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Rapid Communication

First established population of marbled crayfish *Procambarus fallax* (Hagen, 1870) f. *virginalis* (Decapoda, Cambaridae) in Romania

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Abstract

The marbled crayfish, *Procambarus fallax* f. *virginialis*, is an obligate parthenogenetic crayfish species, its spread in the wild being linked to the aquarium pet-trade. Forty-two adult individuals were found for the first time in Romania in the semi-natural ponds in Băile Felix, near Oradea. Nine ovigerous females were captured as evidence that the population is breeding in the wild. They probably originated from the pet trade and were released by hobbyists into the pond. Microsatellite analysis revealed the same allelic patterns as in a previous study, confirming that these marbled crayfish are parthenogenetic and originate from a single individual. The crayfish plague pathogen *Aphanomyces astaci* was not present in the population. The site inhabited by this established marbled crayfish population is supplied with water by thermal underground streams, ensuring a constant high temperature. The nearby Peța Natural Reserve protects several endemic species that could be threatened by the further range extension of marbled crayfish. Special protection measures are therefore urgently needed.

Key words: invasive species, Marmorkrebs, parthenogenesis, pet trade

Introduction

Biological invasions, especially ones triggered by humans, cause damage to the environment (Dorcas et al. 2012; Buckley 2017), and to the economy of the "host" countries (Pimentel et al. 2005; Wittenberg et al. 2006). While some species have been introduced for farming (Holdich 1993) or brought as pets (Chucholl and Wendler 2017), the long-term consequences were underestimated in most cases (Lenteren 1997). Such incidents include various crayfish species, such as *Orconectes limosus* (Rafinesque, 1817), *Pacifastacus leniusculus* (Dana, 1852) and *Procambarus clarkii* (Girard, 1852), which escaped from aquaculture facilities or were introduced into the wild, thus leading to their successful on-going invasion in Europe (Gherardi 2006). All of these species possess impressive invasive prowess through higher growth rates than native species (Kozák et al. 2007), adaptive ability (Buřič et al. 2013), high fecundity (Pârvulescu et al. 2015), and food plasticity (Olsson et al. 2009). They are also resistant carriers of *Aphanomyces astaci* Schikora, 1906 (Strauss et al. 2012; Schrimpf et al. 2013), an oomycete pathogen causing the crayfish plague (Jussila et al. 2014). Its virulence to indigenous crayfish species outside of North America has led to *A. astaci* being classified among the world's 100 worst invasive alien species (Lowe et al. 2004).

Marbled crayfish, also known as Marmorkrebs, are one of the most popular pet crayfish species in the world (Faulkes 2015; Patoka et al. 2017). Their origin is unknown, as the first record of their presence comes from the German aquarium trade (Lukhaup 2001). Martin et al. (2010) regarded this crayfish as

Procambarus fallax (Hagen, 1870) f. *virginalis*. Marbled crayfish is either a result of autopolyploidy (Martin et al. 2016) or hybridization between *P. fallax* (Hagen, 1870) and other species of the genus *Procambarus*. Vogt et al. (2015) proposed elevation of marbled crayfish to the species level, *P. virginalis*.

Many individuals have reached wild environments through human negligence and have occasionally created stable populations (Lipták et al. 2016; Chucholl and Wendler 2017), because one individual can theoretically start a new population via parthenogenesis (Scholtz et al. 2003; Martin et al. 2010). Furthermore, their high spawning rate may be a great advantage in establishing and maintaining wild populations (Chucholl et al. 2012). Marbled crayfish can survive at low temperatures (Veselý et al. 2015; Kaldre et al. 2016), as shown by studies on its establishment in continental Europe (e.g., Chucholl et al. 2012; Patoka et al. 2016). They have been reported in many European countries: Croatia (Samardžić et al. 2014), the Czech Republic (Patoka et al. 2016), Germany (Chucholl et al. 2012), Italy (Vojkovská et al. 2014), Sweden (Bohman et al. 2013), Hungary (Weiperth et al. 2015; Lőkkös et al. 2016), Slovakia (Janský and Mutkovič 2010), Ukraine (Novitsky and Son 2016), and also in Madagascar (Jones et al. 2009) and Japan (Faulkes et al. 2012). This species is also a host to the crayfish plague pathogen, *A. astaci* (Keller et al. 2014).

