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Slovak section of the Danube has its well-established breeding ground of marbled crayfish *Procambarus fallax* f. *virginalis*

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Abstract – Established populations of the non-indigenous parthenogenetically reproducing marbled crayfish *Procambarus fallax* f. *virginalis* have been recently reported from various European countries. The colonised sites are usually lentic and relatively isolated from major watercourses and in such cases the immediate threat of the spread of this taxon is limited. Here we report on a marbled crayfish population that is likely to become a seed for colonisation of the Danube in Slovakia. It is located in a channel within the Slovak capital Bratislava in the immediate vicinity of a pumping station that occasionally releases significant amounts of water into the side arm of the Danube. The population is well established with a high growth potential: numerous adult marbled crayfish individuals were observed at the site in September and October 2016 and the progeny (eggs or first two developmental stages) of 27 berried females exceeded 11 000 individuals. The maximum observed fecundity per female reached 647 juveniles in the second developmental stage. The Danube side arm downstream of the pumping station harbours a population of spiny-cheek crayfish *Orconectes limosus* infected with the crayfish plague pathogen *Aphanomyces astaci*. We presume that marbled crayfish is already present below the pumping station and it is just a matter of effort and time until it is discovered. The investigated specimens of marbled crayfish were found free of *A. astaci*, but horizontal transmission from infected spiny-cheek crayfish may be expected, as well as further spread of marbled crayfish in the Danube.

Keywords: pet trade / aquatic invasion / fecundity / asexual reproduction / Slovakia

Résumé – Une portion slovaque du Danube est un site de reproduction bien établie d'écrevisse marbrée *Procambarus fallax* f.*virginalis*. Des populations établies d'écrevisses marbrées non-indigènes à reproduction parthénogénétique *Procambarus fallax* f. *virginalis* ont récemment été signalées dans différents pays européens. Les sites colonisés sont généralement lentiques et relativement isolés des grands cours d'eau et, dans de tels cas, la menace immédiate de propagation de ce taxon est limitée. Nous rapportons ici sur une population d'écrevisses marbrées qui risque de devenir une source pour la colonisation du Danube en Slovaquie. Elle est localisée dans un canal situé dans la capitale slovaque Bratislava, à proximité immédiate d'une station de pompage qui libère occasionnellement d'importantes quantités d'eau dans le bras latéral du Danube. La population est bien établie avec un fort potentiel de croissance: de nombreux adultes d'écrevisse marbrée ont été observés sur le site en septembre et octobre 2016 et la progéniture (œufs ou deux premiers stades de développement) de 27 femelles grainées dépasse 11 000 individus. La fécondité maximale observée par femelle a atteint 647 juvéniles au deuxième stade de développement. Le bras latéral du Danube en aval de la station de pompage abrite une population d'écrevisses américaines *Orconectes limosus* infectées par l'agent de la peste de l'écrevisse *Aphanomyces astaci*. Nous supposons que les écrevisses marbrées sont déjà présentes au-dessous de la station de pompage et c'est juste une question de prospection et de temps jusqu'à ce qu'elles soient découvertes. Les spécimens étudiés d'écrevisses marbrées ont été trouvés exempts d'*A. astaci*, mais on peut s'attendre à une

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transmission horizontale à partir d'écrevisses infectées et à une propagation accrue des écrevisses marbrées dans le Danube.

Mots-clés : commerce d'animaux de compagnie / invasion aquatique / fécondité / reproduction asexuée / Slovaquie

