This manuscript is contextually identical with the following published paper: Weiperth A; Gál B; Kuříková P; Bláha M; Kouba A; Patoka J (2017) **Cambarellus patzcuarensis in Hungary: The first dwarf crayfish established outside of North America**. - Biologia (Bratislava) 72: (12) pp. 1529-1532.
The original published PDF available in this website: https://www.degruyter.com/view/j/biolog.2017.72.issue-12/biolog-2017-0159/biolog-2017-0159.xml

Cambarellus patzcuarensis in Hungary: The first dwarf crayfish established outside of North America

András Weiperth[1]*, Blanka Gál[1,2], Pavlína Kuříková[3], Martin Bláha[4], Antonín Kouba[4], Jiří Patoka[3]

[1] Centre for Ecological Research, Hungarian Academy of Sciences, Danube Research Institute, Karolina út 29, H-1113 Budapest, Hungary

[2] Doctoral School of Environmental Sciences, Eötvös Loránd University, Pázmány Péter sétány 1/C, H-1117 Budapest, Hungary

[3] Czech University of Life Sciences Prague, Faculty of Agrobiology, Food and Natural Resources, Department of Zoology and Fisheries, Kamýcká 129, CZ-16500 Prague-Suchdol, Czech Republic

[4] University of South Bohemia in České Budějovice, Faculty of Fisheries and Protection of Waters, South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses, Zátiší 728/II, CZ-38925 Vodňany, Czech Republic

**Corresponding author*

E-mail: weiperth.andras@okologia.mta.hu

Tel.: 003612793100/304, 0036203916468

Abstract: In 2017, a new non-indigenous crayfish species was established in Europe. The captured individuals were identified as an orange morph of the Mexican dwarf crayfish *Cambarellus patzcuarensis* Villalobos, 1943. Fifteen adults (including three ovigerous females) and 26 juveniles were collected in a thermal pond in Budapest, Hungary. Two additional adults were caught below the pond's outflow in the adjacent Danube River. To our knowledge, this is the first record of a *C. patzcuarensis* population outside North America, which is also true for the rest of dwarf crayfish (family Cambaridae, subfamily Cambarellinae). With this finding, indigenous crayfish species in Europe are now more than two-fold outnumbered by non-indigenous species. An analysis of the probability of establishment of *C. patzcuarensis* in continental Europe revealed that specific regions in the south of the continent are suitable areas for the establishment of the species. Moreover, as a confirmed carrier of the crayfish plague pathogen, this species should be treated with caution and eradicated if possible.

Key words: non-native species; introduction; biological invasion; climate match; thermal water; dwarf crayfish

Introduction

The international trade in live ornamental animals is a well-known source of non-native species worldwide (Padilla and Williams 2004). Contrary to commercial aquaculture where only a limited number of stakeholders possess large quantities of animals, the pet trade is characterised by limited numbers of exotic species kept by many hobbyists and accordingly higher risk of release in multiple locations. This trend was recently debated in relation to crayfish (Chucholl 2013; Faulkes 2015a).

Around 30 crayfish species are available on the market in countries with long history of trade in aquatic animals for the pet industry, such as the USA (Faulkes 2015b), Germany (Chucholl and Wendler 2017), and the Czech Republic (Patoka et al. 2015); certain species have also been detected as trade animals in Greece (Papavlasopoulou et al. 2014), Hungary (Weiperth et al. 2017), Kazakhstan (Uderbayev et al. 2017), the Russian Federation (Vodovsky et al. 2017), Slovakia (Lipták and Vitázková 2015), Turkey (Turkmen and Karadal 2012), and Ukraine (Kotovska et al. 2016). It is obvious that the propagule pressure of species under trade has increased. Moreover, crayfish are kept not only in indoor aquaria but also in garden ponds (Patoka et al. 2014b; Patoka et al. 2017), and in outdoor aquaria (Perdikaris et al. 2017) or ponds close to the restaurant which advertise crayfish as a delicacy (Chucholl and Daudey 2008). It is not surprising that released or escaped crayfish have been consequently recorded in many countries. The majority of crayfish species under trade belong to the North-American cambarids which often established in the wild (e.g. Chucholl and Daudey 2008; Novitsky and Son 2016; Patoka et al. 2016a).