In Romania, there are three native species of crayfish: *Astacus astacus* (Linnaeus, 1758), *A. leptodactylus* Eschscholtz, 1823, and *Austropotamobius torrentium* (Schrank, 1803). The alien crayfish *O. limosus,* was first documented in the Romanian Danube in 2008 (Pârvulescu et al. 2009) and successfully competes against *A. leptodactylus* populations in occupied habitats (Pârvulescu et al. 2015). The recent growth of the pet trade in Eastern Europe, including Romania (Raghavan et al. 2013), suggests this is the source of many alien species in the country. We investigated individual morphology of non-indigenous crayfish found in a semi-natural pond in Romanian territory; and conducted microsatellite analysis of the same population to compare the allele pattern to individuals from previous studies. We also tested the population for presence of *A. astaci* so that a plan to prevent impact on indigenous crayfish species could be instigated if necessary.

Methods

Field sampling

Sampling was conducted after finding that one or more of the five ponds in Băile Felix, near Oradea, România (Figure 1), could be populated by exotic crayfish. The investigated location is an urban area in Băile Felix-Sânmartin, Bihor County, Romania (Figure 1), containing five semi-natural ponds named "Waterlily lakes" ("Lacul cu nuferi", in Romanian). The central pond is located at 46º59′20.1″N; 21º58′43.3″E. The ponds are cement walled basins with 80–90 cm deep water and a surface varying between ~ 150–400 m^2. The basins are supplied by subterranean warm water springs (39.5 °C on the date of investigation). Water temperature (24.6 °C) and conductivity (595 µS cm^{-1}) were measured in sample site 3 (see the map in Figure 1) using a Hach-Lange multi-parameter (Düsseldorf, Germany). The lowest water temperature in this pond during winter was 15 °C (air temperature −16 °C), and small ice-sheets were observed only at the pond's margins (A. Togor, unpublished data). Two traps were used to catch crayfish five times over five consecutive weeks in April and May of 2017 resulting in five groups of captured individuals. For each capture effort, the traps were baited with fish, left overnight, and checked the following day. The captured crayfish were marked and released in the same pond after a general inspection, except for nine specimens which were preserved and transported in the laboratory for the measurement of the total length (TL), cephalothorax length (CL) and width (CW) to the nearest 0.01 mm using a Black & Decker digital calliper. Wet weight (WW) was recorded using a Kern analytical balance to the nearest 0.01 g. Tissue from these nine crayfish was collected by detaching the last walking leg of each individual and preserving it in 96% ethanol for molecular analyses. Samples for the detection of *A. astaci* consisted of soft abdominal cuticle, walking legs, telson and uropods (Vrålstad et al. 2009), and were stored in 96% ethanol.

Species identification and population genetics

Species diagnosis followed the guidelines of Martin et al. (2010) and Vogt et al. (2015). The key feature for differentiating marbled crayfish and females of *P. alleni* (even at small sizes) is the morphology of the sperm receptacle, the *Annulus ventralis*. Moreover, microsatellite analysis was used to compare the allelic pattern of captured marbled crayfish to individuals from previous studies. Nuclear DNA was extracted from walking legs of nine collected specimens of marbled crayfish with the Qiagen Blood & Cell Culture DNA Kit (Hilden, Germany). The same five primer pairs (PclG-02, PclG-04, PclG-08, PclG-48, PclG-26) and methods as in Vogt et al. (2015) were used. PCR was carried out using a Primus 96 Cycler (Peqlab Biotechnologie, Erlangen, Germany)

