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Marbled crayfish *Procambarus virginalis* invades a nature reserve: how to stop further introductions?

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Abstract

We hereby provide the first documented data on the occurrence of a viable *Procambarus virginalis* population in a nature reserve in Poland and the steps that were taken to prevent further introductions of the species in the country. To date, Poland represents the most north-eastward distribution area in Europe, where the species occurs in ecosystems with natural water temperature regimes. The ecological plasticity of *P. virginalis* and its obligate parthenogenetic reproduction make this crayfish an exceptional invader. The crayfish may have detrimental effects on the native invertebrates, amphibians, and fish, and alter the state of the entire ecosystem. Therefore, we investigated the presence of the species in the nature reserve of Pojezierze Łęczyńskie Landscape Park after a suspected *P. virginalis* individual was found by a local citizen. The nature reserve also includes an area designated to protect native amphibians and turtles. Our study revealed the presence of a thriving population of *P. virginalis* in the protected area and clear indications of its impact on native and invasive species, both in aquatic and terrestrial environments. If no action is taken, the species will likely expand to other water bodies across the country. An extensive sensibilization campaign allowed us to find additional invaded areas and significantly contribute to the effective ban of this and other invasive crayfish species from private and commercial online trade. Raising public awareness and banning invasive crayfish species trade is essential for successfully detecting and preventing further introductions.

Keywords: Alien species, freshwater crustacean, ornamental pet trade, inland waters, Central Europe

Introduction

Biological invasions have severely affected global biodiversity, particularly over the course of the last centuries (Catford et al. 2012; Gallardo et al. 2016; Pyšek et al. 2020). Human activities primarily mediate the introduction of species outside their native ranges, and the number of translocated species is globally increasing (Savini et al. 2010; Early et al. 2016; Seebens et al. 2017). This often results in the establishment of non-native and potentially invasive species. Aquatic ecosystems are particularly vulnerable and are often invaded by multiple alien species that interact with native biota

and alter the ecosystem through additive and multiplying effects (Hudina et al. 2011; Jackson et al. 2015). Moreover, widespread habitat degradation and climate warming are likely to enhance the adverse impact of invasive species (MacDougall & Turkington 2005; Walther et al. 2009).

Invasive crayfish, besides their biological traits, such as rapid growth, fecundity, and tolerance to environmental stress, are among the most destructive of invasive species, with their impact affecting multiple ecological levels of invaded ecosystems (Lodge et al. 2012; Twardochleb et al. 2013;

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Lipták 2019). Decline and extinction of native crayfish through direct and indirect competition, and transmission of the crayfish plague, a disease caused by the oomycete *Aphanomyces astaci*, are among the most alarming effects of non-native crayfish introductions (Lodge et al. 2012; Twardochleb et al. 2013; Svoboda et al. 2017; Ungureanu et al. 2020). Crayfish are generalist omnivores; thus, they may severely affect primary and secondary production (Perry et al. 2000). Moreover, they are ecosystem engineers. Through their grazing and burrowing activity, they increase the rate of leaf-litter breakdown, nutrient cycling, and coarse sediments transport, which coupled with a reduction of benthic algae and macrophyte cover could alter the state of the ecosystem from clear to phytoplankton dominated turbid water (Bobeldyk & Lamberti 2008; Matsuzaki et al. 2009; Johnson et al. 2011; Twardochleb et al. 2013). Crayfish predation on native invertebrates, fish, and amphibians in concomitance with habitat alteration often causes a decline in diversity and abundances of native species (Kats & Ferrer 2003; Correia & Anastácio 2008; Twardochleb et al. 2013). Invasive crayfish are often carrier of pathogens such as *A. astaci* which is lethal for native species (Svoboda et al. 2017), or the chytrid fungus *Batrachochytrium dendrobatidis*, which is pathogenic for amphibians (Oficialdegui et al. 2019) as well as other non-native epibionts including branchiobdellidans, temnocephalans and ostracods (Ohtaka et al. 2005; Aguilar-Alberola et al. 2012; Du Preez & Smit 2013). Hence, non-native crayfish are a major threat to freshwater biodiversity and ecosystem functioning (Twardochleb et al. 2013; Gallardo et al. 2016; Pyšek et al. 2020).

