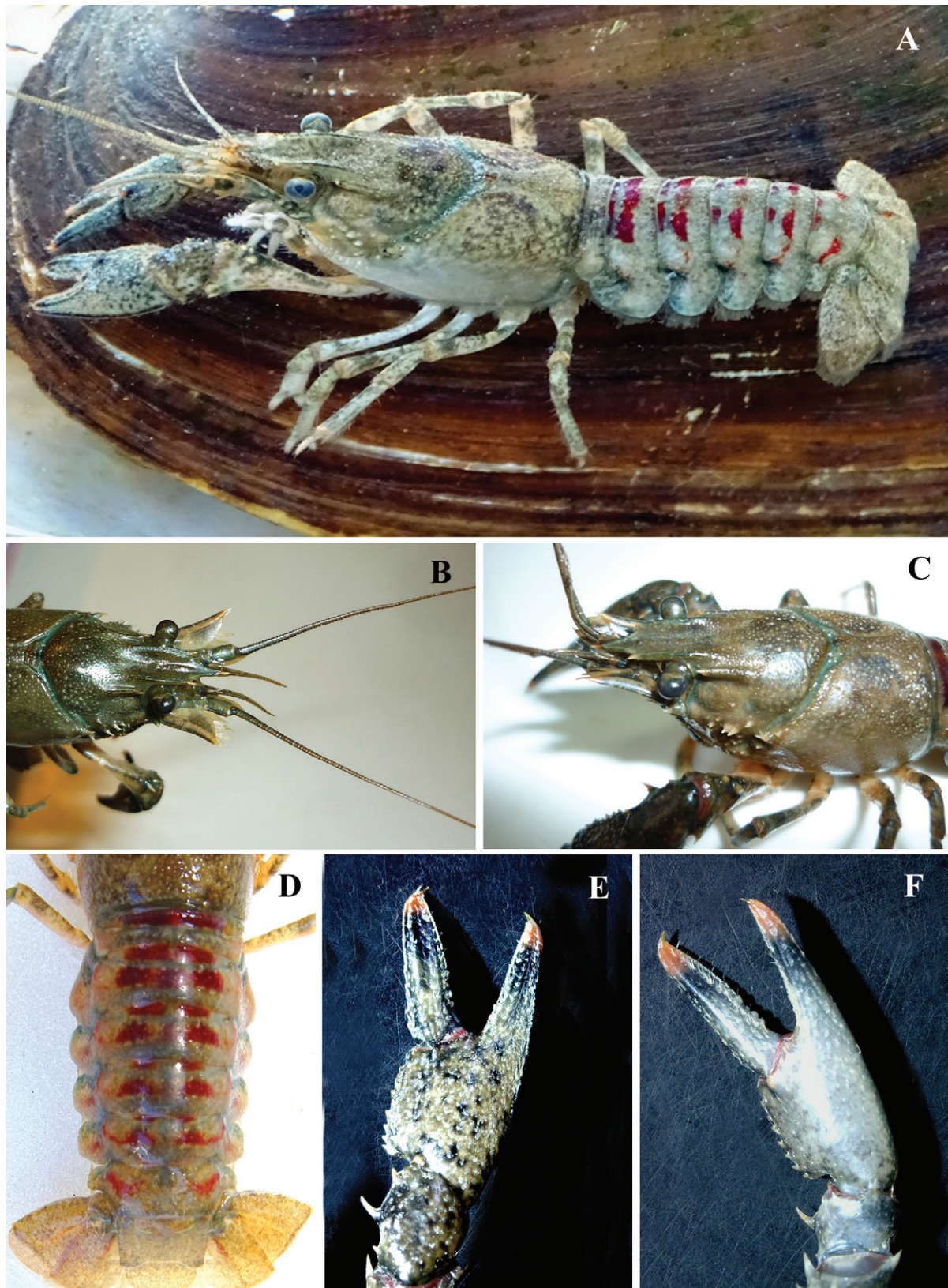


Fig. 3. Morphometric characters distinguishing the spiny-cheek crayfish *Faxonius limosus* from the native crayfish species in Bulgaria: (A) General view; (B) and (C) View of the anterior carapace with characteristic sharp spines, single and long postorbital ridges, a middle-sized rostrum with parallel borders, and a prominent acumen; (D) View of abdominal segments and pleura with characteristic transverse brown-red bands across; (E) and (F) View of upper and lower side of the chela with characteristic orange tips of the fingers with black bands below, and a prominent spur on inferior margin of the cheliped carpus.

2006, HOLDICH et al. 2006, 2009, SCHRIMPF et al. 2012, PÂRVULESCU et al. 2012, KOZÁK et al. 2015). In Croatia, the rapid spread of *F. limosus* through the Danube River catchment has adverse effects on the populations of *P. leptodactylus* (HOLDICH et al. 2009). In the Romanian sector of the Danube River, from 2009 to 2011, the relative abundance of *F. limosus* has steadily increased, while the native *P. leptodactylus* dramatically decreased in abundance. Subsequently, 70–90% of *P. leptodactylus* have been replaced by *F. limosus*. The presence of *A. astaci* DNA was detected in at least 32% of the invasive and 41% of the native crayfish coexisting in the Danube River (PÂRVULESCU et al. 2012). The decrease in population abundance of the native species is mainly due to the fact that *F. limosus* is a carrier of the crayfish plague pathogen, *A. astaci*, to which it is resistant (HOLDICH et al. 2009, PÂRVULESCU et al. 2012, 2015, SCHRIMPF et al. 2012, PANTELEIT et al. 2018). During our study, *F. limosus* was not found in the Danube River main channel, which indicates still low abundance of the species there. At the same time, at most of the sampling sites in the Danube River, the presence and good condition of the population of the native narrow-clawed crayfish *P. leptodactylus* was registered. *Pontastacus leptodactylus* was also found in the Archar River, upstream of Archar Village. According to literature data, the Danube tributaries in the northwestern Bulgaria (e.g. Voinishka River) are some of the few extant localities of the threatened noble crayfish *A. astacus* in the country (TRICHKOVA et al. 2013). Therefore, the further spread of *F. limosus* in the Danube River main channel and upstream of the Danube tributaries may pose real threat to the native crayfish populations at the national level.

Conclusions

We report the first finding and present data on the distribution of the spiny-cheek crayfish *Faxonius limosus* in three tributaries of the Danube River in northwestern Bulgaria, in 2015–2016. Based on the distribution of the species, we assume that it was introduced in Bulgaria through the Danube River, coming downstream from the Serbian and Romanian sectors, where *F. limosus* have been recorded earlier. The comparatively small sizes of the specimens recorded, the relatively low population abundance of the species, and the fact that *F. limosus* was found only in the lower reaches of the Danube tributaries indicate an early stage of establishment of the species in the Bulgarian sector of the Danube River. The further spread of

this invasive alien species upstream of the Danube tributaries and in the inland waters will threaten the native crayfish populations in Bulgaria. Urgent measures in accordance with the Regulation (EU) No 1143/2014 need to be taken at national and regional level in order to prevent the range extension and the negative impact of *F. limosus* in Bulgaria.

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