Research Article

First populations of invasive red swamp crayfish flourish in Slovakia

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Abstract

The red swamp crayfish *Procambarus clarkii* is one of the most prominent invasive crayfish species in Europe. Here, we document the first recording of this species in Slovakia, including evidence of its occurrence in two natural localities, both fed by thermal springs. The first locality is near Turčianske Teplice in Central Slovakia, immediately downstream of a commercial culturing facility for aquatic ornamentals, while the other is located approximately 150 km southwards, close to Komárno. Both *P. clarkii* populations are well-established, with numerous individuals of both sexes, size classes, and ovigerous females. Although none of the 32 screened adult crayfish of either population tested positive for *Aphanomyces astaci*, the causative agent of the crayfish plague, its presence cannot be entirely excluded. So far, *P. clarkii* is confined to short stretches in both sites. However, its independent expansion into nearby watercourses can be expected. Despite the translocation of non-native species of Union concern, such as *P. clarkii*, being strictly forbidden, illegal human actions can facilitate their dispersal. Given the still relatively limited territory conquered by the invasive species in the two localities, urgent eradication and control measures were recently adopted. The ongoing eradication campaigns yielded thousands of *P. clarkii* individuals extracted from both localities in 2023. As thermal springs and thermally polluted waters are known hotspots of non-native and potentially invasive species, monitoring these sites is highly recommended.

Key words: biological invasions, crayfish plague, freshwater ecosystems, management, *Procambarus clarkii*

Introduction

The introduction of non-native species, particularly those with invasive potential, is one of the main threats to the world's biodiversity in the Anthropocene (Turvey and Crees 2019; Pyšek et al. 2020). Freshwater ecosystems are prone to a higher degree of species introductions than other ecosystems, often resulting in biodiversity decline, profound alteration of energy flows, and substantial changes to the physical environment (Gallardo et al. 2016; Havel et al. 2015; Lipták et al. 2019; Haubrock et al. 2020). In freshwater ecosystems, non-native engineering species, such as crayfish, are particularly impactful, as they alter the ecosystem on multiple levels (Reynolds et al. 2013; Kouba et al. 2016).

Crayfish are culturally and economically valuable animals, which have long been utilised in aquaculture (Oficialdegui et al. 2020a; Haubrock et al. 2021) and more recently, in the global pet trade business due to their aesthetic appeal (Faulkes 2015; Patoka et al. 2016; Chucholl and Wendler 2017). However, in many instances, non-native crayfish accidentally escape or are deliberately introduced into new natural environments, where they establish viable populations, spread, and often become problematic (Holdich et al. 2009; Kouba et al. 2014). In the past, non-native and potentially invasive crayfish species were introduced to European inland waters for food purposes, but nowadays, new species introductions are mainly linked to the pet trade (Weiperth et al. 2020; Jussila et al. 2021; Bláha et al. 2022), and Slovakia is not an exception (Lipták et al. 2016, 2017).

Many European native crayfish species have become endangered or locally extinct largely due to interactions with their non-native counterparts and a weak immune defence against the crayfish plague pathogen, *Aphanomyces astaci* Schikora, carried by North American crayfish species (Richman et al. 2015; Svoboda et al. 2016; Wiśniewski et al. 2020; Theissinger et al. 2022; Mojžišová et al. 2022). In Europe, one of the most widespread invasive crayfish species causing severe ecological impacts is the red swamp crayfish *Procambarus clarkii* (Girard, 1852) (Souty-Grosset et al. 2006; Oficialdegui et al. 2020a). Although this North American crayfish species is primarily associated with warm waters in the southern part of the continent (Kouba et al. 2014; Karaouzas et al. 2024), it can also find suitable habitats in thermal waters in central Europe, which helps the species to withstand low winter temperatures (Veselý et al. 2015; Haubrock et al. 2019; Maciaszek et al. 2019). Due to its impacts, the European Union (EU) has listed *P. clarkii* as an invasive species of Union concern (EU 2014, 2016). Consequently, keeping, selling, breeding, importing, translocating and releasing this species is strictly forbidden and should not be executed under any circumstances. The sightings of this species, as well as those of other suspected non-native crayfish, by the general public should be immediately reported to the appropriate institution (e.g., Slovak Nature Conservancy in the case of Slovakia) or experts in the field so early assessments and the necessary management actions can be swiftly undertaken.