In Europe, invasive crayfish are spreading at alarming rates. There are numerous non-native crayfish species with established populations in central and western Europe and others may follow in the near future (Kouba et al. 2014; Weiperth et al. 2017; Collas 2019; Haubrock et al. 2021). Among them, the marbled crayfish *Procambarus virginalis* Lyko, 2017 formerly known as *Procambarus fallax* (Hagen, 1870) f. *virginalis*, is the only decapod crustacean that reproduces by obligate parthenogenesis (Scholtz et al. 2003; Kozák et al. 2015). Nowadays, this crayfish species is well established also outside Europe, in countries such as Madagascar (Andriantsoa et al. 2019) and Japan (Usio et al. 2017). The settling of *P. virginalis* in the wild results from intentional or unintentional releases (Patoka et al. 2017; Hossain et al. 2018). This North American species is one of the most common ornamental crayfish in the pet trade (Patoka et al. 2014; Faulkes 2015; Gutekunst et al. 2021). Despite being

listed as an invasive alien species, *P. virginalis* is still present in aquarium trade within the European Union (EU), which banned the species in 2016. Similarly, the invasive *Procambarus clarkii*, is regularly sold as a popular ornamental species in European pet shops or via Internet (Patoka et al. 2014), including Poland (Maciaszek et al. 2019). Likewise, these species remain broadly available in neighboring non-EU countries such as Ukraine and Russia, where the trade of these decapods has not been yet restricted by law (Kotovska et al. 2016; Vodovsky et al. 2017). The EU Regulation 1143/2014 on invasive alien species requires the notification of the mentioned species for early detection and/or eradication. However, no information had been received by the Notification System of European Alien Species Information Network (EASIN Notsys), and no official monitoring or eradication has been performed to date in Poland, although both *Procambarus* species have been already detected. The invasive *P. virginalis* was first reported in Szczecin (north-west Poland) in 2009. Afterward, other observations followed, mostly of single individuals (Śmietana et al. 2018). The first breeding population was observed in 2019 in the semi-natural pond of Morskie Oko park in Warsaw (Mazurska et al. 2019), where thousands of crayfish have already been caught under Łowca Obcych/ Alien Hunter initiative (Maciaszek 2022). Fortunately, this semi-natural pond is well isolated from other water bodies and to date no overland dispersion of the species has been observed.

The invasive *P. virginalis* is one of major concerns due to its rapid spread, dominance over native and some invasive crayfish species, predation on native invertebrates and vertebrates, the spread of *A. astaci*, and habitat alterations through grazing and burrowing activity (Lipták 2019; Hossain et al. 2020; Kouba et al. 2021). Moreover, *P. clarkii* is a more efficient amphibian predator than European native crayfish species (Gherardi et al. 2001). It could potentially transmit *B. dendrobatidis* and exclude amphibians from potentially suitable reproductive areas as observed in the Iberic peninsula (Cruz et al. 2006; Oficialdegui et al. 2019). For instance, *P. clarkii* was responsible for the disappearance of over half of the amphibian species in a nature reserve in Portugal (Cruz et al. 2008) and the local extinction of tadpole in northern Italy, including the threatened *Rana latastei* (Boulenger, 1879) (Ficetola et al. 2011). Thus, we can expect similar impacts in the case of *P. virginalis* invasion.

In this study, we investigated the presence of *P. virginalis* in the Pojezierze Łęczyńskie Landscape Park (Poland), after being notified by a local citizen

who found the suspected crayfish in the proximity of the Nature Reserve of Ostoja Parczewska. This nature reserve also includes an area designated for the protection of the European fire-bellied toad, *Bombina bombina* (Linnaeus, 1761), an amphibian whose population have declined across the continent (De Cahsan et al. 2019), as well as the European pond turtle, *Emys orbicularis* (Linnaeus, 1758). The presence of native *Astacus astacus* (Linnaeus, 1758), the noble crayfish has also been reported in a nearby area. Our investigation aimed to assess if *P. virginalis* established the first viable population in a Polish nature protected area and its potential impact on native species. Moreover, we share the action taken following our findings as an example that could be implemented elsewhere to prevent further introductions of invasive crayfish species.

Material and methods

Locality

Pojezierze Łęczyńskie Landscape Park is located in eastern Poland and is a part of Łęczyńsko-Włodawskie Lakeland. It is characterized by a relatively flat topography with lakes differing in water purity, shape, trophy, and vegetation type. Bogs and ponds often surround the lakes. On the east side, the park borders the Polesie National Park, which is also protected under the Ramsar Convention. This territory is undoubtedly one of the most valuable natural areas in Poland and is also known for its aquaculture ponds.

Miejskie Lake (N 51.5077, E 22.8847) is a natural shallow lake with a surface area of 49 ha and a depth of ca. 2 m, located near Ostrów Lubelski city in the eastern part of Pojezierze Łęczyńskie Landscape Park. The bottom of the lake is silty and sandy. The lake has no inflow of surface waters. However, it is connected with the neighboring Kleszczów Lake via amelioration ditches. It is surrounded by wet meadows and wetlands with overgrown trees and a pine forest. Miejskie Lake is protected for ecological use and is a known place for recreational activities, including fishing.