Material and methods

During two field surveys in Budapest, Hungary (May 19 and 30, 2017), crayfish were collected using nine (5+4) plastic bottle traps baited with halibut pellets and cyprinid fishmeat and left in the pond for 24 hours. Captured individuals were preserved for later identification in pure (96%) ethanol, and a single walking leg from four adult individuals was collected for genetic analysis. The initial morphological species identification was confirmed by a molecular marker amplified by polymerase chain reaction. A primer pair 1471 (5'–CCTGTTTANCAAAAACAT-3') and 1472 (5'AGATAGAAACCAACCTGG-3') was used for amplification of the 16S gene (Crandall & Fitzpatrick 1996). The DNA extraction and amplification was processed according to Patoka et al. (2016b). The samples were sequenced using the Macrogen sequencing service (www.macrogen.com).

The probability of the establishment of captured crayfish throughout the entire European continent was evaluated 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). Climatic conditions were represented by temperature during the coldest quarter of the year in the analysis. The region which is the native geographic range of the evaluated species was used as the source area. The target area was defined as the territory of Europe containing 1117 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 in accordance with previous studies (e.g., Kotovska et al. 2016; Patoka et al. 2016b).

Results and discussion

We captured 26 juveniles (2+24, total body length < 9 mm, not sexed) and 15 adults (4+11, carapace length 11–17 mm, total body length 29–38 mm, ten males and five females, three of them ovigerous on May 30) were collected in a thermal pond (Fig. 1; 47^{0}31’3.72” N, 19^{0}2’16,11”E). The pond belongs to the complex of the Lukács Thermal Baths and is approximately rectangular in shape, ca. 8 × 14 m. The water temperature in the pond fluctuates from 31 to 37◦C during the year. The second survey was associated with monitoring a 400 m long section of shoreline of the adjacent Danube River (47^{0}31’6.30” N, 19^{0}2’21.93” E), which resulted in two adult males caught close to the mouth of the outflow. Subsequently, the species was identified as an orange morph of the Mexican dwarf crayfish *Cambarellus patzcuarensis* Villalobos, 1943 (Fig. 1). We identified one haplotype, which was a match with already known and available haplotypes in GenBank (Accession Numbers

MF449471, MF449472, MF449473 and MF449474). This is the first record of the species established as an outdoor population outside North America, which is also true for the rest of dwarf crayfish (subfamily Cambarellinae). *Cambarellus patzcuarensis* is an endangered endemic species having only a restricted native range in Mexico (Pedraza-Lara et al. 2012; Faulkes 2015b). Based on available information, we consider the population in the thermal pond established. In light of this finding, the indigenous crayfish species in Europe are now outnumbered by non-indigenous species more than two-fold (cf. Holdich et al. 2009). Although the availability of this crayfish species in the Hungarian market was previously assessed as "rare" (species available occasionally in small quantities; Weiperth et al. 2018), this colour morph also called "CPO" is a very attractive and popular strain among hobby keepers (Patoka et al. 2014b; Faulkes 2015b; Chucholl & Wendler 2017). Because this species is not exploited in commercial aquaculture due to its tiny size (adult total body length is ca. 3.5 cm), we assume that it was intentionally released from aquaria. Since the climate matching for *C. patzcuarensis* in this region was low (Weiperth et al. 2018), its extensive spread outside the thermal pond is not expected. On the other hand, the overwintering ability of several ornamental crayfish initially considered to be "warm-water" species, has been also proved (Veselý et al. 2015). Climate matching of native range of *C. patzcuarensis* and target area of Europe shows that the score of ≥7 was reached in 65 meteorological stations. All of these stations were located in the southern Europe, with the highest probabilities to establish wild populations predicted for Greece, Italy, Portugal and Spain (Fig. 2). Moreover, there are various examples of the occurrence of non-indigenous crayfish species in thermal waters in regions where climatic conditions are unsuitable (von Petutschnig et al. 2008; Jaklič & Vrezec 2011). Similar to other crayfish of North-American origin, *C. patzcuarensis* can serve as a vector of the crayfish plague pathogen, an oomycete *Aphanomyces astaci* Schikora, which is a fatal disease for all crayfish species not originating from the North American continent