Due to its use for aquaculture purposes and attractive colouration (ranging from reddish to various colour morphs in cultured individuals), *P. clarkii* has been introduced into over 40 different countries around the world, becoming one the most successful crayfish invaders worldwide (Oficialdegui et al. 2020a), and particularly in the European continent (Kouba et al. 2014). In Europe, this species was first introduced to southwestern Spain from where it expanded northwards, aided by multiple human-induced secondary introductions (Oficialdegui et al. 2019). Nowadays, the species is found throughout Europe, often in isolated populations, likely as a result of independent releases from various sources during recent decades (Oficialdegui et al. 2020b). *Procambarus clarkii* has become widely available in the pet trade in many European countries (Chucholl 2013; Patoka et al. 2015; Weiperth et al. 2020; Lipták et al. 2023b), including Slovakia (Lipták and Vitázková 2015), which is often a precursor to releases into the wild (Maciaszek et al. 2019; Battisti and Scalici 2020; Weiperth et al. 2020; Karaouzas et al. 2024). Unfortunately, legal actions against the unlawful release of non-native crayfish are rarely implemented and illicit releases and escapes are a common phenomenon (Patoka et al. 2018; Yuliana et al. 2021).

In this paper, we present the first reports of *P. clarkii* in thermal waters in Slovakia at two sites approximately 150 km apart. We describe the specific sites where it has been found and discuss possible introduction pathways, its likely further expansion, and the potential risks this invasion may entail if applied management actions fail.

Materials and methods

Description of the sites

The first site, named Čepčínsky Brook, is located near the town of Turčianske Teplice in central Slovakia (Figure 1), which is famous for its springs with warm water and thermal spas. The thermal brook is seven kilometres long and emerges at an elevation of 517 meters above sea level. The Čepčínsky Brook flows into the Turiec River, a tributary of the Váh River, which has a basin area of 934 m^2 and receives water from more than 70 streams. The temperature of the Čepčínsky Brook measured close to its spring on May 5, 2023 was 28.6 °C and decreased to 23.5 °C 300 m downstream. Inversely, pH increased from 7.2 in the uppermost part to 7.8. The site was inspected at three points (Point 1: 48°51′42.0″N; 18°50′18.8″E, Point 2: 48°52′04.6″N; 18°50′07.1″E and Point 3: 48°52′13.4″N; 18°49′17.7″E). Situated within the spring is a large facility (48°51′42.8″N; 18°50′17.9″E) dedicated to the cultivation and trade of aquatic ornamentals, especially warm-water fish (Figure 1). The inspected section of the brook is fully regulated with a uniform profile and follows a local road.

The second site (henceforth referred to as the “brook near Komárno”), is also a regulated thermal brook, which is situated between the towns of Komárno and Kolárovo in the southern part of Slovakia (Figure 2). The locality (47°51′27.3″N; 18°00′19.6″E) is situated at an elevation of 108 meters above sea level, in an agricultural landscape consisting of crop fields interspersed by irrigation canals. The brook is fed by a nearby natural thermal spring and flows into the Stará Částa stream. This stream then freely discharges into the Kolárovo-Kameničná canal, which is obstructed at its downstream end by a pumping station (47°50′57.4″N; 18°02′02.0″E;