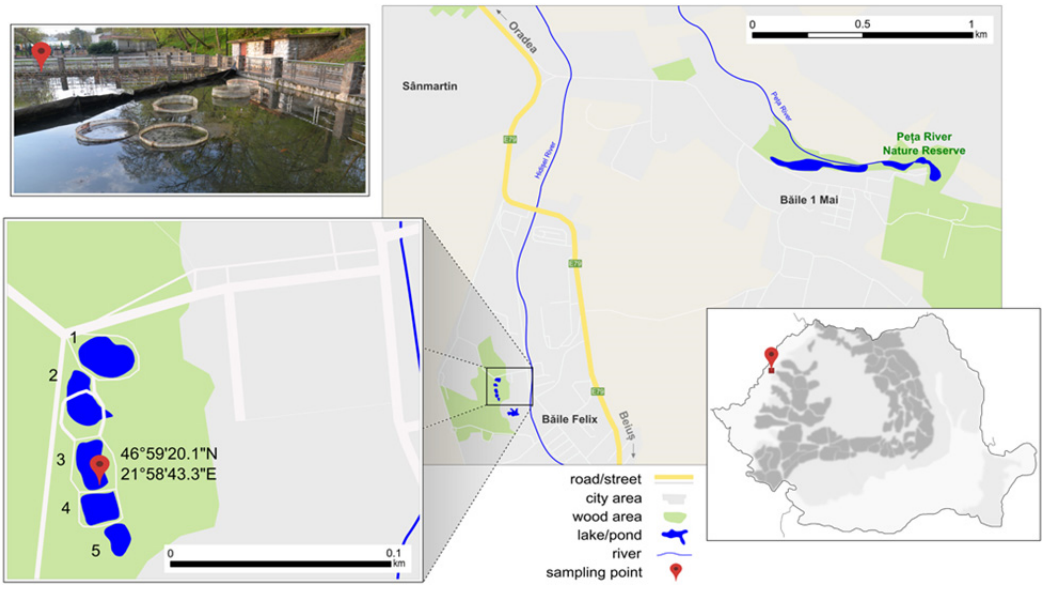

Figure 1. Map showing the location of the established population of marbled crayfish in the semi-natural ponds in Băile Felix, Oradea, Romania with GPS location of pond 3, detailed street-map and photography of the site. Street-map support by OpenStreetMap (https://www.openstreetmap.org).

in two separate batches, A and B. The conditions were as follows: DNA was denatured at 95 °C for 2 min, followed by 35 cycles of denaturation at 95 °C for 30 sec, annealing at 65 °C or 55 °C for batch A and batch B, respectively and elongation at 72 °C for 1 min. A final elongation step at 72 °C for 5 min concluded the PCR. Fragment analysis was performed on a Beckman Coulter CEQ 8000 eight capillary sequencer (Beckman Coulter, Krefeld, Germany) using the Beckman Coulter DNA Size Standard Kit 400 bp. The microsatellite peaks were scored using the Software GeneMarker V. 1.95 (SoftGenetics, Pennsylvania, USA). Juvenile stages were scored according to Vogt et al. (2004).

Aphanomyces astaci *infection status analysis*

DNA from nine crayfish was extracted using the E.Z.N.A. Insect DNA Kit (Omega bio-tek, Atlanta, USA) according to the manufacturer's instructions. To assess the infection status of marbled crayfish, a TaqMan® minor groove binder (MGB) qPCR was conducted, targeting the ITS region as described in Vrålstad et al. (2009) with some modifications according to Schrimpf et al. (2013). An initial Pre-PCR decontamination step was done at 50 °C for 120 sec followed by polymerase activation and template denaturation at 95 °C for 10 min. The PCR itself consisted of 50 cycles of denaturation at 95 °C for 15 sec followed by annealing at 62 °C for 15 sec. A final cooling step was included for 60 sec at 40 °C. Infection status and agent levels were defined according to Vrålstad et al. (2009) based on the number of PCR forming units (PFU), where samples with agent level A0 (0 PFU) and A1 (PFU_{obs} < 5 PFU) are considered uninfected and agent level A2 (5 PFU ≤ PFU_{obs} < 50 PFU) and higher are considered *A. astaci* positive.

Results

In this study, 42 crayfish individuals were captured in ponds 2 to 5, all indentified as marbled crayfish (Figure 2A). No specimens were captured in pond 1 during the investigation. Six individuals carrying eggs and three carrying juveniles in the second developmental stage were found (Figure 2C). The feature used to identify the species was the *Annulus ventralis*, which had a flatter, bell-shaped aspect, without scooped lateral wings on the lateral parts, and no peaked anterior portion (Figure 2B). The microsatellite pattern of the studied samples confirmed the species identification, being identical to the patterns found in Vogt et al. (2015), with clear triploidy (fragment length 267/271/303 bp, respectively) at the marker PclG02. This supports the notion that these marbled crayfish are obligatory parthenogenetic and originate from a single individual.