Kleszczów Lake (N 51.5213, E 22.8932) is a natural, mesotrophic lake with a surface area of 54 ha and a depth of ca. 3 m, also situated in Pojezierze Łęczyńskie Landscape Park. The bottom of the lake is silty and sandy. Wetlands and pine forests surround it. The lake and its immediate surroundings are included in the Special Area of Conservation Ostoja Parczewska PLH 060013 and the Special Protection Area of Lasy Parczewskie PLB 060006. This Natura 2000 habitat zone also

includes an area designated for the protection of *B. bombina* as well as *E. orbicularis*. Same as Miejskie Lake, this ecosystem is protected for ecological use. On the southern part, Kleszczów Lake is connected with the Miejskie Lake, while in the northern part is connected with the Bobrówka river via amelioration ditches. The Bobrówka river is a tributary of the Tyśmienica river, which is a part of the Vistula river basin.

The melioration ditches connecting both lakes and the Kleszczów Lake with the Bobrówka river seem to be neglected remains of past melioration works. They are often dry or covered by swamp vegetation and tree branches, and only in the aftermath of heavy rainfall water fill them up. Amphibians, mainly frogs and toads, widely inhabit these ditches.

Sampling sites designation

Following observations carried out by local citizens in June and July 2020, we investigated the area to assess the possible origin and migration pathways for *P. virginalis* on July 28–29, 2020. All crayfish observed by locals have been found in holiday plots located at the Miejskie Lake. In each case, they were found after heavy rainfalls that occurred in the second part of June and the beginning of July 2020. Most crayfish were found dead or alive on plot lawns and local roads, mainly near the forest. Initial consultations with local anglers confirmed the presence of the species and revealed that no other crayfish than *P. virginalis* had been deliberately introduced in Miejskie Lake. According to local anglers, *P. virginalis* was introduced there approximately three years earlier as a food source for native predatory fish that had been overfished and competitively displaced by the invasive brown bullhead, *Ameiurus nebulosus* (Lesueur, 1819). The population was then kept by anglers in good condition by immediate releasing caught crayfish to the lake. Locals also mentioned the potential occurrence of the spiny-cheek crayfish *Faxonius limosus* (Rafinesque, 1817) and an unidentified crayfish (most likely the native *A. astacus* or the narrow-clawed crayfish *Pontastacus leptodactylus* (Eschscholtz, 1823) known from neighboring water bodies in the region) in both lakes as well as Bobrówka river.

Sampling was conducted in Ostrów Lubelski (7 sampling sites) and Warsaw (1 sampling site) (Figure 1). The sampling sites in Ostrów Lubelski were designated on the basis of reports from the local community and included lakes and ditches (Figure 1, Table I). We sampled along the shoreline of Miejskie (1) and Kleszczów (6) lakes using transects of 100 m in length and 4 m in width (half open

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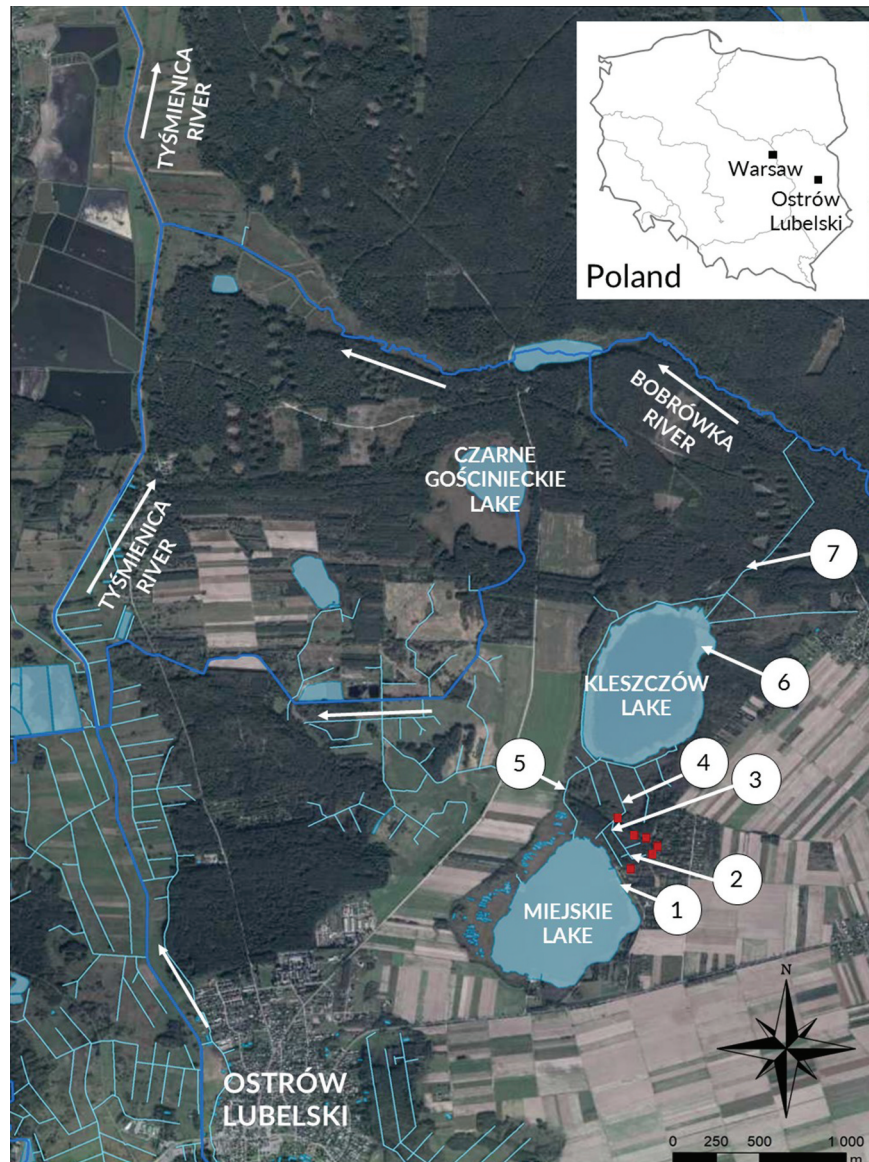