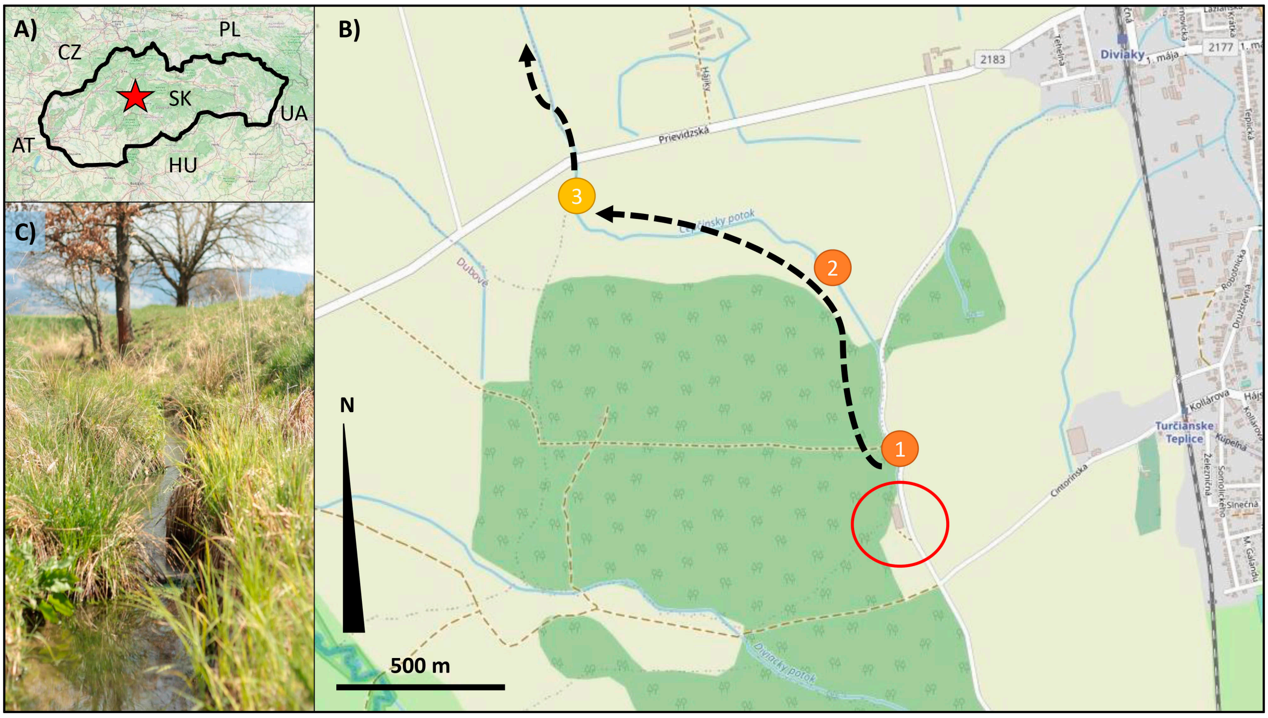

Figure 1. Details of the Čepčínsky Brook site. A) The map indicates the position of the study area at the national scale (background map downloaded from mapy.cz online portal). B) Study area where the red circle indicates the location of the commercial culturing facility, the orange dots (sampling points 1 and 2) indicate places where the *Procambarus clarkii* is currently present, the yellow dot (3) shows the section where the crayfish is still absent. Dotted lines with arrows represent the downstream route of the likely range expansion. C) Habitat in which the *P. clarkii* was found and collected. SK – Slovak Republic, CZ – Czech Republic, PL – Poland, AT – Austria, UA – Ukraine.

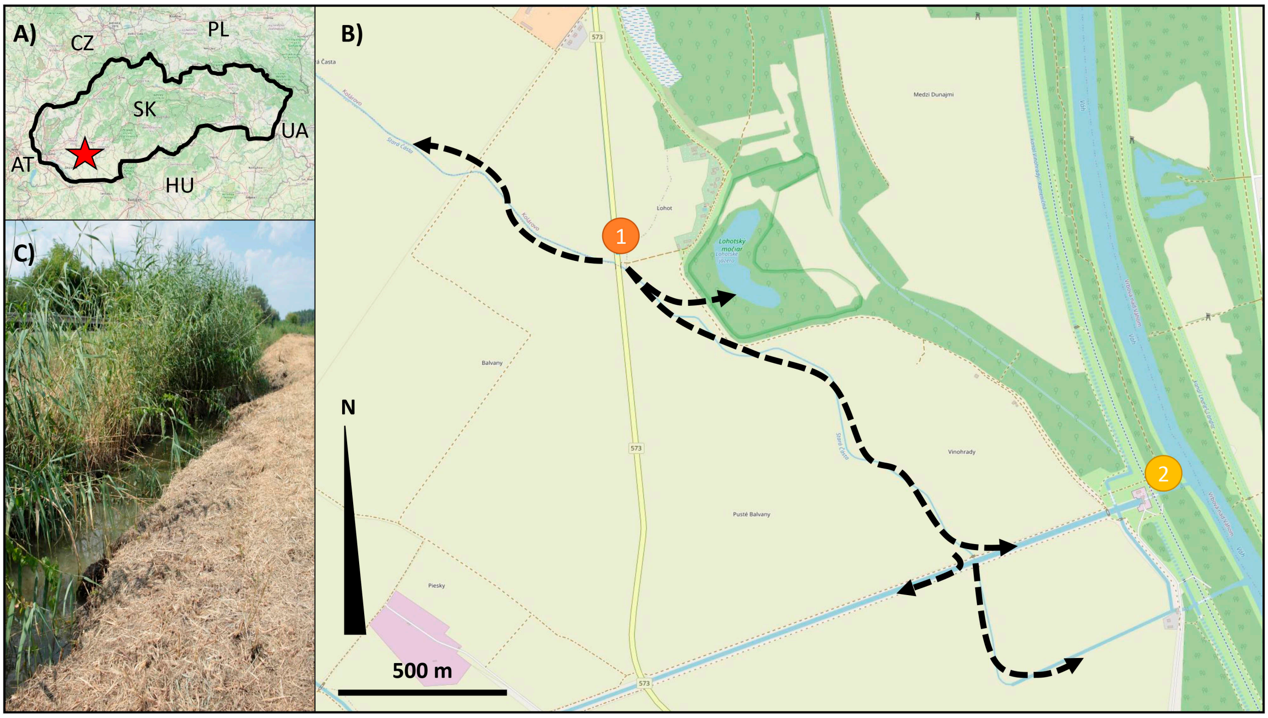

Figure 2. Details of the brook near Komárno site. A) The map indicates the position of the study area at the national scale (background map downloaded from mapy.cz online portal). B) Study area where the orange dot (sampling point 1) indicates the place where *Procambarus clarkii* was discovered, and the yellow dot (point 2) shows the second sampling point, where *P. clarkii* was still absent; dotted lines with arrows represent the likely routes of the species range expansion. C) Habitat in which the *P. clarkii* were collected. SK – Slovak Republic, CZ – Czech Republic, PL – Poland, AT – Austria, UA – Ukraine.

see Figure 2 for details). The pumping station is responsible for expelling excess water from the canal to the Váh River. The Kolárovo-Kameničná canal, like many others, is artificially connected to the Váh River and used to irrigate the surrounding agricultural land. The temperature of the brook on May 25, 2023, was 30.4 °C with a pH of 7.9.