1 Introduction

Biological invasions have devastating consequences on the native biota, which is particularly apparent in freshwater ecosystems (Richman *et al.*, 2015). Introduced non-indigenous crayfish species affect the invaded biotopes, with negative community-level impacts (Moorhouse *et al.*, 2014; Roukonen *et al.*, 2016). Among alien crayfish, the marbled crayfish *Procambarus fallax* f. *virginalis* is an emerging threat, particularly in Europe. It is the only known crayfish reproducing via obligate apomictic parthenogenesis, producing genetically uniform offspring (Martin *et al.*, 2010). This species is characterised by early maturation (Seitz *et al.*, 2005), reproduces throughout the whole year under favourable conditions (Vogt *et al.*, 2004; Seitz *et al.*, 2005), and its high competitiveness for food and shelters has been documented (Jimenez and Faulkes, 2011). Its survival under low temperatures was proven both in the laboratory and the field (Veselý *et al.*, 2015; Lipták *et al.*, 2016). The marbled crayfish was first discovered in the German aquarium trade in the mid-1990s, from where it further dispersed (Scholtz *et al.*, 2003). Its availability at the pet markets is usually high (*e.g.* Kotovska *et al.*, 2016; Vodovsky *et al.*, 2017). At the beginning of the new millennium, reports on occurrence of single specimens from the wild appeared, followed by confirmation of established populations in Germany and Slovakia in 2010; since then, the number of invaded European countries has steadily increased (see Patoka *et al.*, 2016 and references cited therein), and the ability of marbled crayfish to carry the crayfish plague pathogen has been confirmed both in aquarium trade (Mrugała *et al.*, 2015) and in the field (Keller *et al.*, 2014). Due to all these characteristics, the marbled crayfish became listed among the invasive alien species of European Union concern according to recent legislation (EU Regulation No. 1143/2014 and Commission Implementing Regulation No. 2016/1141). Here we report an established marbled crayfish population in Bratislava, Slovakia, which has presumably initiated the colonisation of the Danube.

2 Material and methods

The marbled crayfish was discovered by a chance during research focused on the ecology of another alien species, the yellow-bellied slider *Trachemys scripta scripta* and the red-eared slider *T. s. elegans*, both native to North America. Two marbled crayfish were caught in turtle traps on August 25, 2016, in front of the pumping station in the Chorvátske rameno in Bratislava. Chorvátske rameno is a dead-end artificial canal within the town district Petržalka, which ends at a pumping station (48.0996 N, 17.1306 E) next to a side arm of the Danube (Jarovecké rameno) directly connected to the river (Fig. 1A, B). The canal is approx. 5 km long and 20 m wide with a depth of 2–3 m in its centre. Submerged macrophytes are present in some sections of the canal, and its banks are usually lined with emergent macrophytes. The canal bed is formed by fine gravel mixed with organic detritus.

Two installed pumps at the station in Chorvátske rameno have a capacity of 260 $l \cdot s^{-1}$ and are activated mainly during elevated flow rates (floods) in the Danube and during extensive rainfalls in the area in order to regulate ground waters in this highly populated town district. They are also occasionally activated when being checked for functionality. The pumping activity will transfer any biota in the immediate vicinity of the station into the side arm of the Danube, with no further barriers to dispersal to the river itself.

After accidental finding of marbled crayfish, two additional field samplings followed, the first on September 11, and the second on October 24, 2016. Both samplings focused on the areas just above and below the pumping station, *i.e.*, places where the presumed chance of successful capture of crayfish was highest. The first survey of the Chorvátske rameno canal was performed by a single researcher, who explored 2 m long stony section of the shore for 30 min. The second survey was performed by three researchers on a 10 m stretch. The sampling lasted for 40 min. Thanks to the high abundance of the marbled crayfish and easy access to the site, no crayfish trapping was needed. The Jarovecké rameno side arm is stabilised by heavy stones forming several layers. Thus manual search (ineffective in such conditions) was combined with trapping, using six baited traps exposed overnight during the first survey and 25 traps in the second survey.

Carapace length of sampled crayfish was measured to the nearest 0.1 mm. The eggs and juveniles in the first two developmental stages were counted if present. Juveniles in the third developmental stage become gradually independent and their quantification would be inaccurate. All captured crayfish individuals were preserved in 96% ethanol. Screening of the presence of the crayfish plague pathogen *Aphanomyces astaci*, using the quantitative PCR-based methods of Vrålstad *et al.* (2009), was conducted on all adult crayfish captured at both investigated sites (Chorvátske rameno and Jarovecké rameno). Details of the laboratory protocols are described in Mrugała *et al.* (2015) and Lipták *et al.* (2016).

3 Results

During the two field sampling events, altogether 39 adult marbled crayfish (11 + 28 females) and 9 spiny-cheek crayfish *Orconectes limosus* (7 + 2 individuals of both sexes) were captured. All marbled crayfish were caught above the pumping station in the Chorvátske rameno canal, while all spiny-cheek crayfish individuals were caught into traps below the pumping station in the Jarovecké rameno side arm (Fig. 1B).

