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Risk assessment of pet-traded decapod crustaceans in Hungary with evidence of *Cherax quadricarinatus* (von Martens, 1868) in the wild

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Abstract. The pet trade is one of the most important sources of introduction of freshwater non-native decapod crustaceans. Precise and timely identification of potentially hazardous species is necessary for the effective prevention of new introductions. Here, we present a list of species of ornamental freshwater decapod crustaceans pet-traded in Hungary and their risk assessment, including the probability of establishment based on climate matching. The list contains 13 shrimps, eight crayfish, two crabs, and one hermit crab. Three crayfish, *Cherax destructor*, *Procambarus clarkii*, *P. virginalis*, and one crab, *Eriocheir sinensis*, were classified in the high-risk category. During field sampling, we found three individuals of *C. quadricarinatus* that were probably released or escaped from aquaria. These are the first records of this species in the wild of Carpathian Basin. We strongly recommend further educating hobbyists about the risks related to the escapes and releases of high-risk taxa, as well as monitoring of the region for their occurrence.

Key words: Carpathian Basin, crayfish, crab, hermit crab, invasiveness, ornamental animal, redclaw, shrimp

Introduction

Thousands of freshwater species are involved in the international pet trade, a rapidly growing sector of the aquacultural industry (Tlusty 2002). In contrast with commercial aquaculture, the pet trade of ornamental freshwater decapod crustaceans has long been overlooked as a potential source of invasive species. Recently, various examples of crayfish and shrimps released or escaped from indoor aquaria and garden ponds highlighted the pet trade as one of the most important pathways for introductions of non-native decapod crustaceans (Belle et al. 2011, Klotz et al. 2013, Patoka et al. 2016a).

This issues is particularly apparent and best described in case of ornamental crayfish. Marbled crayfish, *Procambarus virginalis* Lyko 2017, was previously used as a model taxon in prediction of non-indigenous crayfish species spread (Feria & Faulkes 2011, Perdikaris et al. 2012, Chucholl 2014). It is one of three known invasive and also very popular pet-traded crayfish species (Chucholl 2013, Patoka et al. 2015). Together with *P. clarkii* (Girard, 1852) and *Cherax destructor* (Clark, 1936), they were not thought to be able to well survive winters in temperate region (e.g. Capinha et al. 2012, Chucholl et al. 2012, Holdich & Sibley 2009). Nevertheless, they are capable to successfully overwintered in water temperatures relevant at least for stagnant waters of Central Europe (Veselý et al. 2015). Established populations of the marbled crayfish were recently found also outside the estimated range (Chucholl 2014) in Europe (Lipták et al. 2016, Novitsky & Son 2016, Patoka et al. 2016b). Besides crayfish, also other pet-traded freshwater decapods, especially shrimps, were introduced outside their native range (e.g. Mitsugi et al. 2017, Yeo 2010). In temperate zone, these species are known inhabiting thermal localities. Sometimes even dispersion to the adjacent waters with a regular temperature regime is hypothesized (Klotz et al. 2013).

Taking into account that ornamental crustaceans are very commonly available and sold at low prices at pet trade markets (Patoka et al. 2015), all environmentally suitable regions in Europe can be potentially inhabited. Availability on market and risk assessment primarily focused on crayfish has been analysed for leading European regions in the pet trade of decapod crustaceans – Germany (Chucholl 2013) and the Czech Republic (Patoka et al. 2014). Further studies refer to Greece (Papavlasopoulou et al. 2014), Italy (Tricarico et al. 2010), Slovakia (Lipták & Vitázková, 2015), Ukraine (Kotovska et al. 2016), and the Caspian region in the Russian Federation (Vodovsky et al. 2017). Data from other European countries are mostly lacking, despite the fact that the pet trade of these animals is probably well developed, with the exception of countries where trade is totally banned such as Sweden, Switzerland, Republic of Ireland, United Kingdom of Great Britain and Northern Ireland (with the exception of *Cherax quadricarinatus* (von Martens, 1868), in the case of England and Wales) (Chucholl 2014, Peay 2009). Despite the regulation, ornamental crayfish species are also known from natural habitats in regions where they are banned (c.f. Hefti & Stucki 2006, Bohman et al. 2013, Faulkes 2014, Kouba et al. 2014). Contribution by Uderbayev et al. (2017) is an example of non-European study providing risk assessment of pet-traded decapods extended also on shrimps and crabs.