Crayfish sighting and monitoring

Monitoring conducted on May 31, 2022, by the staff of the National Park Veľká Fatra (led by Mária Apfelová) confirmed the presence of the crayfish in Čepčínsky Brook. Subsequently, a barrage of investigations was launched at the locality to collect and analyse crayfish individuals in detail. Sampling took place on April 22, May 8, July 19, and August 28 and 29, 2023, and was stretched along three different sampling points indicated in Figure 1. On all occasions, the site was visited during the daytime, potential hiding places of the crayfish were inspected, and the crayfish were collected by a hand-held aquarist net. Minikin data loggers (Environmental measuring systems, Brno, Czech Republic) for the continuous monitoring of temperature were installed at the three sampling points at Čepčínsky Brook (Figure 1) on May 8, 2023, and temperatures were registered hourly until June 25, 2023.

The second site (Figure 2) was inspected for the first time on May 25, 2023, during an ichthyological survey performed by the staff of the Natural History Museum Bratislava (led by Ján Kautman), aiming at the detection of potential non-native fish species in thermal water bodies. Following the confirmation of crayfish presence by electrofishing (24 crayfish were captured, including 13 adult males, 10 adult females, and one juvenile crayfish), a second visit was organized on July 18, 2023, to inspect the site in detail. During the second visit, the location was inspected at two sample points during the day, and crayfish were collected by a hand-held aquarist net.

All captured crayfish individuals were morphologically identified based on the available literature (i.e., Souty-Grosset et al. 2006). Crayfish were measured (carapace length) using a calliper, weighed, and sexed. In addition, different crayfish tissues (see below) were preserved in 96% ethanol for subsequent molecular analyses targeting crayfish species identification and the possible presence of *A. astaci*.

Molecular analyses

DNA used for crayfish identification (3 individuals from Čepčínsky Brook and 20 from the brook near Komárno) was isolated from the ethanol-preserved muscle tissue, while that used for *A. astaci* screening (12 and 20 individuals, respectively) was isolated from mixed-tissue samples (soft abdominal cuticle, one uropod, an eye stalk, and a walking leg joint) using a modified salt precipitation protocol (Grabner et al. 2015). The final volume of the extracted DNA was dissolved in 100 uL of TE buffer. Molecular identification of crayfish was obtained with the universal eukaryotic primers pair LCO1490/HCO2198 (5'-GGTCAACAAATCATAAAGATATTGG-3' and 5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer et al. 1994) targeting the cytochrome *c* oxidase subunit I (COI). PCR conditions for the primer pair LCO1490/HCO2198 were as follows: 1-minute initial denaturation at 94 °C, followed by five cycles of 1 minute at 94 °C, 90 seconds at 45 °C, and 90 seconds at 68 °C, followed by further 40 cycles of 1 minute at 94 °C, 90 seconds at 50 °C, and 1 minute at 68 °C, with a final extension of 5 minutes at 72 °C. For the molecular detection of *A. astaci*, we used the primer pair 42/640 (5'-GCTTGTGCTGAGGATGTTCT-3' and 5'-CTATCCGACTCC GCATTCTG-3') targeting the internal transcribed spacer (ITS) regions and the 5.8 rRNA gene of *A. astaci* (Oidtmann et al. 2006). PCR conditions for the primer pair 42/640 were: 5 minutes of initial denaturation at 96 °C, followed by 45 cycles of 1 min at 96 °C, 1 min at 59 °C, 1 min at 72 °C, followed by a final extension step of 10 min at 72 °C. All PCR reactions consisted of 20 µL composed of 10 µL of 2 × AccuStart II PCR ToughMix (Quantabio), 0.5 µM of each primer, and one µL of DNA template. PCR products were sent to Microsynth Seqlab (Germany) for Sanger sequencing. Since *A. astaci* infection in non-symptomatic carriers may be below the limit of detection of conventional PCR, the results were validated at the Department of Ecology, Charles University, Prague, using an improved species-specific qPCR assay developed by Strand et al. (2023). This qPCR assay has similar sensitivity as the established *A. astaci* qPCR assay of Vrålstad et al. (2009), which is about tenfold more sensitive than the set of primers here used for conventional PCR (Tuffs and Oidtmann 2011).