The TL of the nine individuals analysed in the laboratory ranged from 71.8 to 94.9 mm, with a mean

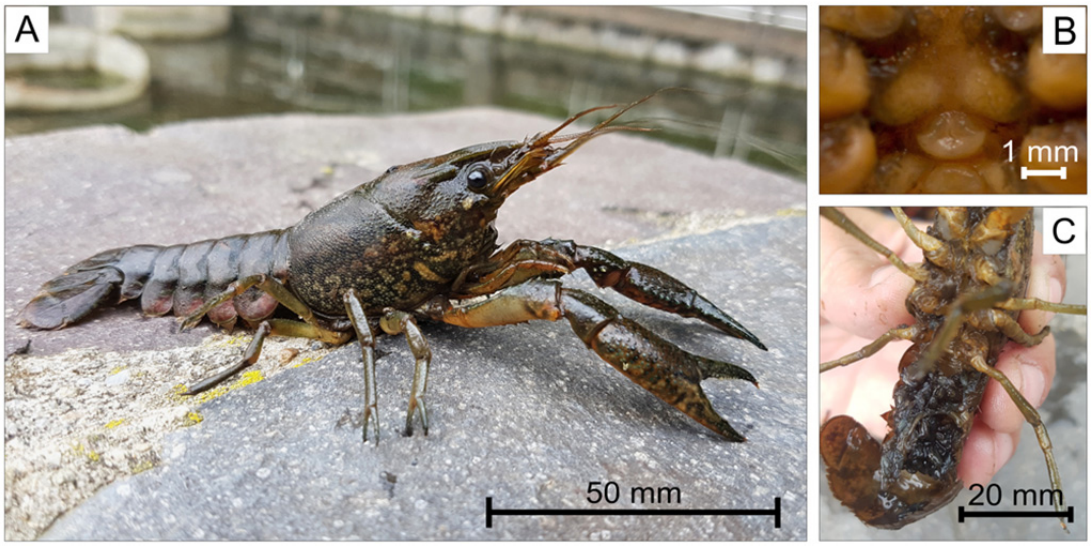

Figure 2. Pictures showing a general view of a specimen of marbled crayfish collected in the semi-natural ponds in Băile Felix, Oradea, Romania (**A**), a close up of the *Annulus ventralis* (**B**), and carried stage two juveniles (**C**). Photo by A. Togor (**A**, **C**), L. Pârvulescu (**B**).

Table 1. Biometric data measured for marbled crayfish individuals collected in the semi-natural ponds in Băile Felix, Oradea, Romania, and inspected in the laboratory. Abbreviations: TL - total length, CL - cephalothorax length, CW - cephalothorax width, WW - wet weight.

Order	TL	CL	CW	WW	General observations
1	94.25	43.57	20.60	20.91	fresh molted
2	84.06	38.91	18.44	15.12	dark and elongated pleopods
3	73.83	35.38	16.70	10.48	fresh molted
4	71.83	34.62	15.99	9.31	fresh molted
5	92.45	43.59	20.67	19.25	dark and elongated pleopods
6	92.05	42.36	20.04	17.95	116 juveniles, stage 2
7	94.93	44.39	20.77	21.16	dark and elongated pleopods
8	84.32	39.31	18.68	15.69	dark and elongated pleopods
9	87.44	40.69	19.71	17.32	dark and elongated pleopods
Mean	86.13	40.31	19.07	16.35	
SD	8.5	3.6	1.8	4.2	

of 86.1 mm (SD = 8.5). The largest weight was 21.16 g (Table 1). The egg-carrying marbled crayfish in this capture measured 92.5 mm in TL, and weighed 17.95 g without its clutch. Other females presented dark and elongated pleopods which suggests they had probably bred before (Hopkins 1967).

None of the nine samples analysed for presence of *A. astaci* tested clearly positive for DNA of the pathogen. All samples had the agent level A1, thus being below the limit of detection.

Discussion

The established population of marbled crayfish in Romania is in a recreational promenade area, including five interconnected water bodies close to Hidişel River (Figure 1), with a roughly constant temperature of around 25 °C provided by warm springs (Tenu et al. 1981). The area is frequently visited by tourists because of the local attraction, the thermal lotus *Nymphaea lotus* f. *thermalis*. This lotus species is endemic in the thermal waters of the nearby Peţa River Nature Reserve (Figure 1), while the semi-natural ponds are populated artificially. The crayfish species probably found its way into this pond by being abandoned thre, like many other exotic species, such as *Trachemys scripta*, *Carassius* spp., *Colisa* spp., *Xiphophorus* spp. (A. Togor, pers. comm.). Alongside the thermal lotus, these warm waterbodies are inhabited by two other endemic taxa: the fish *Scardinius racovitzai* Müller, 1958 and the mollusc *Melanopsis parreyssi* (Philippi, 1847), both of which are critically endangered species (Freyhof and Kottelat 2008; Fehér 2011).

