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Occurrence of two exotic decapods, *Macrobrachium nipponense* (de Haan, 1849) and *Procambarus virginalis* Lyko, 2017, in Ukrainian waters

Mikhail O. Son[1], Halyna Morhun[2], Roman O. Novitskyi[3], Serhii Sidorovskyi[2], Maryna Kulyk[2] and Serge Utevsky[2,*]

[1] Institute of Marine Biology of the National Academy of Sciences of Ukraine, Department of water quality, 37 Pushkinska str., 65148 Odesa, Ukraine
[2] V.N. Karazin Kharkiv National University, Department of Zoology and Animal Ecology, 4 Maidan Svobody, 61022 Kharkiv, Ukraine
[3] Dnipro State Agrarian and Economic University, Department of Water Bioresources and Aquaculture, 25 Serhii Yefremov str., 49600 Dnipro, Ukraine

Abstract – New information on the occurrence of two alien decapod crustaceans, *Macrobrachium nipponense* and *Procambarus virginalis*, was summarized for Ukraine. The invasive potential of both species was estimated in the context of local environmental and socio-economic issues. Their history as non-native species is marked by the early use of alien decapods as a natural resource by local residents. Both intentional and unintentional introductions occurred for *M. nipponense*, while the ornamental trade was an important source of non-native *P. virginalis*. New records of *M. nipponense* and *P. virginalis* from open freshwater habitats in the Odesa and Kharkiv provinces suggest continuing expansion of these decapods. We conducted a molecular phylogenetic analysis of *COI* sequences, which confirmed morphological identifications of invasive *M. nipponense*. This reinforces the presumed pathway of this species, including its pathway from China to south-western Ukraine. *Procambarus fallax* was found to be paraphyletic in relation to *P. virginalis*, which was probably caused by analyzing only one mitochondrial gene, which could be impacted by the recent emergence of the latter species.

Keywords: Decapoda / *Macrobrachium nipponense* / *Procambarus virginalis* / Ukraine / invasive species

Résumé – Présence de deux décapodes exotiques, *Macrobrachium nipponense* (de Haan, 1849) et *Procambarus virginalis* Lyko, 2017, dans les eaux ukrainiennes. De nouvelles informations sur la présence de deux crustacés décapodes exotiques, *Macrobrachium nipponense* et *Procambarus virginalis*, ont été compilées pour l'Ukraine. Le potentiel d'invasion des deux espèces a été estimé dans le contexte des problèmes environnementaux et socio-économiques locaux. Leur histoire en tant qu'espèces non indigènes est marquée par l'utilisation précoce des décapodes exotiques comme ressource naturelle par les résidents locaux. Des introductions intentionnelles et non intentionnelles ont eu lieu pour *M. nipponense*, tandis qu'un commerce ornemental a été une source importante de *P. virginalis* non indigène. De nouveaux signalements de *M. nipponense* et de *P. virginalis* dans des habitats d'eau douce ouverts dans les provinces d'Odesa et de Kharkiv suggèrent une expansion continue de ces décapodes. Nous avons effectué une analyse phylogénétique moléculaire des séquences COI, qui a confirmé les identifications morphologiques de *M. nipponense* envahissante. Cela renforce la voie présumée de cette espèce, y compris sa trajectoire de la Chine vers le sud-ouest de l'Ukraine. On a constaté que *Procambarus fallax* était paraphylétique par rapport à *P. virginalis*, ce qui est probablement dû à l'analyse d'un seul gène mitochondrial, qui pourrait être affecté par l'émergence récente de cette dernière espèce.

Mots-clés : Décapode / *Macrobrachium nipponense* / *Procambarus virginalis* / Ukraine / espèce envahissante

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*Corresponding author: serge.utevsky@karazin.ua

Table 1. Samples used in our study and main characteristics of localities.

Locality	Coordinates	Date	Salinity (PSU)	Substrate	Collectors
Procambarus virginalis					
1. Kharkiv, pond "Rodnyk"	50.030453, 36.348038	10.10.2019	No data	Sand and clay	S.A. Sidorovskyi, M.O. Kulyk
2. Dnipro, Kotlovan Lake	48.506056, 35.102197	13.11.2015	0.957	Sand	R.O. Novitskyi, A. Artemenko
Macrobrachium nipponense					
3. Fish pond (Akkarzhanka River Basin)	46.3441966, 30.5963854	23.07.2018 20.08.2018 23.08.2018	2.920	Mud	M.O. Son, H. Morhun, S.Utevsky
4. Olexandrivka Reservoir (Akkarzhanka River Basin)	46.336806, 30.625972	02.09.2018	1.555	Mud	M.O. Son
5. Reservoir in the Great Adzhalyk Liman Basin	46.610917, 30.878972	13.03.2020	1.286	Mud with reed fragments	M.O. Son

1 Introduction

Over the past decades, expansions of five non-native decapods, *Rhithropanopeus harrisii* (Gould, 1841), *Eriocheir sinensis* Milne-Edwards, 1853, *Macrobrachium nipponense* (De Haan, 1849), *Orconectes limosus* (Rafinesque, 1815) and *Procambarus virginalis* Lyko, 2017 have been recorded in Ukraine's inland waters (Son *et al.*, 2013; Novitsky and Son, 2016; Kudryashov, 2020). While invasive crabs *R. harrisii* and *E. sinensis* are common along the Azov-Black Sea coast and penetrate river basins through estuarine systems, *O. limosus*, *M. nipponense* and *P. virginalis* are distributed exclusively in inland waters (Son *et al.*, 2013; Novitsky and Son, 2016; Kudryashov, 2020).