Obtained sequences were quality-checked and edited using Geneious v2023.1.2 (Biomatters) and aligned with the MAFFT v7.490 algorithm with standard settings (Katoh et al. 2019). For the initial identification of hosts and pathogens, obtained sequences were compared against records contained in GenBank using megablast (Morgulis et al. 2008). Then, sequences belonging to *P. clarkii* were compared with those of Oficialdegui et al. (2019) to identify the corresponding haplotype(s). Since the 600 bp fragment of mtDNA was identical for all crayfish in both sites, we used only one mitochondrial sequence (i.e., haplotype) per site to construct a Maximum likelihood phylogenetic tree with bootstrap support values (1000 replicates) in IQ-Tree 2.2.0 (Minh et al. 2020). The TIM+F+G4 substitution model was selected based on Bayesian information criterion scores. The native noble crayfish *Astacus astacus* (Linnaeus, 1758) (Genbank accession number MW726635) was used as an outgroup.

Statistical analyses

Given the relatively low number of samples screened for *A. astaci*, an exact binomial probability estimation with a 95% confidence of interval was

Figure 3. Representative of *Procambarus clarkii* individual in the catch at the Čepčínsky Brook. Photo: Boris Lipták.

performed with the package *exactci* in the open-source software R (version 4.2.3) to estimate the probable infection rate among the background population at both sites.

Results

At the first site (Čepčínsky Brook), all the crayfish (Figure 3) were observed upstream in the section containing the warmer water (i.e., sampling points 1 and 2), while we did not find any crayfish in the colder water occurring downstream at sampling point 3 in any of the samplings conducted in 2023 (see Figure 1 and Figure 4). The second site (brook near Komárno) was investigated at two points, with the presence of the crayfish confirmed only at the thermally influenced section (sampling point 1). Point 2, located behind the pumping station, was also inspected for crayfish presence, with negative results (see Figure 2 for details).

Altogether 88 crayfish individuals were collected from the two locations. Of these, 47 were females, and 41 were males. Overall, 54 individuals were captured from Čepčínsky Brook (28 males and 26 females) and 34 from the brook near Komárno (13 males and 21 females). The relationships between the carapace length and the body weight of crayfish (males and females separately) for both sites are represented in Figure 5. The mean weight of the individuals was around 10 g at both sites, with females being slightly heavier on average, and some individuals exceeding 20 g (Table 1).

Between August 28 and 29, 2023, at the Čepčínsky Brook, three females carrying juveniles were captured, one of which had 209 juveniles attached. Three depigmented and completely white crayfish individuals were also observed and collected from the site during the last visit.

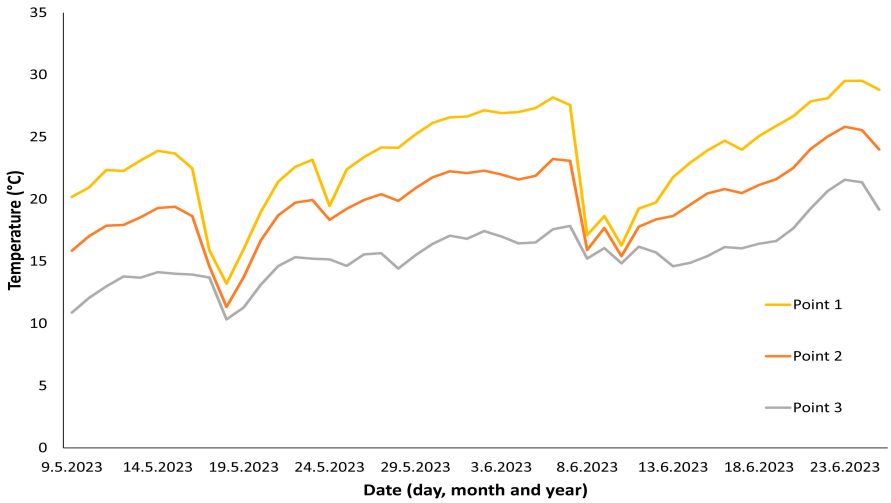

Figure 4. Continuous temperature monitoring at the Čepčínsky Brook. Temperature monitoring points correspond with the sampling points (see Fig. 1).