The ponds are not directly connected to natural river systems. Still, the risk of further expansion seems high because the area is frequently visited by uninformed

public who could translocate specimens from the ponds to the nearby Hidişel and Peţa rivers. As marbled crayfish consume plants (VanArman 2011), they pose a potential threat to the thermal lotus, which decreased in population size in 2017 (A. Togor, unpublished data). We suspect that crayfish might damage the lotus plants by eating the bulbs and roots, and/or the fragile sprouts in spring.

Considering the evidence found in other studies (Chucholl et al. 2012), this crayfish species seems less able to colonise large water courses. Consequently, we believe that the expansion of marbled crayfish does not represent a major threat for native crayfish populations, which are well represented in the mountain and submountain areas of the region by *A. astacus* and *A. torrentium*, the nearest at ~ 50 km, upstream on the Criş River (for maps see Pârvulescu and Zaharia 2013, 2014). The mean multiannual temperature (Fick and Hijmans 2017) in the upstream area of Criş rivers inhabited by native crayfish species is 4 to 7 °C, much colder than the area of the ponds inhabited by marbled crayfish at 10–11 °C.

Many pet crayfish species are carriers of the crayfish plague pathogen *A. astaci* (Mrugała et al. 2014; Panteleit et al. 2017). In this study, no infection with *A. astaci* could be detected in the marbled crayfish population. It should not, however, be assumed that the population is disease-free. Marbled crayfish can carry the pathogen and tolerate the infection like *P. fallax* (Keller et al. 2014). The detection of agent level A1 in this study is not enough to confirm the absence of *A. astaci*. Thus, this population may be a latent reservoir for the pathogen. We suggest authorities take active measures against the introduction of animals into the ponds (Vrålstad et al. 2011), but also warn tourists and local people not to transfer plants or animals from the site.

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References

Bohman P, Edsman L, Martin P, Scholtz G (2013) The first Marmorkrebs (Decapoda: Astacida: Cambaridae) in Scandinavia. *BioInvasions Records* 2: 227–232, https://doi.org/10.3391/bir.2013.2.3.09

Buckley YM (2017) Invasion ecology: Unpredictable arms race in a jam jar. *Nature Ecology & Evolution* 1: 0028, https://doi.org/10.1038/s41559-016-0028

Buřič M, Kouba A, Kozák P (2013) Reproductive plasticity in freshwater invader: from long-term sperm storage to parthenogenesis. *PLoS ONE* 8: e77597, https://doi.org/10.1371/journal.pone.0077597

Chucholl C, Morawetz K, Groß H (2012) The clones are coming–strong increase in Marmorkrebs [*Procambarus fallax* (Hagen, 1870) f. *virginalis*] records from Europe. *Aquatic Invasions* 7: 511–519, https://doi.org/10.3391/ai.2012.7.4.008

Chucholl C, Wendler F (2017) Positive selection of beautiful invaders: long-term persistence and bio-invasion risk of freshwater crayfish in the pet trade. *Biological Invasions* 19: 197–208, https://doi.org/10.1007/s10530-016-1272-5

Dorcas ME, Willson JD, Reed RN, Snow RW, Rochford MR, Miller MA, Meshaka WE, Andreadis PT, Mazzotti FJ, Romagosa CM, Hart KM (2012) Severe mammal declines coincide with proliferation of invasive Burmese pythons in Everglades National Park. *Proceedings of the National Academy of Sciences* 109: 2418–2422, https://doi.org/10.1073/pnas.1115226109

Faulkes Z (2015) Marmorkrebs (*Procambarus fallax* f. *virginalis*) are the most popular crayfish in the North American pet trade. *Knowledge and Management of Aquatic Ecosystems* 416: 20, https://doi.org/10.1051/kmae/2015016