Hungary is a medium-sized state of the European Union (EU) situated in Carpathian Basin (also known as Pannonian Basin), with two main waterways, the Danube and Tisza rivers. Hungary has a continental climate with an average annual temperature of 9.7 °C, but there are also numerous thermal springs with water temperature above 30 °C year round (Bélteky 1972). The Danube river is one of the busiest shipping routes in Europe, providing excellent opportunities for the spread and range extensions of non-native aquatic species (Bódis et al. 2012, Pârvulescu et al. 2012, Lipták et al. 2017). Moreover, this waterway was artificially connected with other catchments by the Rhine-Main-Danube Canal, which is the southern man-made corridor facilitating migra-

tion of aquatic animals among river basins in Europe (Bij de Vaate et al. 2002).

Three indigenous crayfish species occur in Hungary: *Astacus astacus* (L., 1758), and *Austropotamobius torrentium* (Schrank, 1803) (Kouba et al. 2014), *Pontastacus leptodactylus* (Eschholtz, 1823). All of them are classified as endangered and therefore protected (Kozubíková et al. 2010, Puky et al. 2005). Non-native decapod crustaceans include the crayfish species *Faxonius limosus* (Rafinesque, 1817), *Pacifastacus leniusculus* (Dana, 1852), *Procambarus clarkii*, and *P. virginalis* and *Cambarellus patzcuarensis* Villalobos, 1943 (Kovács et al. 2015, Lőkkös et al. 2016, Weiperth et al. 2015, 2017), and the crab *Eriocheir sinensis* (H. Milne Edwards, 1853) (Puky et al. 2005). Their presence may have negative community-level impacts (Moorhouse et al. 2014, Ruokonen et al. 2014). Among others, the non-native crayfish species can be very harmful to native counterparts for instance due to the spread of *Aphanomyces astaci* (Schikora), a pathogen causing crayfish plague, which is a fatal disease for European native crayfish (Keller et al. 2014, Mrugała et al. 2015, Svoboda et al. 2017). Recent studies also suggest that even freshwater shrimps and crabs may became alternative hosts of this pathogen (Svoboda et al. 2014a,b, Putra et al. 2018). Transmission of *A. astaci* from infected *E. sinensis* to the susceptible *A. astacus* has been already confirmed (Schrimpf et al. 2014).

Although pet trade of freshwater decapods has not been evaluated in Hungary, some crustaceans were imported to this country from the Czech Republic (Kroupa M., pers. comm., 2016), the hub for freshwater ornamental animals in Europe (Kalous et al. 2015, Patoka et al. 2015). Therefore, we surveyed the market in Hungary, based on climate match performed species distribution models, and conducted risk assessment for the species found. This output was accompanied by sampling in the field focused on the potential presence of new decapods introduced via the pet trade.

Material and Methods

Data collection

Information about decapod species within the pet trade, their availability, and origin in the Hungarian market was collected from March 2015 to October 2016. Interviews were conducted with 6 wholesalers, 76 pet shop owners, 13 online shops, and 23 local producers. Furthermore, 4 pet bazaars (places where people can privately sell or change pet animals) were also visited. Advertised species were recorded and photographed for later identification. Collected records were subsequently clarified during personal visits and/or provided photo-documentation, and misnomers, as well as alternative trade names, were eliminated.

Market availability for the species found to be pet-traded in Hungary

Market availability was estimated for each species according to Chucholl (2013), using the following criteria: (i) "very rare": species available only for a short period and in small quantities, (ii) "rare": species available occasionally in small quantities, (iii) "common": species available frequently in small quantities, and (iv) "very common": species always available in large quantities. Even if this method is to a certain degree heuristic, it is applicable for a rough estimate of species availability in the market (Kotovska et al. 2016).