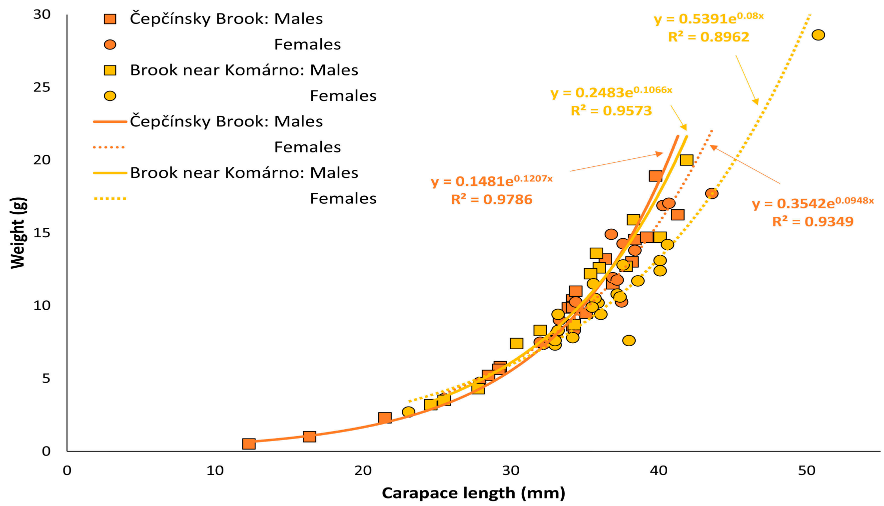

Figure 5. Relationship between carapace length (mm) and weight (g) in male and female *Procambarus clarkii* individuals obtained from the first site – Čepčínsky Brook and the second site – Brook near Komárno in Slovakia.

Table 1. Sampling size, sex, and biometry of captured *Procambarus clarkii* individuals in both Slovak localities (April 22, May 8, and July 18–19, 2023).

Sampling site	No.	Sex	CL (mm) ± SD	Weight (g) ± SD
Čepčínsky Brook	28	Male	31.1 ± 7.2	9.6 ± 5.0
	26	Female	31.5 ± 6.9	11.0 ± 4.3
Brook near Komárno	13	Male	33.8 ± 5.4	10.5 ± 5.1
	21	Female	36.3 ± 5.0	10.7 ± 4.8

DNA barcodes confirmed that the collected individuals (Genbank accession number OR462185 and OR462186) were *P. clarkii* with 100% similarity to haplotype 4 (Genbank accession number MK026674) *sensu* Oficialdegui et al. (2019), a central haplotype with a worldwide distribution (Figure 6). On the other hand, none of the individuals screened with the conventional PCR and qPCR were positive for *A. astaci*. We estimated the 95% confidence interval of the infection prevalence by *A. astaci* in the screened populations up to 27% in the Čepčínsky Brook and 17% in the brook near Komárno.

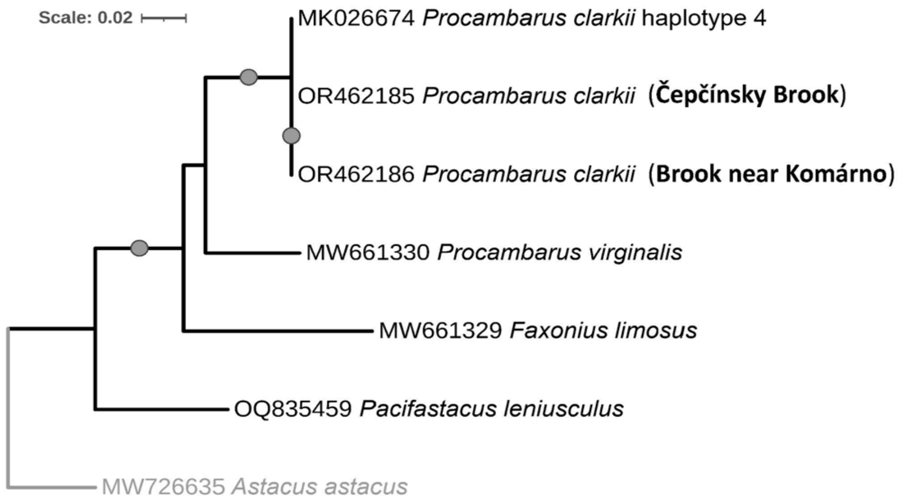

Figure 6. Maximum likelihood phylogenetic tree including *Procambarus clarkii* found in this study, and three other invasive crayfish already established in Slovakia, namely *P. virginalis*, *Faxonius limosus*, and *Pacifastacus leniusculus*. The native *Astacus astacus* was used as an outgroup. The phylogenetic tree was constructed using an IQ-Tree 2.2.0 and the TIM3 + F + G4 substitution model. The grey dots represent bootstrap branch support values (1000 replicates) above 90%.