Faulkes Z, Feria TP, Muñoz J (2012) Do Marmorkrebs, *Procambarus fallax* f. *virginalis*, threaten freshwater Japanese ecosystems? *Aquatic Biosystems* 8: 13, https://doi.org/10.1186/2046-9063-8-13

Fehér Z (2011) *Melanopsis parreyssii*. The IUCN Red List of Threatened Species 2011: e.T155737A4835365. http://dx.doi.org/10.2305/IUCN.UK.2011-1.RLTS.T155737A4835365.en (accessed on August 2017)

Fick SE, Hijmans RJ (2017) Worldclim 2: New 1-km spatial resolution climate surfaces for global land areas. *International Journal of Climatology* 37: 4302–4315, https://doi.org/10.1002/joc.5086

Freyhof J, Kottelat M (2008) *Scardinius racovitzai*. The IUCN Red List of Threatened Species 2008: e.T19948A9113563 http://dx.doi.org/10.2305/IUCN.UK.2008.RLTS.T19948A9113563.en (accessed on August 2017)

Gherardi F (2006) Crayfish invading Europe: the case study of *Procambarus clarkii*. *Marine and Freshwater Behaviour and Physiology* 39: 175–191, https://doi.org/10.1080/10236240600869702

Holdich DM (1993) A review of astaciculture: freshwater crayfish farming. *Aquatic Living Resources* 6: 307–317, https://doi.org/10.1051/alr:1993032

Hopkins CL (1967) Breeding in the freshwater crayfish *Paranephrops planifrons* White. *New Zealand Journal of Marine and Freshwater Research* 1: 51–58, https://doi.org/10.1080/00288330.1967.9515191

Janský V, Mutkovič A (2010) Marbled crayfish—*Procambarus* sp. (Crustacea: Decapoda: Cambaridae)—first find in Slovakia. *Acta Rerum Naturalium Musei Natuionalis Slovenici* 56: 64–67

Jones JP, Rasamy JR, Harvey A, Toon A, Oidtmann B, Randrianarison MH, Raminosoa N, Ravoahangimalala OR (2009) The perfect invader: a parthenogenic crayfish poses a new threat to Madagascar's freshwater biodiversity. *Biological Invasions* 11: 1475–1482, https://doi.org/10.1007/s10530-008-9334-y

Jussila J, Makkonen J, Vainikka A, Kortet R, Kokko H (2014) Crayfish plague dilemma: how to be a courteous killer? *Boreal Environment Research* 19: 235–245

Kaldre K, Meženin A, Paaver T, Kawai T (2016) A preliminary study on the tolerance of marble crayfish *Procambarus fallax* f. *virginalis* to low temperature in Nordic climate. In: T Kawai, Z Faulkes, G Scholtz (eds), Freshwater Crayfish: A Global Overview, Boca Raton, CRC Press, pp 54–62

Keller NS, Pfeiffer M, Roessink I, Schulz R, Schrimpf A (2014) First evidence of crayfish plague agent in populations of the marbled crayfish (*Procambarus fallax* forma *virginalis*). *Knowledge and Management of Aquatic Ecosystems* 414: 15, https://doi.org/10.1051/kmae/2014032

Kozák P, Buřič M, Policar T, Hamáčková J, Lepičová A (2007) The effect of inter-and intra-specific competition on survival and growth rate of native juvenile noble crayfish *Astacus astacus* and alien spiny-cheek crayfish *Orconectes limosus*. *Hydrobiologia* 590: 85–94, https://doi.org/10.1007/s10750-007-0760-0

Lenteren JV (1997) Benefits and risks of introducing exotic macrobiological control agents into Europe. *EPPO Bulletin* 27: 15–27, https://doi.org/10.1111/j.1365-2338.1997.tb00611.x

Lipták B, Mrugała A, Pekárik L, Mutkovič A, Gruľa D, Petrusek A, Kouba A (2016) Expansion of the marbled crayfish in Slovakia: beginning of an invasion in the Danube catchment? *Journal of Limnology* 75: 305–312, https://doi.org/10.4081/jlimnol.2016.1313

Lőkkös A, Müller T, Kovács K, Várkonyi L, Specziár A, Martin P (2016) The alien, parthenogenetic marbled crayfish (Decapoda: Cambaridae) is entering Kis-Balaton (Hungary), one of Europe's most important wetland biotopes. *Knowledge and Management of Aquatic Ecosystems* 417: 16, https://doi.org/10.1051/kmae/2016003