Climate match for the species found to be pet-traded in Hungary

Climatic conditions were represented in our analysis by temperature during the coldest quarter of the year as the variable. The climate match between source and target area was compared using the Climatch tool (v.1.0; Invasive Animals Cooperative Research Centre, Bureau of Rural Sciences, Australia, http://data.daff.gov.au:8080/Climatch/climatch.jsp). As the source area, we used the native geographic range of a given evaluated taxon. The native region of *Procambarus virginalis* is not yet known, therefore the source region was taken, in accordance with Chucholl (2014), as the verified European localities where it is currently established (Patoka et al. 2016b). The target area was defined as the territory of Hungary containing 14 climatic stations from the database of the WorldClim project (Hijmans et al. 2005). Where the climate match between the source area and the climatic station in the target area reached a score of ≥ 7.0, this was interpreted as there is no environmental barrier to survival (Kalous et al. 2015).

Risk assessment

To evaluate the invasive potential of decapod crustaceans pet-traded in Hungary, we used the Freshwater Invertebrate Invasiveness Scoring Kit produced by the UK Centre for Environment, Fisheries & Aquaculture Science (FI-ISK, v.1.19, Tricarico et al. 2010). Based on the FI-ISK score, each evaluated species was subsequently classified as (i) low-risk (score <1), (ii) medium-risk (score ≥1 but <16), or (iii) high-risk (score ≥16) from a Hungarian perspective.

Field sampling

Selected localities were sampled for potential occurrence of pet-traded decapods from January 2015 to March 2017. It covered a range of both thermal localities (warm-water ponds and their outflows, sometimes connected with thermal spas; n = 11) and those possessing regular temperature conditions (n = 17) in the Danube river, its side arms and tributaries. Since then, several new populations of exotic aquatic species and potential habitats were identified (Takács et al. 2017, Weiperth et al. 2015, 2016). We sampled 1 km long section of each selected waterbody using five baited traps set every two weeks from spring to autumn. Thermal localities were additionally monitored during the winter. The traps were exposed and checked for two consecutive days. The morphological identification of captured crayfish followed Holthuis (1949) and Souty-Grosset et al. (2006).

Genetic analysis

Due to confusion in the taxonomy of some *Cherax* species (see Austin 1996, Bláha et al. 2016), we included DNA analysis in our study. The importance of DNA identification has been previously highlighted for several non-native crayfish species (Filipová et al. 2011).

The morphological identification of each captured specimen was confirmed by a polymerase chain reaction (PCR) of a selected gene (mitochondrial cytochrome oxidase subunit I – COI), utilizing universal primer pair LCO1490 (5′-GGTCAACAAATCATAAAGATATTGG-3′) and HCO2198 (5′-TAAACTTCAGGGTGACCAAAAAATCA-3′) (Folmer et al. 1994). The samples were sequenced using the Macrogen sequencing service in the Netherlands (www.macrogen.com). Chromatograms were assembled and checked for potential errors. Edited sequences were aligned using Clustal W, as implemented in the BioEdit software package (Hall 1999).

Results

We recorded 24 species of decapod crustaceans pet-traded in Hungary (Table 1). This set contained 13 shrimp species (12 from family Atyidae and one from family Palaemonidae), eight crayfish species (five from family Cambaridae and three from family Parastacidae), two crab species (one from family Sesarmidae and one from family Varunidae), and one hermit crab species (family Coenobitidae). Three shrimps (*Caridina breviata* Ng and Cai, 2000, *C. multidentata* Stimpson, 1860, *Neocaridina zhangjiajiensis* Cai, 1996) and two crayfish

Table 1. List of pet-traded species of decapod crustaceans in Hungary, their families, availability in the market (VC = very common, C = common, R = rare, VR = very rare), potential invasiveness (FI-ISK score), as low-risk (score < 1), medium-risk (score ≥ 1 and <16), and high-risk (score ≥ 16), and risk category (FI-ISK category), nursery environment required for larval or juvenile development, environment for adult individuals (B = brackish water, F = freshwater, M = marine, T = terrestrial); and origin (D = domestic production, I = import).