(Mrugala et al. 2015; Svoboda et al. 2017). *Cambarellus patzcuarensis* is, compared to the Hungarian trade, more frequently available in the market of freshwater ornamental animals in other countries: e.g. in USA (Faulkes 2015b), Germany (Chucholl & Wendler 2017), the Czech Republic (Patoka et al. 2014a), and Ukraine (Kotovska et al. 2016). Moreover, the abundance of *C. patzcuarensis* in aquaria may increase in the future because it is usually proposed by pet shop owners to replace recently banned, and previously the most traded and kept crayfish *Procambarus clarkii* (Girard, 1852) and *P. fallax f. virginalis* Martin et al., 2010 in European Union (Regulation No. 1143/2014). Even if the bright orange colouration disadvantages this morph in the wild due the higher visibility to predators (Faulkes 2015b), the risk of crayfish plague exists. Since there are no available data on the crayfish pet trade in most regions of southern Europe, we propose this species to the attention of conservationists, wildlife managers and policymakers of European countries. We also recommend further surveys of the aquaria pet market and conducting a risk assessment of invasiveness based on this finding.

Acknowledgements

This study was supported by the Internal Grant Agency of the Czech University of Life Sciences Prague "CIGA", project No. 20152007 and by the Ministry of Education, Youth and Sports of the Czech Republic – projects "CENAKVA" (No. CZ.1.05/2.1.00/01.0024) and "CENAKVA II" (No. LO1205 under the NPU I program). The English was proofread by Andrew Hamer, University of Melbourne.

References

Chucholl C. 2013. Invaders for sale: trade and determinants of introduction of ornamental freshwater crayfish. Biol. Invasions **15:** 125-141.

Chucholl C. & Daudey T. 2008. First record of *Orconectes juvenilis* (Hagen, 1870) in eastern France: update to the species identity of a recently introduced orconectid crayfish (Crustacea: Astacida). Aquat. Invasions **3:** 105-107.

Chucholl C. & Wendler F. 2017. Positive selection of beautiful invaders: long-term persistence and bio-invasion risk of freshwater crayfish in the pet trade. Biol. Invasions **19:** 197-208.

Crandall K.A. & Fitzpatrick J.J. 1996. Crayfish molecular systematics: Using a combination of procedures to estimate phylogeny. Syst. Biol. **45:** 1-26.

Faulkes Z. 2015a. The global trade in crayfish as pets. Crustacean Research **44:** 75-92.

Faulkes Z. 2015b. Marmorkrebs (*Procambarus fallax* f. *virginalis*) are the most popular crayfish in the North American pet trade. Knowl. Manag. Aquat. Ec. **416:** 20.

Hijmans R.J., Cameron S.E., Parra J.L., Jones P.G. & Jarvis A. 2005. Very high resolution interpolated climate surfaces for global land areas. Int. J. Climatol. **25:** 1965-1978.

Holdich D.M., Reynolds J.D., Souty-Grosset C. & Sibley P.J. 2009. A review of the ever increasing threat to European crayfish from non-indigenous crayfish species. Knowl. Manag. Aquat. Ec. **394-395:** 11.

Jaklič M. & Vrezec A. 2011. The first tropical alien crayfish species in European waters: the redclaw *Cherax quadricarinatus* (Von Martens, 1868) (Decapoda, Parastacidae). Crustaceana **84:** 651-665.

