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Predictions of marbled crayfish establishment in conurbations fulfilled: Evidences from the Czech Republic

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Abstract: The marbled crayfish (*Procambarus fallax* f. *virginalis*) has become one of the potentially most dangerous non-indigenous crayfish species spreading in European countries and elsewhere. This taxon reproduces parthenogenetically and recently has been verified as a vector of the crayfish plague pathogen. Here, we report on two established populations of marbled crayfish in the Czech Republic. The marbled crayfish was observed during autumn 2015 in an urban pond connected by sewer piping with the Rokytka brook near its mouth to the Vltava River in Prague. Subsequently, three adult females, two of them having well-developed glair glands and oocytes, were captured in this pond during spring 2016, suggesting successful overwintering of the local population. Furthermore, four adult females were captured in an artificial pond at the Radovesická lignite spoil heap in the vicinity to the industrial conurbation of Bílina in summer 2016; one of them carried eggs. We tested these for the presence of the crayfish plague pathogen *Aphanomyces astaci*, with negative results. The introduction pathway for both populations is most likely a release from private aquaria, as these sites are popular for recreation activities. Our findings substantiate previous predictions that conurbations are likely to be the primary areas for marbled crayfish introductions.

Key words: *Procambarus fallax* f. *virginalis*; biological invasion; first record; pet trade; Marmorkrebs; urban pond; post-mining site

Introduction

The introduction, establishment, and subsequent spread of non-indigenous crayfish species (NICS) are known to constitute one of the main factors seriously affecting abundance of European indigenous crayfish species (Peay 2009; Gherardi et al. 2011; Perdikaris et al. 2012; Chucholl 2014). The trade for ornamental purposes (pet trade) has been considered an important source of new alien species introductions worldwide (Padilla & Williams 2004; Duggan 2010; Chucholl 2013; Patoka et al. 2015a, 2016). The pet trade in imported crayfish started to expand around 1995, and consequently production of crayfish for ornamental purposes developed in various countries (Vogt et al. 2004; Faulkes 2010; Patoka et al. 2015b). Germany, currently with 28 NICS available for sale (Chucholl & Wendler 2016), and the Czech Republic, with 27 NICS (Patoka et al. 2014a, b), have been identified as the leading European countries in this regard.

The marbled crayfish (Marmorkrebs in German) has been identified as one of the most dangerous of ornamental NICS from a European perspective (Scholtz et al. 2003; Chucholl 2014; Patoka et al. 2014a; Kotovska et al. 2016; Souty-Grosset et al. 2016). Although it cannot be excluded that the origin of this triploid parthenogenetic crayfish could have been a hybridization between *Procambarus fallax* (Hagen, 1870) and some other species of the genus *Procambarus* (Martin et al. 2016), it is usually regarded as a parthenogenetic form of the former (*P. fallax* forma *virginalis* Martin et al., 2010). Although the native geographical distribution of this form, if at all present in the wild, is still unknown (Martin et al. 2010, 2016; Kouba et al. 2014), the sexually reproducing *P. fallax* occurs in Florida and Georgia (Taylor et al. 2007).

Populations of the marbled crayfish are exclusively composed of females, which reproduce by obligatory apomictic parthenogenesis. Thus, a single female can theoretically be sufficient to establish a viable popula-