Discussion

The North American *P. clarkii*, reported in this study, is the fourth invasive crayfish species recorded in Slovakia, after the discovery of established populations of marbled crayfish *P. virginalis* (Lyko, 2017) in 2010 (Janský and Mutkovič 2010), previously established spiny-cheek crayfish *Faxonius limosus* (Rafinesque, 1817) (Janský and Kautman 2007) and signal crayfish *Pacifastacus leniusculus* (Dana, 1852) (Petrusek and Petrusková 2007). Thus, invasive crayfish species now outnumber their three native counterparts, namely the *A. astacus*, narrow-clawed crayfish *Pontastacus leptodactylus* (Eschscholtz, 1823), and stone crayfish *Austropotamobius torrentium* (von Paula Schrank, 1803) (Lipták 2013; Lipták et al. 2016, 2017). The presence of the aforementioned invasive crayfish species will likely further exacerbate the decline of native crayfish, e.g., the narrow-clawed crayfish, whose populations are still present mainly in southern parts of Slovakia, where conditions such as temperature, are best suited to the requirements of *P. clarkii* (Stloukal and Gruľa 2013; Lipták 2014).

Our molecular analyses did not enable us to trace the introduction routes of these individuals, as all sequenced crayfish shared the most common haplotype both in native and non-native range. The presence of a single haplotype, namely haplotype 4 *sensu* Oficialdegui et al. (2019), suggests a common origin for both sites, which might be related to the crayfish originally available in the pet trade (Lipták and Vitázková 2015). For instance, the species is already established in neighbouring Poland (Maciaszek et al. 2019), Austria (Kouba et al. 2014) and thrives especially in Hungary (Mojžišová et al. 2024). In Europe, this haplotype was broadly found over the Iberian Peninsula, South France, and Italy, less so in the central and western parts of Europe, where haplotype 11 was more prevalent (Oficialdegui et al. 2019). Although an extensive screening of pet traded and feral *P. clarkii* populations in central and eastern Europe is ongoing (Oficialdegui et al. *in prep*.), the singular presence of haplotype 4 in Slovakia is somewhat unexpected. Natural barriers such as mountain ranges and lack of connectivity with the Iberian, French, and Italian populations suggest human-induced translocation or escapes from culturing facilities. Accordingly, *P. clarkii* found in Čepčínsky Brook likely escaped from a nearby aquaculture facility dedicated to farming and selling aquatic ornamentals. However, we cannot entirely rule out that the presence of the crayfish in the Čepčínsky Brook may be linked to individual introductions of crayfish by enthusiasts. The crayfish individuals found in the brook near Komárno may, with high probability, be the result of an illegal release(s) of *P. clarkii* by hobbyists.

The *P. clarkii* specimens at both localities have established self-sustaining populations. This is supported by the presence of numerous juvenile and mature individuals, including ovigerous females. Later investigations discovered that local citizens had been aware of the occurrence of unspecified crayfish species in the Čepčínsky Brook since 2019, but no relevant authorities were informed to investigate the situation in greater detail. Since both populations are likely to be present in the respective areas for some time, *P. clarkii* may have been already illegally translocated by humans to other water bodies. In addition to secondary introductions facilitated by humans and natural spread primarily downstream, an expansion of *P. clarkii* can also be facilitated by other dispersal vectors such as birds, by attaching to their feet and feathers (Anastácio et al. 2014), or via overland movements (Thomas et al. 2019). The species' presence in warm water conditions with exposure to cold water over time may facilitate adaptation to cold water by increasing tolerance or acquiring behavioural properties as documented in the case of another warm-water pet pet-traded crustacean in Germany (Schneider et al. 2022).

The introduction of this species into other sites may occur due to often limited knowledge of the crayfish fauna, specifically, the inability to discern between invasive and native species and the common belief among the general public that all crayfish species are native (perceived cultural value) and thus protected by law. Moreover, crayfish are generally perceived as aesthetically attractive and indicators of good water quality, and thus, considered suitable candidates to be introduced into freshwater environments (Lipták et al. 2023a). The spread of *P. clarkii* toward the Váh River, the longest and arguably most important river in Slovakia (besides the Danube River), is highly concerning as native crayfish populations of *A. astacus* inhabit its tributaries (Janák et al. 2015). Furthermore, reaching the Váh River would also provide a gateway to the Danube River and other native crayfish populations along its basin, similarly as is the case with the ongoing invasion of the *P. virginalis* (Lipták et al. 2016, 2017).