Kotovska G., Khrystenko D., Patoka J. & Kouba A. 2016. East European crayfish stocks at risk: arrival of non-indigenous crayfish species. Knowl. Manag. Aquat. Ec. **417:** 37.

Lipták B. & Vitázková B. 2015. Beautiful, but also potentially invasive. Ekológia (Bratislava) **34:** 155-162.

Mrugała A., Kozubíková-Balcarová E., Chucholl C., Resino S.C., Viljama-Dirks S., Vukić J. & Petrusek A. 2015. Trade of ornamental crayfish in Europe as a possible introduction pathway for important crustacean diseases: crayfish plague and white spot syndrome. Biol. Invasions **17:** 1313-1326.

Novitsky R.A. & Son M.O. 2016. The first records of Marmorkrebs [*Procambarus fallax* (Hagen, 1870) f. *virginalis*] (Crustacea, Decapoda, Cambaridae) in Ukraine. Ecologica Montenegrina **5:** 44-46.

Padilla D.K. & Williams S.L. 2004. Beyond ballast water: aquarium and ornamental trades as sources of invasive species in aquatic ecosystems. Front. Ecol. Environ. **2:** 131-138.

Papavlasopoulou I., Perdikaris C., Vardakas L. & Paschos I. 2014. Enemy at the gates: introduction potential of non-indigenous freshwater crayfish in Greece via the aquarium trade. Centr. Eur. J. Biol. **9:** 1-8.

Patoka J., Bláha M., Kalous L. & Kouba A. 2017. Irresponsible vendors: non-native, invasive and threatened animals offered for stocking garden ponds. Aquat. Conserv. **27:** 692-697.

Patoka J., Buřič M., Kolář V., Bláha M., Petrtýl M., Franta P., Tropek R., Kalous L., Petrusek A. & Kouba A. 2016a. Predictions of marbled crayfish establishment in conurbations fulfilled: Evidences from the Czech Republic. Biologia **71:** 1380-1385.

Patoka J., Kalous L. & Kopecký O. 2014a. Risk assessment of the crayfish pet trade based on data from the Czech Republic. Biol. Invasions **16:** 2489-2494.

Patoka J., Kalous L. & Kopecký O. 2015. Imports of ornamental crayfish: the first decade from the Czech Republic's perspective. Knowl. Manag. Aquat. Ec. **416:** 4.

Patoka J., Petrtýl M. & Kalous L. 2014b. Garden ponds as potential introduction pathway of ornamental crayfish. Knowl. Manag. Aquat. Ec. **414:** 13.

Patoka J., Wardiatno Y., Yonvitner, Kuříková P., Petrtýl M. & Kalous L. 2016b. *Cherax quadricarinatus* (von Martens) has invaded Indonesian territory west of the Wallace Line: evidences from Java. Knowl. Manag. Aquat. Ec. **417:** 39.

Pedraza-Lara C., Doadrio I., Breinholt J.W. & Crandall K.A. 2012 Phylogeny and evolutionary patterns in the dwarf crayfish subfamily (Decapoda: Cambarellinae). PLoS One **7:** e48233.

Pedraza-Lara C., Doadrio I., Breinholt J.W. & Crandall K.A. 2012. Phylogeny and evolutionary patterns in the dwarf crayfish subfamily (Decapoda: Cambarellinae). PLoS One 7: e48233. DOI: 10.1371/journal.pone.0048233

Perdikaris C., Konstantinidis E., Georgiadis C. & Kouba A. 2017. Freshwater crayfish distribution update and maps for Greece: combining literature and citizen-science data. Knowl. Manag. Aquat. Ecosyst. 418: Article No. 51. DOI:10.1051/kmae/2017042

Svoboda J., Mrugała A., Kozubíková-Balcarová E. & Petrusek A. 2017. Hosts and transmission of the crayfish plague pathogen *Aphanomyces astaci*: A review. J. Fish. Dis. **40:** 127-140.