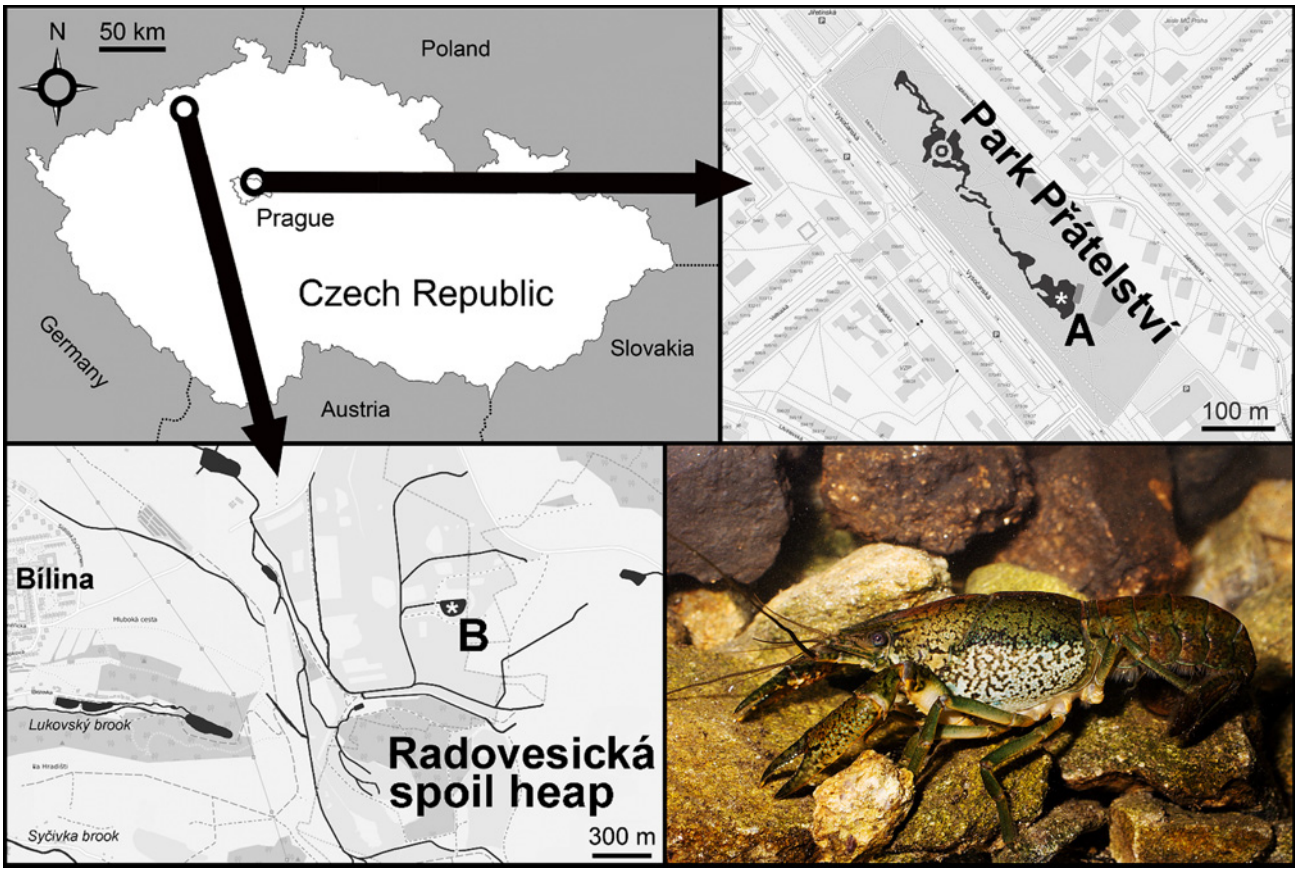

Fig. 1. Map showing the location of the Přátelství Park in Prague (A), and the Jiřina pond (B) at the Radovesická spoil heap, Czech Republic; both indicated by white asterisks. The crayfish female on the photo was captured by funnel trapping in the Jiřina pond. The basis for the maps are available under the Open Database License (www.openstreetmap.org).

tion (Scholtz et al. 2003). This reproduction strategy, together with low intraspecific aggressiveness, a generation time of only 6 months, and high fecundity, frequently lead to population explosion in a very short time (Scholtz et al. 2003; Vogt et al. 2004).

This crayfish has been recorded in the wild in several European countries, including Croatia, Germany, Hungary, Italy, the Netherlands, Slovakia, Sweden, and Ukraine (Nonnis Marzano et al. 2009; Chucholl & Pfeiffer 2010; Janský & Mutkovič 2010; Soes & Koese 2010; Bohman et al. 2013; Samardžić et al. 2014; Vojkovská et al. 2014; Weiperth et al. 2015; Lipták et al. 2016; Lőkkös et al. 2016; Novitsky & Son 2016), although the establishment success in some of those countries is not clear. As one of the negative effects to native biodiversity, ornamental animals can serve as important hosts and vectors of exotic commensals (Patoka et al. 2015a) and pathogens (e.g., Martinez-Murcia et al. 2008; Kalous et al. 2015). Indeed, marbled crayfish has been recently confirmed as a vector of the crayfish plague pathogen *Aphanomyces astaci* Schikora (Keller et al. 2014; Mrugała et al. 2015), which still threatens European indigenous crayfish species (Holdich et al. 2009). Due to its invasion potential as well as disease carrier status, marbled crayfish has been recently included, together with four other invasive crayfish of North American origin, in the list of 37 Invasive Alien Species of European Union Concern (EU Regulation No. 1143/2014; Commission Implementing Regulation No. 2016/1141).