Lowe S, Browne M, Boudjelas S, De Poorter M (2004) 100 of the world's worst invasive alien species. A selection from the Global Invasive Species Database. The Invasive Species Specialist Group (ISSG), a specialist group of the Species Survival Commission (SSC) of the IUCN, Gland, Switzerland

Lukhaup C (2001) *Procambarus* sp. – Der Marmorkrebs. *Aquaristik Aktuell* 7–8: 48–51

Martin P, Dorn NJ, Kawai T, van der Heiden C, Scholtz G (2010) The enigmatic Marmorkrebs (marbled crayfish) is the parthenogenetic form of *Procambarus fallax* (Hagen, 1870). *Contributions to Zoology* 79: 107–118

Martin P, Thonagel S, Scholtz G (2016) The parthenogenetic Marmorkrebs (Malacostraca: Decapoda: Cambaridae) is a triploid organism. *Journal of Zoological Systematics and Evolutionary Research* 54: 13–21, https://doi.org/10.1111/jzs.12114

Mrugała A, Kozubíková-Balcarová E, Chucholl C, Cabanillas Resino S, Viljamaa-Dirks S, Vukić J, Petrusek A (2014) Trade of ornamental crayfish in Europe as a possible introduction pathway for important crustacean diseases: crayfish plague and white spot syndrome. *Biological Invasions* 17: 1313–1326, https://doi.org/10.1007/s10530-014-0795-x

Novitsky RA, Son MO (2016) The first records of Marmorkrebs [*Procambarus fallax* (Hagen, 1870) f. *virginalis*] (Crustacea, Decapoda, Cambaridae) in Ukraine. *Ecologica Montenegrina* 5: 44–46

Olsson K, Stenroth P, Nyström P, Granéli W (2009) Invasions and niche width: does niche width of an introduced crayfish differ from a native crayfish? *Freshwater Biology* 54: 1731–1740, https://doi.org/10.1111/j.1365-2427.2009.02221.x

Panteleit J, Keller NS, Kokko H, Jussila J, Makkonen J, Theissinger K, Schrimpf A (2017) Investigation of ornamental crayfish reveals new carrier species of the crayfish plague pathogen (*Aphanomyces astaci*) *Aquatic Invasions* 12: 77–83, https://doi.org/10.3391/ai.2017.12.1.08

Patoka J, Buřič M, Kolář V, Bláha M, Petrtýl M, Franta P, Tropek R, Kalous L, Petrusek A, Kouba A (2016) Predictions of marbled crayfish establishment in conurbations fulfilled: Evidences from the Czech Republic. *Biologia* 71: 1380–1385, https://doi.org/10.1515/biolog-2016-0164

Patoka J, Bláha M, Kalous L, Kouba A (2017) Irresponsible vendors: Non-native, invasive and threatened animals offered for garden pond stocking. *Aquatic Conservation: Marine and Freshwater Ecosystems* 27: 692–697, https://doi.org/10.1002/aqc.2719

Pârvulescu L, Paloş C, Molnar P (2009) First record of the spiny-cheek crayfish *Orconectes limosus* (Rafinesque, 1817) (Crustacea: Decapoda: Cambaridae) in Romania. *North-Western Journal of Zoology* 5: 424–428

Pârvulescu L, Pîrvu M, Moroşan LG, Zaharia C (2015) Plasticity in fecundity highlights the females' importance in the spiny-cheek crayfish invasion mechanism. *Zoology* 118: 424–432, https://doi.org/10.1016/j.zool.2015.08.003

Pârvulescu L, Zaharia C (2013) Current limitations of the stone crayfish distribution in Romania: Implications for its conservation status. *Limnologica* 43: 143–150, https://doi.org/10.1016/j.limno.2012.07.008

Pârvulescu L, Zaharia C (2014) Distribution and ecological preferences of noble crayfish in the Carpathian Danube basin: biogeographical insights into the species history. *Hydrobiologia* 726: 53–63, https://doi.org/10.1007/s10750-013-1751-y

Pimentel D, Zuniga R, Morrison D (2005) Update on the environmental and economic costs associated with alien-invasive species in the United States. *Ecological Economics* 52: 273–288, https://doi.org/10.1016/j.ecolecon.2004.10.002