Turkmen G. & Karadal O. 2012. The survey of the imported freshwater decapod species via the ornamental aquarium trade in Turkey. J. Anim. Vet. Adv. **11:** 2824-2827.

Uderbayev T., Patoka J., Beisembayev R., Petrtýl M., Bláha M. & Kouba A. 2017. Risk assessment of pet-traded decapod crustaceans in the Republic of Kazakhstan, the leading country in Central Asia. Knowl .Manag. Aquat. Ec. **418:** 30.

Veselý L., Buřič M. & Kouba A. 2015. Hardy exotics species in temperate zone: can "warm water" crayfish invaders establish regardless of low temperatures? Sci. Rep. **5:** 16340.

Vodovsky N., Patoka J., Kouba A. 2017. Ecosystem of Caspian Sea threatened by pet-traded non-indigenous crayfish. Biol. Invasions **19:** 2207-2217.

von Petutschnig J., Honsig-Erlenburg W., Pekny R. 2008. Zum aktuellen Flusskrebs- und Fischvorkommen des Warmbaches in Villach. Carinthia II **198:** 95-102.

Weiperth A., Gál B., Kuříková P., Langrová I., Kouba A. & Patoka J. 2018. Risk assessment of pet-traded decapod crustaceans in Hungary with evidences of *Cherax quadricarinatus* (von Martens) in the wild. North-West J. Zool. **14**: e171303