We present here the first records of marbled crayfish from open waters in the Czech Republic from two different, geographically distant sites, in which it seems to have established populations. In an urban pond located in the city of Prague, we could confirm a successful overwintering of the population. In a pond on a post-mining spoil heap close to Bílina, several adult animals were captured, one of which carried eggs in late summer. Perdikaris et al. (2012), Chucholl (2014), and Souty-Grosset et al. (2016) had noted that the occurrence of some NICS in European waters, including the marbled crayfish, is entirely driven by propagule pressure in relation to the pet trade, and that crayfish are usually released into the nearest ponds or streams in the vicinity of conurbations. Our observations support this view.

Material and methods

Study sites

The first site, Přátelství Park, is situated in the town district Prosek, Prague, Czech Republic (GPS 50°07′21″ N, 14°29′43″ E) at the altitude 288–295 m a.s.l. (Fig. 1A). The park was founded for recreational purposes of local inhabitants. The park contains a system of small ponds connected with cascades and channels. This system, which was renovated in 2008, has a total length of 450 m and volume of

1800 m^3. The water is circulated through the vegetation season and kept at a low level. The majority of the system is completely drained at the end of vegetation season, with the exception of a pond (denoted as A in Fig. 1). In late autumn, the water is discharged into the water sewer connected with the Rokytka brook close to its confluence with the Vltava River (Elbe basin). The bottom and banks of ponds and channels are made from concrete, and the bottom is covered by a thin layer of mud and detritus, mainly formed by leaf litter. Aquatic macrophytes are represented at the site by three patches of water lilies (*Nymphaea alba* L.). These plants are taken out of the water over winter.

The second site, Radovesická spoil heap (Fig. 1B), is situated in the North Bohemian lignite basin. The whole area is heavily affected by opencast lignite mining, resulting also in numerous larger heaps of spoil consisted mainly of tertiary clays. The Radovesická spoil heap is a relatively large heap (ca 12.5 km^2) formed between 1964 and 2003 in a vicinity to the Bílina conurbation. Its majority has been technically reclaimed with artificial creation of several pools and ponds supplemented by numerous pools formed spontaneously in the terrain depressions (Harabiš et al. 2013). The Jiřina pond (0.6 ha; GPS 50°33′08″ N, 13°48′53″ E; altitude 340 m a.s.l.; denoted as B in Fig. 1) is a middle-sized artificial pond created in the early 1990s for the future recreation of Bílina inhabitants. Although still officially inaccessible, it is already relatively popular for fishing and swimming. It has no affluent, but there is an outlet conducting water after strong rains or snowmelt to a drainage channel system flowing into the Bílina River (Elbe basin). The pond banks and bottom are formed by clays and are regularly rounded and highly homogeneous. Its littoral zone is very narrow (max. 1 m) because the bottom steeply descends to the depth of over 2 m; it is dominated by dense common reed [*Phragmites australis* (Cav.) Trin. ex Steud.] and much sparser bladderwort (*Utricularia* sp.).

Data collection

Marbled crayfish was first observed to occur in open waters in the Czech Republic during October 2015, when three individuals were captured in the Přátelství Park by Jakub Friedl, a student of the Czech University of Life Sciences Prague. These crayfish were photographed and subsequently released to the pond. On 16 March 2016, we intensively sampled the pond by electrofishing, using a backpack electroshocker (Bednář; www.r-bednar.cz). Supplementary sampling included small seine-netting and sweep-netting in detritus.

In the Radovesická spoil heap, the first marbled crayfish was recorded on 30 July 2016. A female entered one of five funnel traps (80 × 27.5 × 27.5 cm, 0.5 cm green mesh nylon, 3.5 cm of an entrance diameter) exposed for 24 hours in the littoral zone for the purpose of aquatic insect monitoring. In the night of 3 September 2016, we exposed eight funnel traps in the littoral zone close to the first record. We also actively searched for crayfish by snorkelling, by sweep-netting of detritus and littoral vegetation, and exploring potential shelters.