Invasive crayfish species themselves have the potential to outcompete native counterparts and cause substantial environmental damage (McCarthy et al. 2006; Scordo et al. 2023). Furthermore, associated pathogens, such as *A. astaci*, might have negative consequences when introduced alongside their hosts (Svoboda et al. 2014; Ungureanu et al. 2020). *Procambarus clarkii* is a well-known carrier of the crayfish plague pathogen (Svoboda et al. 2016; Putra et al. 2018), which can decimate local populations of native crayfish stocks (Mrugała et al. 2015; Mrugała et al. 2017; Mojžišová et al. 2020; Casabella-Herrero et al. 2023). Although none of the 32 screened *P. clarkii* individuals resulted positive for the oomycete *A. astaci*, we cannot exclude its presence from the investigated populations because a low *A. astaci* prevalence is not uncommon in European *P. clarkii* populations (Tilmans et al. 2014; Sieber et al. 2022). Although none of the analysed specimens tested positive for *A. astaci*, the wide 95% confidence intervals (up to 27% in the Čepčínsky Brook and 17% in the brook near Komárno) indicate that populations cannot be declared free of the crayfish plague pathogen without a higher sampling effort (Schrimpf et al. 2013). So far, *P. clarkii* seems to be confined to small, restricted areas (i.e., brooks and canals); however, spores of *A. astaci* might disperse downstream or be transported by animals feeding on fish and crayfish, potentially spreading them in the broader region (Kozubíková et al. 2009; Svoboda et al. 2020). For example, *P. clarkii* and other invasive crayfish species infected by different *A. astaci* haplogroups have been documented in Hungarian regions (Mojžišová et al. 2024). Although crayfish plague outbreaks have not been extensively monitored in Slovakia, the known non-native crayfish plague carriers in the country are the signal crayfish *P. leniusculus* (Kozubíková et al. 2011) and the spiny-cheek crayfish *F. limosus* (Lipták et al. 2017). Closer monitoring including environmental DNA is therefore imperative (Mojžišová et al. 2024).

The current findings highlight the role of naturally thermal and thermally polluted water in the establishment of invasive species in temperate-cold environments and the need for their close monitoring. These habitats are known hotspots of non-native biodiversity in Europe, where warm-adapted pet trade-linked biota, including their parasites, find a suitable environment (Emde et al. 2016; Maciaszek et al. 2019; Weiperth et al. 2020; Bláha et al. 2022). This might facilitate the adaptation of warm water species (Vodovsky et al. 2017) and the associated pathogens to the colder environment of the surroundings (Schneider et al. 2022), which could become a reason for concern. A warming climate might also play a significant role by enhancing non-native species invasive potential through increased water temperatures in the coldest months (Kouba et al. 2021; Bohatá and Patoka 2023).

Education of the general public (Lipták et al. 2024) and regular surveys of potential introduction hotspots are highly recommended. Furthermore, there is an imperative need to prevent the escape of non-native species into the natural environment from facilities. Legal actions must be more rigorously implemented, as the breeding of invasive crayfish species listed under Union concern is strictly forbidden in the EU and their member states (Jussila and Edsman 2020; Schwindt et al. 2024). This is because of their detrimental impacts on the invaded environment accompanied by usually ineffective time- and resource-demanding eradication actions, overall leading to severe monetary costs (Krieg et al. 2020; Kouba et al. 2022). Thus, preventative actions, when accurately done, remain the best way to deal with invasive crayfish species.

The recent finding, coupled with the restricted size of both brooks and the relative isolation of the *P. clarkii* populations, renders the sites suitable for the eradication of this invasive species. Alongside the described monitoring, the Veľká Fatra National Park Administration proceeded with the regular monitoring and intensive removal of *P. clarkii* at the Čepčínsky Brook, and by the end of 2023, more than 12,000 individuals were removed, yet many still remain at the site (State Nature Conservancy of the Slovak Republic 2023a). After the discovery of the second population at the brook near Komárno, a similar eradication effort was undertaken, resulting in the removal of over 3,000 crayfish individuals by the end of November 2023 (State Nature Conservancy of the Slovak Republic 2023b). As seen, the eradication of introduced populations of invasive crayfish from the natural environment is not an easy task and thus, a more drastic and large-scale intervention is required, ideally with the support of local citizens and stakeholders.

Conclusions

In conclusion, the establishment of *P. clarkii* populations in Slovakia is another example of our societal failure to prevent new introductions of invasive species. It highlights the necessity to closely monitor relevant introduction pathways and enhance the recognition of responsible pet ownership. Well exemplified by the case of *P. clarkii* in the Čepčínsky Brook, a broader involvement of citizens in reporting the occurrence of suspicious species is critical in the timely action of responsible authorities. Considering the possible negative impacts of *P. clarkii* on the native biota, the presence of a national park near the Čepčínsky Brook, and the rich faunal and flora elements of the Váh River and nearby water-bodies of the brook near Komárno, a continuation the ongoing management activities aiming to eradicate both crayfish populations is imperative. Moreover, we strongly recommend an education campaign aimed at the sensibilization of the general public on the issues related to biological invasions.

Authors' contribution

BL: research conceptualization, sample design and methodology, investigation and data collection, data analysis and interpretation, writing – original draft, writing – review and editing; SP: sample design and methodology, data analysis and interpretation, writing – review and editing; FJO: investigation and data collection, data analysis and interpretation, writing – review and editing; MA: investigation and data collection; SP: investigation and data collection; JK: investigation and data collection; VJ: investigation and data collection; AK: research conceptualization, data analysis and interpretation, sample design and methodology, writing – review and editing.

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