Figure 1. Sampling locations where *Procamburus virginalis* was detected by local citizens prior to our survey (red squares) in Ostrów Lubelski. Highlighted water bodies represent lakes and ponds (light blue areas), rivers (dark blue lines), and amelioration ditches (light blue lines).

Other transects of 200 m in length and 2 m in width were used in wet (3, 5, 7) and dry (2, 4) ditches. Individuals collected in Warsaw were used as the reference for morphological and genetic analyses.

Crayfish detection and identification

Live crayfish were caught in water using hydrobiological nets or were gathered by hand during day and night time on August 1–2, 2020. On land, they were tracked following dead crayfish bodies as well as indications of their activities, like burrows or migration channels. When found, they were hand-

gathered or excavated from the burrows. All collected specimens were then measured using total and carapax lengths (Kozák et al. 2015). Further evaluated parameters included incidence of pre- and post-molting individuals and egg-carrying females. Species identity was confirmed by combining morphological (Kozák et al. 2015) and molecular methods.

The DNA was isolated from the muscle tissue of six *P. virginalis* collected in Ostrów Lubelski and Warsaw, using the Chelex method (Casquet et al. 2012). The COI (cytochrome oxidase I) fragment was amplified with the primer pair LCO JJ and

Table I. Sampling sites examined for crayfish presence.

	No.	Type/Name	Coordinates	Crayfish presence
Ostrów Lubelski	1	Miejskie Lake	N 51.5077	Detected
	2	Dry ditch	N 51.5084	Detected
	3	Wet ditch	N 51.5099	Detected
	4	Dry ditch	N 51.5107	
	5	Wet ditch	N 51.5120	
	6	Kleszczów Lake	N 51.5213	Detected
	7	Wet ditch	N 51.5235	
Warsaw	-	Morskie Oko	N 52.2063	Detected

HCO JJ (Astrin & Stüben 2008) in PCR reaction following the protocol by Hou et al. (2007). The PCR products were purified with exonuclease I (Exo I) and alkaline phosphatase (FastAP) and then sent for sequencing to Macrogen Inc., Europe. Obtained sequences were blasted against GenBank records (Altschul et al. 1990). The sequences were aligned and trimmed to the same length of 624 bp using Geneious 10.0.2 software (Kearse et al. 2012). Obtained sequences were deposited in the BOLD Systems (Ratnasingham & Hebert 2007), as well as in GenBank (Benson et al. 2005) with accession numbers MW661998, MW662001, MW662002 (*P. virginalis* from Warsaw) and MW661999, MW662000, MW662003 (*P. virginalis* from Ostrów Lubelski). The COI haplotypes were designated in DnaSPv5 software (Librado & Rozas 2009). The phylogenetic tree based on the HKY model (Hasegawa et al. 1985) was constructed with the Maximum Likelihood (ML) method in MEGA X software (Kumar et al. 2018). The validity of nodes was estimated with the bootstrap test (1000 replicates) (Felsenstein 1985). To supplement the tree following sequences were taken from GenBank collection: *P. virginalis* of the accession number LR884234, deposited by Halfmaerten (unpublished), *P. virginalis* (acc. no. HM358012 and HQ171459) deposited by Martin et al. (2010), *P. virginalis* (acc. no. LC228303) deposited by Usio et al. (2017), and *P. clarkii* (acc. no. KT959364) deposited by Aguilar et al. (unpublished).

Statistical analysis

The total and carapax length of *P. virginalis* collected at different sites were expressed as mean \pm SD (mm). The significance of differences between these groups was assessed using the analysis of variance (ANOVA, post hoc tests). All statistical calculations were performed using IBM SPSS Statistics 27.