All animals captured in both surveyed sites were identified immediately after their capture and subsequently, with exception of crayfish, were returned to the pond.

Identification

The identity of crayfish specimens was verified by both morphological and genetic analyses. Morphological analyses followed Martin et al. (2010). The mitochondrial gene for the cytochrome c oxidase subunit I (COI) from one captured female per each site was sequenced using the universal primer pair LCO1490/HCO2198 (Folmer et al. 1994) and following the protocols described in Mrugała et al. (2015).

Testing for the presence of the crayfish plague pathogen

As the marbled crayfish may host the crayfish plague pathogen *Aphanomyces astaci* (Keller et al. 2014; Mrugała et al. 2015), we analysed individuals captured at the Radovesická spoil heap for the presence of its DNA, following the protocols in Mrugała et al. (2015). We dissected the soft abdominal cuticle and telson of each individual, and isolated DNA from the mixed subsample of these tissues (ca 50 mg per individual) by DNeasy tissue kit (Qiagen) to the volume of 200 µl. Then, TaqMan minor groove binder quantitative PCR (qPCR) assay modified from Vrålstad et al. (2009), which specifically and with high sensitivity detects *A. astaci* DNA, was run on iQ5 real-time PCR detection system (Bio-Rad), using 5 µl of the DNA isolate in 25 µl reaction. Further details of the protocol are provided in Mrugała et al. (2015) and Svoboda et al. (2014).

Results

Three females were captured in the pond A of the Přátelství Park (Fig. 1A) in March 2016. The first individual, with cephalothorax length (CL, measured from tip of rostrum to posterior end of cephalothorax) of 35 mm and total body length (BL, measured from tip of rostrum to posterior edge of telson) of 73 mm was captured by electrofishing and had a carapace surface densely covered by periphyton. The other two females (CL/BL of 33/70 and 32/70 mm) were captured by sweep-netting in detritus and had well-developed glair glands and oocytes.

Four females were captured in the Jiřina pond at the Radovesická spoil heap (Fig. 1B). In July 2016 one individual (CL/BL of 56/115 mm) was captured by funnel trapping. In September 2016, one female entered a funnel trap at the same location as the first one, and two females were found in shelters under a stone and a sunken concrete block close to the pond bank (CL/BL of 38/83, 42/90, and 42/90 mm). One of the two larger individuals carried three eggs.

Morphological characteristics of the crayfish corresponded with the description given in Martin et al. (2010). The DNA barcoding confirmed the morphological identification of the captured crayfish as *P. fallax* f. *virginalis*. The obtained COI fragments matched completely each other and the reference sequences for marbled crayfish publicly available from GenBank (acc. nos. KC107813, HM358011, JF438007; Filipová et al. 2011; Martin et al. 2010; Shen et al. 2013). The qPCR assay did not detect DNA of *A. astaci* in any of the tested individuals.

Fish stock captured by electroshocking and seine-netting in the pond A of the Přáteství Park included the following potential crayfish predators: catfish *Silurus glanis* L., 1758 (2 subadults), perch *Perca fluviatilis* L., 1758 (2 adults), carp *Cyprinus carpio* L., 1758 (1 adult). Also, five pairs of mallard ducks (*Anas platyrhynchos* L., 1758) were observed feeding at the site. In the Jiřina

pond at the Radovesická spoil heap, we have observed several (sub)adults of eel *Anguilla anguilla* (L., 1758).

Discussion

We discovered and subsequently surveyed two populations of marbled crayfish occurring within an urban pond system in Prague and at a larger artificial pond on a reclaimed spoil heap. Based on our results, we consider both populations to be established. The repeated findings of marbled crayfish in the Přátelství Park suggest that the local population successfully overwintered in the pond that had not been drained at the onset of winter. Although the marbled crayfish has been considered a warm water taxon (Chucholl & Pfeiffer 2010), its established populations occur in European temperate zone (Chucholl et al. 2012; Lipták et al. 2016) and tolerance to winter water temperatures was confirmed also experimentally (Veselý et al. 2015). However, an alternative scenario that animals found in the Přátelství Park after winter originated from repeated introductions of marbled crayfish from aquaria cannot be entirely ruled out without a continuous monitoring or a capture-recapture study. The reproductive potential of the local population is indicated by the fact that the captured females had well-developed glair glands.