The carapace length (totalling *ca.* 50% of the body size) of marbled crayfish specimens ranged from 21.8 to 48.1 mm, with a mean of 39.2 mm (Fig. 1C). In total, 27 marbled crayfish (69% of the catch) carried eggs or juveniles. The quantity of the offspring ranged between 147 and 647 (on average 420) eggs or juveniles per female, with a positive correlation with

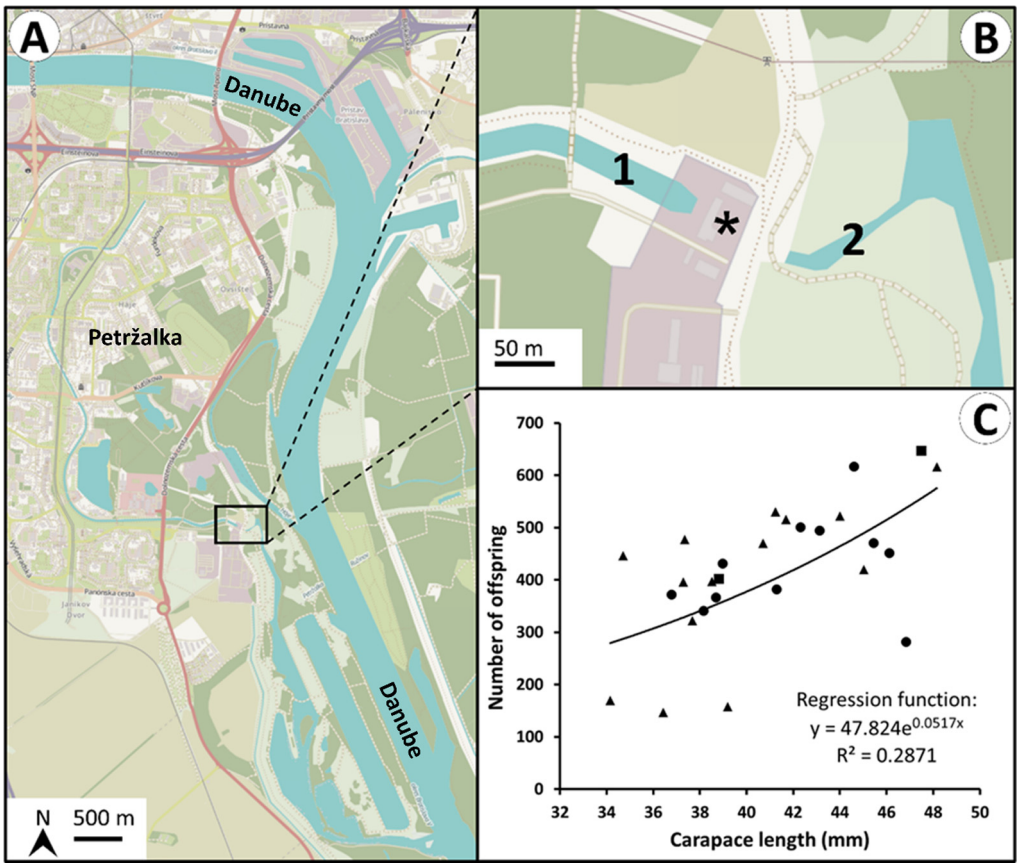

Fig. 1. Map showing the marbled crayfish *Procambarus fallax* f. *virginalis* occurrence in Bratislava, Slovakia – general view (A) and detailed location (B). Asterisk refers to the pumping station, while 1 and 2 to the locations in the Chorvátske rameno canal and the Jarovecke rameno side arm where marbled crayfish and spiny-cheek crayfish *Orconectes limosus* were found, respectively. Fecundity of marbled crayfish females (C) expressed as the number of eggs (circles), stage 1 (triangles) and stage 2 juveniles (squares), respectively, and the relationship between female carapace size and the number of offspring (exponential regression, with all three age categories pooled). The basis for the maps is available under the Open Database License (www.openstreetmap.org).

the size of the mother (Fig. 1C). Altogether, the 27 captured berried females carried 11 348 offspring. No trace of *A. astaci* DNA was detected in any analysed marbled crayfish.

Of the spiny-cheek crayfish (6 males, 3 females, carapace length 25.0–52.1 mm, mean 42.6 mm), one specimen was confirmed as being infected with *A. astaci* (agent level A3, according to the method of Vrålstad *et al.*, 2009).