Results

A total of 715 crayfish were collected in Ostrów Lubelski sites, of which 714 were identified as *P. virginalis* and a single individual as *F. limosus* (Figure S1). All specimens were females. The vast majority of *P. virginalis* (84.90%) were collected in a wet ditch (Figure 2), while the remaining individuals were collected in a dry ditch (13.01%) as well as along the shoreline of Lake Kleszczów (1.96%) and Lake Miejskie (0.13%). The only specimen of *F. limosus* was collected along the shoreline of Lake Kleszczów. No native crayfish has been captured or

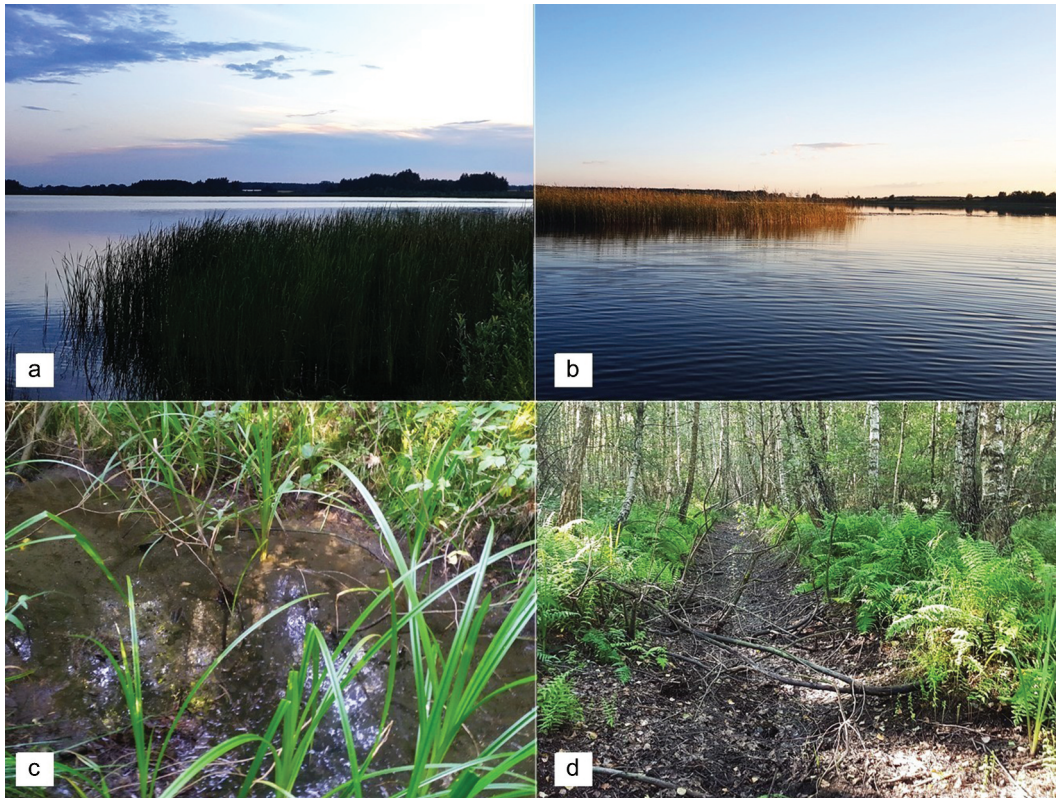


Figure 2. Locations in Ostrów Lubelski where live specimens of *Procambarus virginalis* were detected: Miejskie Lake – 1 (a), Kleszczów Lake – 6 (b), wet ditch – 3 (c), dry ditch – 2 (d).