All females from the Radovesická spoil heap were large adults, which are likely to reproduce *in situ*. One was observed to carry a few eggs at the end of summer, when no other crayfish species in Central Europe breed (Reynolds 2002). We presume this was a remainder of originally larger clutch, considering that the typical size at maturity of this species (at least in laboratory conditions) is less than half of the size of captured females, female fecundity increases with body size, and brood sizes even for very small females usually exceed 45 eggs and may reach up to hundreds of eggs (Seitz et al. 2005; Kouba et al. 2015).

At present, marbled crayfish apparently does not reach high density in either of the studied sites. We hypothesise that the low number of captured individuals in the Přátelství Park could be caused by draining of the channels, and concentration of water only into the pond A over the winter season. The low water level can be very harmful for crayfish due to intensive predation pressure from mallard ducks (Malone 1965) as well as by fish (Syväranta et al. 2010), which are abundant at the location. In the Radovesická spoil heap, we presume a strong predation pressure by the relatively abundant European eel, which has a strong potential to decrease the crayfish population density (Aquiloni et al. 2010). Furthermore, the presence of these predators likely drives the crayfish into little accessible shelters, making the manual surveys less effective.

However, it must be underscored that without rapid management activity focusing on extirpation or control of these populations, we expect a substantial potential of the spread of marbled crayfish from both sites. In the Přátelství Park, crayfish may drift through the water outlet to the nearby adjacent water bodies. Although the Jiřina pond at the Radovesická spoil heap is not permanently connected with any other waters, its outlet is filled by water after strong rains or during a spring melting when superfluous water flows into the drainage system, including more artificial ponds and finally flowing into the Bílina River. Thus, the establishment of marbled crayfish in some adjoining water bodies is also possible. At the spoil heap, both stagnant (Harabiš et al. 2013; Vojar et al. 2016) and flowing (Tichanek & Tropek 2015, 2016) waters are known to harbour unusually rich freshwater biodiversity, which can be potentially threatened by the expanding marbled crayfish. In particular, some of the local ponds have been recently stocked by the native noble crayfish *Astacus astacus* (L., 1758) after a rescue transfer, as the area has been considered free of alien species that may directly affect this protected species vulnerable to crayfish plague. Although we did not detect *A. astaci* in captured crayfish, the low number of tested crayfish does not guarantee absence of the pathogen in the studied site; furthermore, even if the population is pathogen-free at the moment, infection by *A. astaci* sometimes in the future cannot be entirely ruled out.

Both aquarium and garden fishkeeping are traditionally popular and widespread in the Czech Republic. Although the release of non-native organisms is illegal, irresponsible or uninformed hobbyists frequently release their pets, including crayfish, into the wild (Patoka et al. 2014b). Hence, the propagule pressure (*sensu* Duggan et al. 2006) is high. As noted by Patoka et al. (2015b), marbled crayfish originate almost exclusively from domestic production in quantities estimated to be as high as 100,000 individuals annually in the Czech Republic. In comparison with recorded retail prices of other traded crayfish species (€ 2.0 to 25.92), the marbled crayfish is very inexpensive and thus widely accessible; the lowest recorded price was € 0.55 per individual (Patoka et al. 2015b). In addition to its low price, the species is popular, is easy to keep and breed, reproduces rapidly and asexually, and has a short generation time (Faulkes 2015). These facts support the assumption that the discovered population originated due to intentional release from aquaria.

Chucholl et al. (2012) and Chucholl (2014) have predicted that the number of established populations within European territory will further increase due to release by hobbyists of surplus and unwanted crayfish at new sites near conurbations. This suggestion is supported by our both findings from Prague and the vicinity of Bílina, together with other recent records of marbled crayfish occurrence, such as in Budapest, Hungary (Weiperth et al. 2015), and Dnepropetrovsk and Odessa, Ukraine (Novitsky & Son 2016). Populations established in conurbations may serve as the source for further, spontaneous spread. The North American NICS have been evaluated as more dangerous for native European astacofauna than traded crayfish species originating from elsewhere in the world (Patoka et al. 2014a). In agreement with other authors (Peay 2009; Magalhães & Andrade 2014), we recommend further

education of the general public and sharing of information which will improve knowledge as to how dangerous are NICS and how important is conservation of indigenous crayfish species. As both discovered Czech populations of marbled crayfish are in isolated water bodies, their eradication before the further expansion of crayfish should be attempted.

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