			FI-ISK		Environment		
Species	Family	Availability	Score	Category	Nursery	Adulthood	Origin
Shrimps							
Atya gabonensis	Atyidae	C	2	medium	B	F	I
Atyopsis moluccensis	Atyidae	C	2	medium	B	F	I
Caridina babaulti	Atyidae	R	1	medium	F	F	I
Caridina breviata	Atyidae	VC	1	medium	F	F	I
Caridina cantonensis	Atyidae	C	1	medium	F	F	D/I
Caridina glaubrechti	Atyidae	VR	1	medium	F	F	I
Caridina gracilirostris	Atyidae	C	1	medium	B	F	I
Caridina multidentata	Atyidae	VC	4	medium	B	F	I
Caridina serratirostris	Atyidae	R	1	medium	B	F	I
Caridina spongicola	Atyidae	R	-3	low	F	F	I
Neocaridina heteropoda	Atyidae	C	7	medium	F	F	D/I
Neocaridina zhangjiajiensis	Atyidae	VC	1	medium	F	F	I
Macrobrachium lanchesteri	Palaemonidae	C	-3	low	F	F	I
Crayfish							
Cambarellus patzcuarensis	Cambaridae	R	2	medium	F	F	D/I
Procambarus alleni	Cambaridae	VC	11	medium	F	F	I
Procambarus clarkii	Cambaridae	VC	28	high	F	F	D/I
Procambarus cubensis	Cambaridae	VR	7	medium	F	F	I
Procambarus virginalis	Cambaridae	C	30	high	F	F	D/I
Cherax destructor	Parastacidae	R	19	high	F	F	I
Cherax holthuisi	Parastacidae	VR	3	medium	F	F	I
Cherax quadricarinatus	Parastacidae	R	9	medium	F	F	I
Crabs							
Perisesarma bidens	Sesarmidae	C	1	medium	B	B	I
Eriocheir sinensis	Varunidae	VR	28	high	B/M	F	I*
Hermit crabs							
Coenobita clypeatus	Coenobitidae	VR	1	medium	F/T	M	I

* traded mainly for human consumption

(*Procambarus alleni*, Faxon, 1884 and *P. clarkii*) were found very commonly in the market. Five species, *C. glaubrechti* von Rintelen and Cai, 2009, *Cherax holthuisi* Lukhaup and Pekny, 2006, *Coenobita clypeatus* (Fabricius, 1787), *Eriocheir sinensis*, and *P. cubensis* (Erichson, 1846), were found very rare on the market.

Based on interviews with wholesalers, we found that approx. 70% of shrimps were imported from Thailand and Vietnam, 20% from Germany, Italy, and Slovakia, and 10% produced domestically. In total, 95% of crayfish were imported from abroad with suppliers identified from the Czech Republic, Germany, Slovakia, Thailand, and Vietnam. The remaining 5% were produced domestically. The crab *Perisesarma bidens* (De Haan, 1835) was imported from South America, and the crab *Eriocheir sinensis* was imported from China and west-European countries, usually for human consumption.

Climatic data showed the highest probability of establishment for two shrimps *C. multidentata* and *N. heteropoda* Liang, 2000, two crayfish *P. clarkii* and *P. virginalis*, and crab *E. sinensis* (score ≥ 8 over the entire target area) followed by shrimp *N. zhangjiajiensis* and crayfish *C. destructor* (score = 7 over part of the target area).

The FI-ISK score ranged between values of -3 and 30. Two shrimp species were assessed as low-risk (*C. spongicola* Zitzler and Cai, 2006, and *Macrobrachium lanchesteri*, de Man, 1911); 11 shrimp species, five crayfish, one crab, and the hermit crab were classified as medium-risk; and three crayfish and one crab were classified in high-risk category (Table 1). The probability of establishment based on climatic conditions of the four most hazardous species within Hungary, *P. virginalis*, *P. clarkii*, *E. sinensis*, and *C. destructor*, is shown in Figure 1.

During field sampling on September 22, 2016, the crayfish *C. quadricarinatus* was first recorded in Hungary, as well as within the whole Carpathian Basin. We identified one haplotype, which did match with already known and available haplotypes in GenBank (Acc. No. MF449471). An adult female (cephalothorax length, CL = 46 mm, total body length, TL = 87 mm, Fig. 2) was captured in a side arm of the Danube river (rkm 1649), Kopaszi-gát, in Budapest (GPS N47°27'46.69", E19°3'31.20"). Subsequently, two more adult females were found: one in a thermal spring at the Fényes-forrás outflow near Tata (CL = 49 mm, TL = 94 mm, GPS N47°40'13.19", E18°18'20.51") on November 9, 2016, and one in a thermal spa at the Harkány outflow named Melegvíz-csatorna, which flows into the Drava river (CL = 31 mm, TL = 59 mm, GPS N45°50'29.54", E18°13'54.28") on November 15, 2016 (Fig. 3).