Raghavan R, Dahanukar N, Tlusty MF, Rhyne AL, Kumar KK, Molur S, Rosser AM (2013) Uncovering an obscure trade: threatened freshwater fishes and the aquarium pet markets. *Biological Conservation* 164: 158–169, https://doi.org/10.1016/j.biocon.2013.04.019

Samardžić M, Lucić A, Maguire I, Hudina S (2014) The first record of the marbled crayfish (*Procambarus fallax* (Hagen, 1870) f. *virginalis*) in Croatia. *Crayfish News* 36: 4–4

Scholtz G, Braband A, Tolley L, Reimann A, Mittmann B, Lukhaup C, Steuerwald F, Vogt G (2003) Parthenogenesis in an outsider crayfish. *Nature* 421: 806, https://doi.org/10.1038/421806a

Schrimpf A, Chucholl C, Schmidt T, Schulz R (2013) Crayfish plague agent detected in populations of the invasive North American crayfish *Orconectes immunis* (Hagen, 1870) in the Rhine River, Germany. *Aquatic Invasions* 8: 103–109, https://doi.org/10.3391/ai.2013.8.1.12

Strauss A, White A, Boots M (2012) Invading with biological weapons: the importance of disease-mediated invasions. *Functional Ecology* 26: 1249–1261, https://doi.org/10.1111/1365-2435.12011

Tenu A, Constantinescu T, Davidescu F, Nuti S, Noto P, Squarci P (1981) Research on the thermal waters of the Western Plain of Romania. *Geothermics* 10: 1–28, https://doi.org/10.1016/0375-6505(81)90021-3

VanArman PG (2011) Role of native crayfish, *Procambarus alleni* (Faxon) and *Procambarus fallax* (Hagen), in everglades food webs: a literature review and conceptual model. *Florida Scientist* 74: 100–125

Veselý L, Buric M, Kouba A (2015) Hardy exotics species in temperate zone: can "warm water" crayfish invaders establish regardless of low temperatures? *Scientific Reports* 5: 16340, https://doi.org/10.1038/srep16340

Vogt G, Tolley L, Scholtz G (2004) Life stages and reproduction components of the Marmorkrebs (marbled crayfish), the first parthenogenetic decapod crustacean. *Journal of Morphology* 261: 286–311, https://doi.org/10.1002/jmor.10250

Vogt G, Falckenhayn C, Schrimpf A, Schmid K, Hanna K, Panteleit J, Helm M, Schulz R, Lyko F (2015) The marbled crayfish as a paradigm for saltational speciation by autopolyploidy and parthenogenesis in animals. *Biology Open* 4: 1583–1594, https://doi.org/10.1242/bio.014241

Vojkovská R, Horká I, Tricarico E, Ďuriš Z (2014) New record of the parthenogenetic marbled crayfish *Procambarus fallax* f. *virginalis* from Italy. *Crustaceana* 87: 1386–1392, https://doi.org/10.1163/15685403-00003365

Vrålstad T, Knutsen AK, Tengs T, Holst-Jensen A (2009) A quantitative TaqMan® MGB real-time polymerase chain reaction based assay for detection of the causative agent of crayfish plague (*Aphanomyces astaci*). *Veterinary Microbiology* 137: 146–155, https://doi.org/10.1016/j.vetmic.2008.12.022

Vrålstad T, Johnsen SI, Fristad RF, Edsman L, Strand D (2011) Potent infection reservoir of crayfish plague now permanently established in Norway. *Diseases of Aquatic Organisms* 97: 75–83, https://doi.org/10.3354/dao02386

Weiperth A, Csányi B, Gál B, György ÁI, Szalóky Z, Szekeres J, Tóth B, Puky M (2015) Egzotikus rák-, hal-és kétéltűfajok a Budapest környéki víztestekben [Exotic crayfish, fish and amphibian species in various water bodies in the region of Budapest]. *Pisces Hungarici* 9: 65–70

Wittenberg R, Kenis M, Blick T, Hänggi A, Gassmann A, Weber E (2006) Invasive alien species in Switzerland: an inventory of alien species and their threat to biodiversity and economy in Switzerland. Invasive alien species in Switzerland: an inventory of alien species and their threat to biodiversity and economy in Switzerland, 155 pp