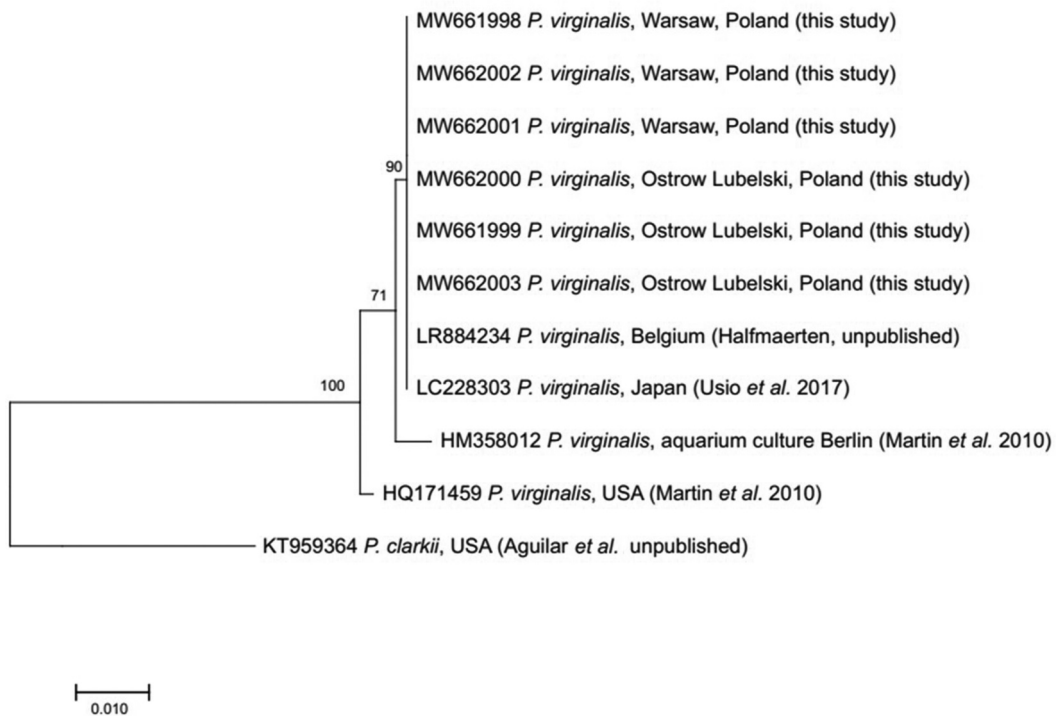


Figure 3. Maximum Likelihood phylogenetic tree including sequences obtained from collected *Procambarus virginalis* and GenBank stocked sequences.



Figure 4. Indications of *Procambarus virginalis* in the terrestrial and littoral zone: intact dead specimens found on forest roads (a); dead crayfish in freshly dried ditch (b); crayfish burrows: in compacted forest soil (c), in moist sand soil (d), in moist clay (e), in leaves litter (f); used crayfish migration channels connecting puddles in wet the ditch (g, h).

noticed. All crayfish collected in Warsaw were morphologically identified as *P. virginalis*.

All barcoded specimens represented only one haplotype, which was also common with the haplotype reported from Belgium and Japan (Figure 3). BOLD assigned investigated specimens to only one, non-unique Barcode Index Number (BIN) ACG1580 (dx.doi.org/10.5883/BOLD:ACG1580), together

with other representatives of the species deriving from private data deposited in this database.

In the wet ditch, crayfish were mostly observed underwater, in puddles, and migration channels (Figure 4). Single individuals were also observed on land in the proximity of the ditch. At least one specimen was found in each migration channel and burrow. On one occasion, 276 individuals

Table II. The number of crayfish individuals inhabiting burrows in wet and dry ditches.

Crayfish inhabitants	Wet ditch burrows		Dry ditch burrows		Total	
	n	%	n	%	n	%
0			21	43.8	21	34.4
1	7	53.8	16	33.3	23	37.7
2	2	15.4	2	4.2	4	6.6
3			3	6.3	3	4.9
5	2	15.4	1	2.1	3	4.9
7	1	7.7	2	4.2	3	4.9
10	1	7.7			1	1.6
11			1	2.1	1	1.6
14			1	2.1	1	1.6
17			1	2.1	1	1.6
Total	13	100.0	48	100.0	61	100.0

were caught from a single puddle of ca. 2 m² surface area and 15 cm depth. A total of 237 individuals were collected in another puddle built in an abandoned fox burrow and connected to an underwater lair system comprising four burrows of maximum length of 70 cm built rather horizontally. Increased activity of crayfish in the ditch was observed 3 hours before and to 6 hours after sunset. Only several crayfish were found freshly dead, most likely due to unsuccessful molting.

The vast majority of crayfish collected in the dry ditch was extracted from burrows. Here, burrows were present in higher numbers compared to the wet ditch. These were often inhabited by a single individual (33.3%); however, in few instances, burrows hosted up to 17 crayfish (Table II). Only a few individuals were observed outside the burrows, some of which were dead. Increased activity (crayfish moving on the ground), in this case, was observed 3 hours after sunset.

In both lakes, all crayfish were caught individually in open water. No specimens were observed in the

Table III. Size classes and number of collected *Procambarus virginalis* individuals (n), their proportion (%), average total length (AVG) in mm ± SD.

Total length (mm)	Location								Total	
	Miejskie Lake		Kleszczów Lake		Wet ditch		Dry ditch			
	N	%	n	%	n	%	n	%	n	%
<60					9	1.5	1	1.1	10	1.4
60–64					21	3.5			21	2.9
65–69					44	7.2	1	1.1	45	6.3
70–74			1	7.1	97	16.0	9	9.7	107	15.0
75–79					144	23.7	19	20.4	163	22.8
80–84			4	28.6	158	26.0	23	24.7	185	25.9
85–89			2	14.3	85	14.0	24	25.8	111	15.5
90–94			5	35.7	35	5.8	14	15.1	54	7.6
95–99	1	100.0	2	14.3	9	1.5	2	2.2	14	2.0
≥100					5	0.8			5	0.7
Total	1	100.0	14	100.0	607	100.0	93	100.0	715	100.0
AVG ± SD			87.2 ^{aB} ±6.6		78.0 ^{AB} ±8.4		81.9 ^{Aa} ±7.3		78.7 ± 8.4	

Values statistically significant—same letters (A,B— $p < 0.01$, a— $p < 0.05$).