Discussion

The importance of effective preventing new introductions of

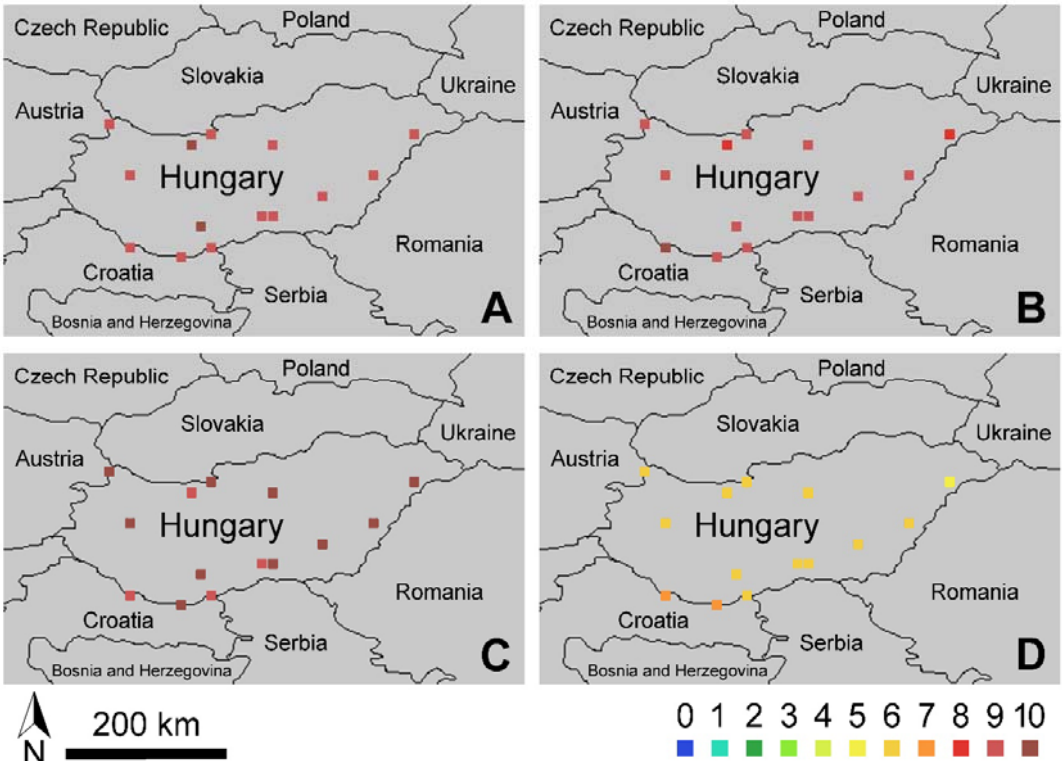

Figure 1. Climate match map of Hungary showing colour-coded regions with a different probability of establishment of the most hazardous species evaluated, A = *Procambarus virginalis*, B = *Procambarus clarkii*, C = *Eriocheir sinensis*, and D = *Cherax destructor*; scores of ≥ 7.0 were interpreted as where there is no environmental barrier to species survival.

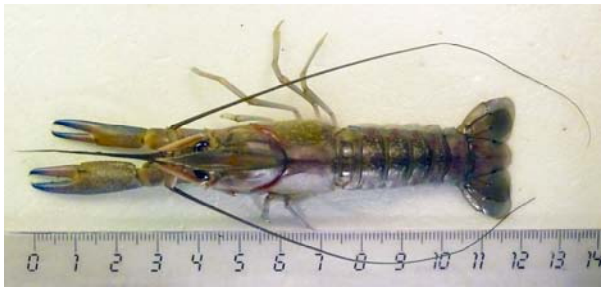

Figure 2. Female *Cherax quadricarinatus* captured in Kopaszi-gát, Budapest on September 22, 2016. This is the first record of the species in Hungary as well as in the whole Carpathian Basin.