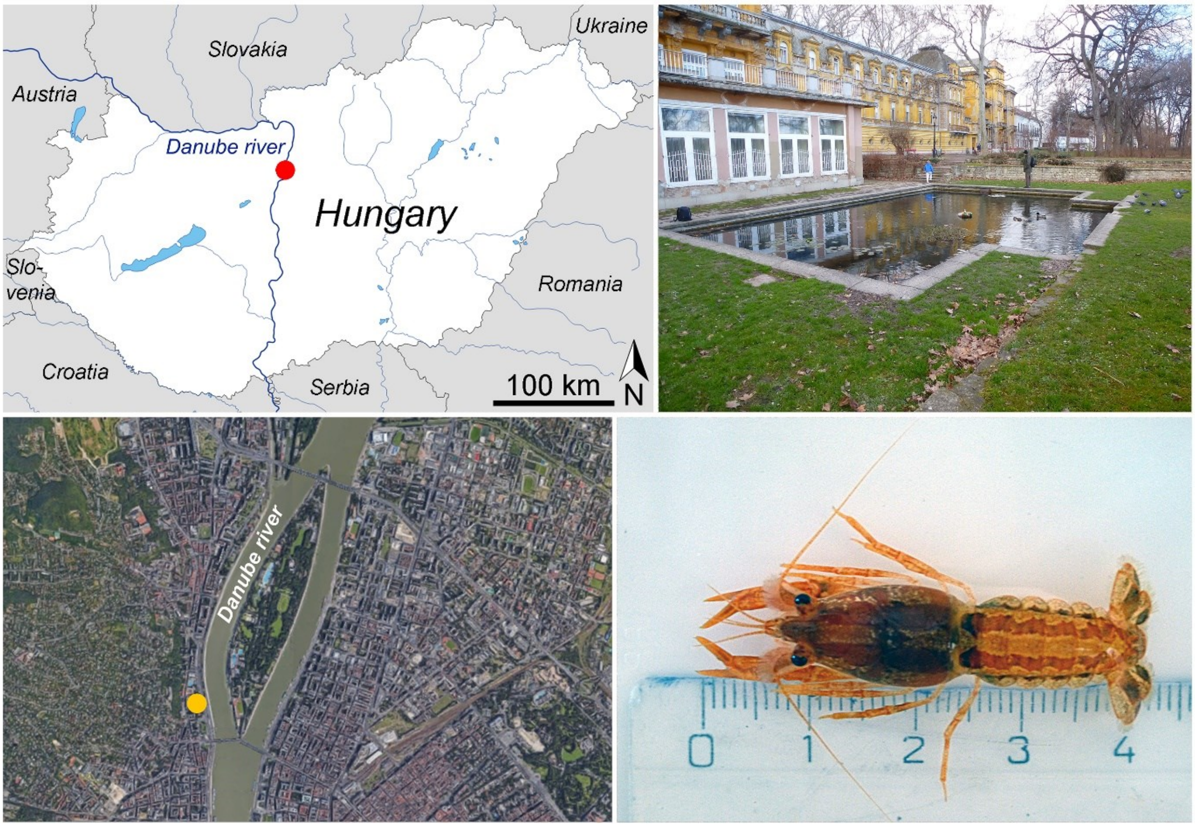

Fig. 1 Map showing the location of the thermal pond in Budapest, Hungary (indicated as coloured dots) with the view on the locality and an example of a captured adult *Cambarellus patzcuarensis* female.

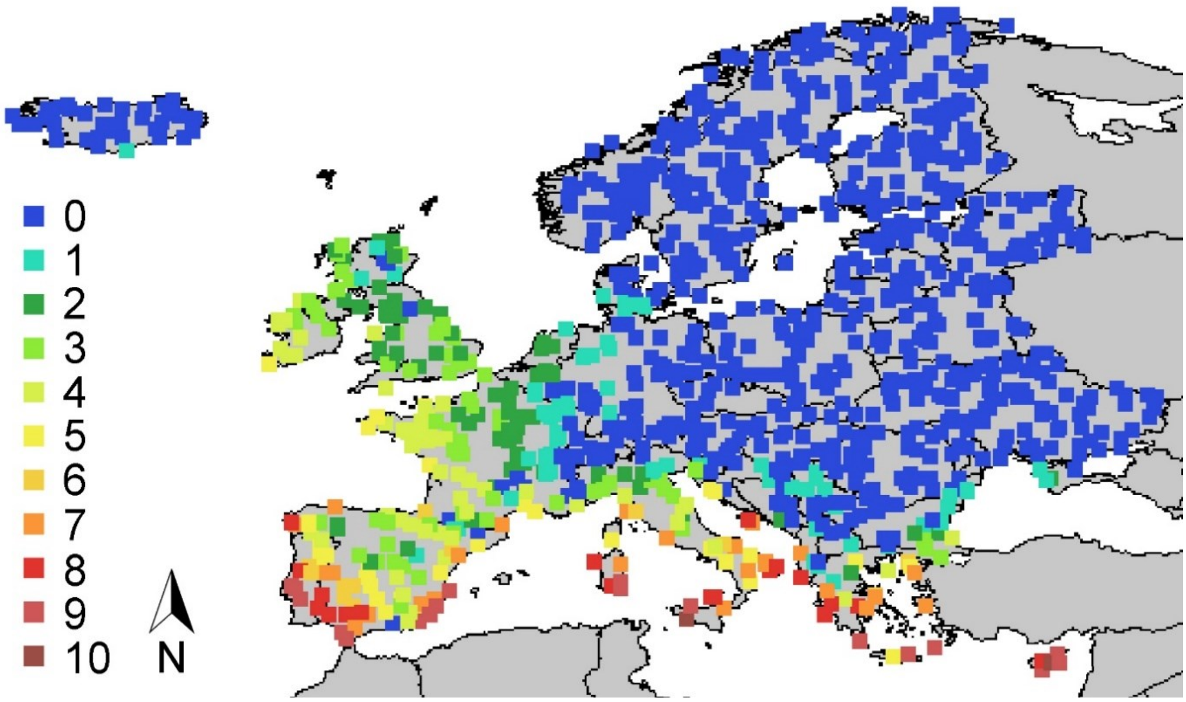

Fig. 2 Climate match map of Europe showing colour-coded regions with a different probability of establishment of *Cambarellus patzcuarensis*; scores of ≥ 7.0 were interpreted as there is no environmental barrier to survival.