Table IV. The presence of pereiopods, moulting and egg-carrying individuals.

Description	Location							
	Miejskie Lake		Kleszczów Lake		Wet ditch		Dry ditch	
	n = 1		n = 14		n = 607		n = 93	
	n	%	n	%	n	%	n	%
Claws								
none					7	1.2		
one missing					48	7.9	7	7.5
both present	1	100	14	100	552	90.9	86	92.5
Other walking legs counts								
5					3	0.5		
6					5	0.8	1	1.1
7					12	2.0		
8	1	100	14	100	587	96.7	92	98.9
Moulting individuals					95	15.7	1	1.1
Females with eggs					2	0.3	2	2.2

water depth exceeding 1.5 m. Increased crayfish activity was observed three hours before and after sunset and ceased immediately after *A. nebulosus* juveniles came closer to the shore. In all examined areas, no evidence of predation on crayfish by native or non-native predators was observed. In contrast to the lakes, no amphibians or mollusks were observed in the ditches. Also, no sign of crayfish presence (e.g. burrows) was observed in water or on land, in lakes. All detected dead crayfish presumably died of natural causes, as the bodies were found mostly intact.

The majority of collected *P. virginalis* (Table III) specimens had a total length of 80–84 mm (25.9%). However, individuals collected in the lakes were significantly bigger (87.2 mm) than those collected from the ditches (81.9 mm and 78.0 mm). The average carapax length of crayfish collected from the lake and dry ditch (Tab. S1) was similar (40.2–41.4 mm), while the significantly smaller individuals (37.7 mm), were collected from the wet ditch. No individuals below 30 mm were found. The total length of a single caught *F. limosus* was 112 mm, while its carapax length was 55 mm.

Most of the specimens (97.2%) had all ten pairs of pereiopods. Their claws were usually intact, the number of individuals missing one and both claws were 55 (7.7%) and 7 (0.9%), respectively. In contrast to crayfish collected in ditches, those from lakes did not have missing pereiopods (Table IV). The specimen of *F. limosus* had all pereiopods. Only 13.4% of the *P. virginalis* specimens recently underwent molting and were found primarily in wet

ditches (99.0%). Two females (0.3%) in the wet ditch and two (2.2%) in the dry ditch incubated eggs. In contrast to the wet ditch, those in the dry ditch incubated a far lower amount of eggs. No females with attached crayfish juveniles were found.

Discussion

We hereby provide the first documented data on the occurrence of viable *P. virginalis* population in a nature-protected area in Poland and the steps that were taken to detect and prevent further introduction of the species in the country. The new site is located approximately 150 km from the nearest stable population of *P. virginalis* of Morskie Oko park in Warsaw. The new localization in Ostrów Lubelski represents the most north-eastward distribution area of the species in European waters with a natural water temperature regime. Despite originating from warmer climates, experimental studies indicate that *P. virginalis* is cold tolerant (Veselý et al. 2015; Haubrock et al. 2019) thus a future north-eastward expansion can be expected. To date, the closest wild populations in temperate waters outside Polish borders are those found in the Czech Republic, Germany, Slovakia, and Ukraine (Maiakovska et al. 2021). The only confirmed established population of *P. virginalis* located further north-east is known from heated waters of the Narva power plant in Estonia (approx. 900 km; Ercoli et al. 2019).

The high density and size variation of *P. virginalis* individuals collected is a clear indication that the

species is well established both in wet and dry habitats. As *P. virginalis* was found primarily in ditches, it is plausible that the crayfish initially introduced to Miejskie Lake used the ditches to invade the neighboring Lake Kleszczów. However, interference of anglers or the local community focused on expanding the range of the species cannot be excluded. Its migration to other water bodies and ditches could have also arisen from a need to find more favorable places and escape predation from *A. nebulosus*. The exclusive presence of ovigerous females in ditches may also be an indication of predator avoidance. Several crayfish species are known to undergo local adaptations in their behavior and resource use in response to predator pressure (Reisinger et al. 2020; Wood & Moore 2020); hence, a trade-off between resource availability and predation risk can be expected. Unfortunately, according to local knowledge, these ditches are also the preferred habitat for amphibians, including the threatened *B. bombina*.

The introduction of *P. virginalis* in Pojezierze Łęczyńskie Landscape Park might have significantly impacted both native crayfish and amphibian communities. *Procambarus virginalis* is a known carrier of the crayfish plague pathogen *A. astaci* which is one of the main driver of declines and local extinctions of native European crayfish populations (Keller et al. 2014; Mrugała et al. 2015) and likely can also transmit the chytrid fungus *B. dendrobatidis* which is pathogenic for amphibians (Oficialdegui et al. 2019). The two native crayfish species *A. astacus* and *P. leptodactylus*, which are threatened by the crayfish plague, were not recorded in the study area. Likewise, amphibians were only found in ditches where the crayfish was absent. Although pathogens may have been partly responsible for the local disappearance of native fauna, other factors such as predation and competition could have potentially played an important role (Kats & Ferrer 2003; Cruz et al. 2006; Oficialdegui et al. 2019).