4 Discussion

Due to irresponsible or uninformed hobby breeders, marbled crayfish are intentionally released into the wild and become established, as documented across Europe (Chucholl *et al.*, 2012; Kouba *et al.*, 2014). Most of the sites with well-documented established populations are lentic habitats relatively isolated from the main watercourses. However, records from some sizeable rivers (*e.g.* the Rhine in Germany or the Po delta in Italy) were also reported, although their recent population status remains unclear (Chucholl *et al.*, 2012; Vojkovská *et al.*, 2014; Patoka *et al.*, 2016 and literature cited therein). Weiperth *et al.* (2015) refer to several specimens of various sizes detected in thermal ponds and their outflows including adjacent Danube in Budapest, Hungary. Evaluation of the population status in the river is an issue of on going research (Weiperth A., pers. comm., 2017).

The newest discovered site with the marbled crayfish in Bratislava, Slovakia, also occurs in the immediate vicinity of the Danube, separated only by a pumping station that occasionally releases its waters to one of the river arms. This section of the Danube is already colonised by the non-indigenous spiny-cheek crayfish which invaded this river section in the last two decades and, recently, also by signal crayfish *Pacifastacus leniusculus* (Lipták and Vitázková, 2014). We have not confirmed syntopic occurrence of marbled crayfish with these species yet, but we consider that confirmation of marbled crayfish in the side arm of the Danube is just a matter of time and search effort. Water pumping, intentional translocation of marbled crayfish by humans, or active migrations of marbled crayfish, are factors that can transfer (or may have already transferred) the species into the Danube (Chucholl *et al.*, 2012; Lipták *et al.*, 2016).

The conditions in the side arm of the Danube, Jarovecké rameno, are favourable for crayfish, as indicated by the locally present spiny-cheek crayfish population. The documented

presence of *A. astaci* in that species corresponds to its infection status elsewhere in the Danube (Kozubíková *et al.*, 2010; Pârvulescu *et al.*, 2012). Upon contact of marbled crayfish with infected spiny-cheek crayfish, we may expect a horizontal transmission of *A. astaci* between the two host species (see James *et al.*, 2017). This means that thereafter the marbled crayfish expansion in the Danube catchment will be very likely accompanied by the expansion of the crayfish plague pathogen, which causes mass mortalities of indigenous crayfish stocks in Europe (Holdich *et al.*, 2009).

Any attempts to eradicate this marbled crayfish population are likely to be ineffective because of its obligate parthenogenetic reproduction mode, when even a single survivor may re-establish the whole population. Its remarkable reproductive capacity and extremely high fecundity, low-temperature tolerance and high competitiveness (Vodovsky *et al.*, 2017 and literature cited therein) all suggest that the marbled crayfish will become a permanent part of the Danube ecosystem, with great potential for an extension of its range, with largely unknown consequences so far. Some of its life history characteristics (*e.g.* higher fecundity, earlier maturation, supposedly faster growth and more reproduction events per year) provide significant advantages, even compared to other non-indigenous crayfish species already present in this section of the Danube, the spiny-cheek crayfish and the signal crayfish (Lipták and Vitázková, 2014).

To conclude, we expect that marbled crayfish might be already present in a side arm of the Danube, where horizontal infection with crayfish plague pathogen originating from spiny-cheek crayfish will occur. We presume that the marbled crayfish will spread actively further (mainly downstream), but its range extension may be accelerated by the occasional floods where successful reproduction of even single dispersed specimens is not limited. A competition with this new invader might have severe consequences for remaining stocks of the indigenous narrow-clawed crayfish *Astacus leptodactylus*, already under pressure of spiny-cheek crayfish (*cf.* Pârvulescu *et al.*, 2012, 2015). Successful competition of marbled crayfish with other non-indigenous crayfish species already present in the Danube may also be expected. However, given the role of crayfish in ecosystems in general and characteristics of marbled crayfish in particular, the spread of marbled crayfish has the potential for significant consequences for much broader range of taxa. This is a serious issue since the Danube possesses habitats for diverse biota, being a unique ecosystem of European importance. Future monitoring of marbled crayfish in the Danube is warranted, but at early phases of establishment may be methodologically challenging in such large river course. Utilisation of eDNA methods might be an useful tool in this regard.

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