Figure 3. Map showing localities where *Cherax quadricarinatus* was collected: Kopaszi-gát (indicated by a red triangle), Fényes-forrás outflow (red cross), and Melegvíz-csatorna outflow (red asterisk).

potential invaders is obvious, and agrees with the EU Regulation No. 1143/2014 and Commission Implementing Regulation No. 2016/1141 on the prevention and management of the introduction and spread of invasive alien species. From all recorded pet-traded decapod crustaceans in Hungary, we identified four species as being hazardous for native ecosystems in general and indigenous crayfish species in particular (e.g. Dittel & Epifanio 2009, Mrugała et al. 2015, 2016, Souty-Grosset et al. 2016). Three of these high-risk species, *Procambarus clarkii*, *P. virginalis*, and *Eriocheir sinensis* have been reported in the wild of Hungary (reviewed by Ludányi et al. 2016). They are also listed among invasive species of EU concern. Their introduction, trade, culture, and keeping are totally banned. However, even such measures might be ineffective as is shown by this study and which, at times, can be also related to an incorrect determination of marketed or kept animals (Patoka et al. 2014). Despite the ban, mentioned species are still being traded and sold, and most probably remain kept by hobbyists in indoor aquaria. Their deliberate release or accidental escapes could pose a serious threat to aquatic ecosystems.

The risk related to the establishment of *P. clarkii* and *P. virginalis* in the wild is well known and described elsewhere. See Souty-Grosset et al. (2016) and Vodovsky et al. (2017) and literature cited therein for review. *Eriocheir sinensis* is also prominent invader of aquatic ecosystems. However, pet trade plays rather a marginal role in its further spread due to rarity of this species at the market (Table 1). Both crabs and shrimps were suggested alternative hosts of crayfish plague pathogen (Svoboda et al. 2014a,b, Putra et al. 2018). Despite this, shrimps seems to be environmentally the least problematic pet-traded decapods in studied region (Table 1).

We provide the first evidence of *C. quadricarinatus* pres-

ence in the wild of Hungary as well as in the entire Carpathian basin. Despite its sensitivity to the crayfish plague (Hsieh et al. 2016), we consider this finding alarming. This crayfish is a large and robust species with documented broad environmental plasticity (Jones et al. 2000, Lin et al. 1999). On the other hand, temperature is a limiting factor and it is probably the reason why this crayfish is not able to successfully overwinter in the temperate zone (Veselý et al. 2015). The species was successfully introduced to and established feral populations within several tropical and subtropical regions worldwide (Ahyong and Yeo 2007, Bortolini et al. 2007, De Moor 2002, Todd 2002). The only known established population in the temperate zone occurs in Europe, inhabiting a thermal oxbow of the Sava river in Slovenia (Jaklič & Vrezec 2011). It is obvious that thermal waterbodies can serve as a suitable habitats for this species; therefore, Hungarian thermal springs may be potentially invaded by this species. Because *C. quadricarinatus* is not commercially produced in Hungarian aquaculture, its releases were probably intentionally or accidentally mediated by hobby keepers.

Previously highlighted socio-economic predictors of non-indigenous crayfish introductions and establishment (Chucholl 2014) seem to be important together with environmental factors within Europe. The pet trade with decapod crustaceans is evidently well developed in many countries, independent of their economic ranking (Kotovska et al. 2016, Patoka et al. 2015). We strongly recommend educating hobbyists in the future about potential risks related to the release of identified high-risk taxa. Although there is no evidence of established populations of *C. quadricarinatus* in Hungary, intensive monitoring focused on the occurrence and distribution of this crayfish is also strongly recommended. Further educating people working in pet shops about which species are prohibited to sell seems to be crucial. Certain freshwater decapod crustaceans are able to quickly spread via the Danube river and colonize the Carpathian Basin many kilometers downstream and also upstream, as documented in the case of the invasive crayfish *Faxonius limosus* (Lipták & Vitázková 2014, Pârvulescu et al. 2009). Therefore, the Hungarian pet trade is of concern in adjoining countries of the Carpathian Basin and in the whole Danube river catchment as a gateway for non-native crustaceans.

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