Procambarus virginalis, like *P. clarkii*, is a polytrophic consumer, which can efficiently predate a wide variety of invertebrates and vertebrates, including amphibians (Veselý et al. 2021). Following the introduction of *P. virginalis* in the Pojezierze Łęczyńskie Landscape Park, the local community confirmed a remarkable shift in frog croaking activity, with the forest becoming increasingly quiet. Accordingly, in areas invaded by *P. clarkii*, exclusion or even local extinction of amphibians have been observed (Cruz et al. 2006, 2008; Ficetola et al. 2011). Unfortunately, no previous amphibian survey has been conducted in the same area. Therefore, we cannot quantify the decline caused by the marbled crayfish. Furthermore, predation

pressure by high crayfish density might explain the absence of mollusks in the ditches.

The absence of native species and the presence of only one individual of *F. limosus* might be the result of *P. virginalis* dominance in antagonistic interactions: faster growth, earlier maturation, more frequent reproduction and higher fecundity (Kouba et al. 2021). Native crayfish are commonly outcompeted by both invasive species. However, *P. virginalis* is able to outcompete other invasive crayfish including *F. limosus* (Linzmaier et al. 2018). Compared to *F. limosus*, *P. virginalis* is a more recent invader in European waters, thus it is more likely that native species might have suffered from two subsequent invasions, the first perpetrated by *F. limosus* and the second by *P. virginalis*, in concomitance with anthropogenic disturbance. However, due to the lack of prior information, we cannot assert with certainty the correct sequence of events.

Besides negatively impacting the native fauna of *P. virginalis* might cause further damages to the environment with its burrowing activity. The extent of burrowing observed in this study suggests that this crayfish can hamper water retention and stability of banks, and alter sediment dynamics. Such damages might result in severe economic costs as seen for *P. clarkii* (Souty-Grosset et al. 2016; Kouba et al. 2022). Moreover, the ability to burrow may reduce exposure to predators and provide shelter during periods of droughts as well as winters enhancing overall invasion success (Kouba et al. 2016).

If no action is taken, a further spread of the species is to be expected, as the population in Pojezierze Łęczyńskie Landscape Park is already out of control. In such a scenario, it is plausible that *P. virginalis* will share the invasion corridors used by *F. limosus* and colonize most of the Polish waterbodies. Therefore, organized actions, including early detection and eradication as well as education of local communities, are necessary as we cannot truly stop the spread of the species.

We hereby present the steps taken to prevent the further spread of *P. virginalis* as an example that can be implemented elsewhere to contrast current and future introductions of non-native crayfish species. Firstly, we removed all the crayfish we could get ahold of in the ditches while conducting our study. After our finding, we immediately redacted reports of the species for all regional and national nature conservation institutions, city governments, and angler associations. We provided identification keys for the species that were subsequently distributed via local media following an official statement of the Polesie National Park. Soon after, information about the invasion of *P. virginalis* was also broadcasted throughout the country. Workshops with live specimens presentations were

organized for all the entities involved in nature conservation and for local citizens. Education on native and invasive crayfish species was introduced in local schools. Identification keys for all six species known from Polish waters have been provided in educational nature boards located at the lakes. Thanks to these actions, *P. virginalis* became one of the most recognizable invasive alien species in the country. Tourists notified another breeding population of *P. virginalis* on August 22, 2020, in Białka Lake (51.5337, 23.0157), ca. 8 km west from Kleszczów Lake and ca. 7 km from the borders of Polesie National Park. Information on the occurrence of *P. virginalis* in Pojezierze Łęczyńskie Landscape Park and Białka Lake was published in EASIN Notsys database and becoming the first record of the species from Poland. Moreover, our actions played a significant role in banning *P. virginalis*, *P. clarkii*, and other crayfish listed as invasive species in the European Union from private and commercial online trade since December 2020, where they were still present. Banning the trade of invasive species, coupled with increased awareness of the threats posed by non-native crayfish species, is a fundamental step to prevent further introductions.

To sum up, *P. virginalis* is well adapted to withstand climatic conditions of temperate areas and is likely to expand its distribution area further. Its presence in the Pojezierze Łęczyńskie Landscape Park may have impacted native and invasive species both in aquatic and terrestrial environments. Sensibilization of public opinion and local knowledge is essential for the early detection and eradication of invasive species.

EASIN Detection: After acceptance of this paper, it will be attached to detection details of “*Procambarus fallax* f. *virginalis* - Poland #1” in EASIN database: <https://easin.jrc.ec.europa.eu/notsys/PUB/Detections/Details/5a6035f9-e131-4343-b3b6-632bc4e4b480>.

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Supplementary material

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