This is rather a large number of non-native species, considering that the native Ukrainian freshwater fauna includes only four decapod species, *Astacus leptodactylus* (Eschscholtz, 1823), *Astacus astacus* (Linnaeus, 1758), *Astacus pachypus* (Rathke, 1837) and *Potamon ibericum* (Bieberstein, 1808), of which the last three are not widespread and are considered to be vulnerable (Akimov, 2009; Policar *et al.*, 2018).

Macrobrachium nipponense is a widespread Indo-Pacific species that occurs in estuarine and fresh waters (Yu and Miyake, 1972; Cai and Ng, 2002). It has been introduced to natural habitats in Bangladesh, Singapore, the Philippines, Kazakhstan, Uzbekistan, Iraq, Iran, Moldova and Ukraine (Alekhnovich and Kulesh, 2001; Cai and Shokita, 2006; De Grave and Ghane, 2006; Salman *et al.*, 2006; Son *et al.*, 2013). In addition, the species is cultivated in cooling reservoirs of thermal power stations in Russia, Belarus and Moldova (Alekhnovich and Kulesh, 2001).

The marmorkrebs (*P. virginalis*) is a triploid parthenogenetic species that was originally found in aquariums (Martin *et al.*, 2010a, 2016). This crayfish is viewed as a recent descendant of *Procambarus fallax* (Hagen, 1870), which is a diploid sexually reproducing species known from Florida and Georgia, USA (Taylor *et al.*, 1996).

The emergence of the marmorkrebs is explained by a very recent, in terms of evolution, macromutation in *P. fallax* that resulted in instant reproductive isolation (Vogt *et al.*, 2015). This reproductive isolation is the basis for the assignment of the invasive marmorkrebs to its own species (Lyko, 2017). This crayfish is known as a non-native species with a high invasive potential, and it occurs throughout Europe (Martin *et al.*, 2010b; Bohman *et al.*, 2013; Chucholl, 2014; Vojkovská *et al.*, 2014; Lőkkös *et al.*, 2016; Lipták *et al.*, 2017; Pârvulescu *et al.*, 2017; Ercoli *et al.*, 2019).

This research was aimed at tracing the invasion history of *M. nipponense* and *P. virginalis* in Ukraine over the last few years based on all currently available data. Both morphological and molecular characters were used to confirm the species identity and to reveal phylogenetic relationships of their populations in Ukraine.

2 Material and methods

2.1 Samples and sampling methods

In 2018–2019, decapod specimens were collected in different types of habitats in three of Ukraine's provinces representing a variety of climatic and ecological conditions of the country (Tab. 1, Figs. 1 and 2).

In a former quarry called Kotlovan Lake (Dnipropetrovsk Region), which was transparent enough to see objects of interest situated under water, samples were simply collected by hand when diving. A transect video was used to estimate population density during this collection. In waterbodies in the Akkarzhanka River Basin, a kick-net (ISO 7828) was dragged along the shore over 5 m distances. A collector moved in zigzags to cover depths from the water edge to a depth of one meter for 15 min, with 3–4 replicates. In the Great Adzhalyk Liman Basin, one specimen was sampled by hand. Similarly, in Kharkiv, crayfish samples were collected by hand in a man-made pond.

Collected specimens were immediately preserved in 96% ethanol for further morphological examination and molecular analysis. The specimens are stored in the collections of invertebrates at the Institute of Marine Biology, National Academy of Sciences of Ukraine, V. N. Karazin Kharkiv

Fig. 1. Habitats of exotic decapodes. A, Olexandrivka Reservoir in a small river basin; B, urban pond "Rodnyk" (Kharkiv); C, former quarry Kotlovan Lake (Dnipro); D, artificial ornamental pond (Odesa) during the renovation that caused the eradication of *Procambarus virginalis*.

National University and the Department of Invertebrate Zoology and Hydrobiology (University of Lodz, Poland). *Procambarus virginalis* is kept as a living culture at the Dnipro State Agrarian and Economic University.

2.2 Morphological identification

Procambarus. virginalis (Fig. 3a–c) differs from the rest of alien cambarids of Europe in its specific marble coloring and very small chelipeds. Since *P. virginalis* belongs to the family Cambaridae, it can be reliably distinguished from all local crayfishes (Astacidae), which bear a median carina of the rostrum (Fig. 3d).

Macrobrachium nipponense (Fig. 3e–g) can be distinguished from the congeneric *M. rosenbergii* (De Man, 1879) by its relatively straight rostrum. The latter species has a narrow arcuate rostrum, where the tip is distinctly bent up. *Macrobrachium nipponense* differs from related local native palaemonid shrimps in a double row of setae along the inferior margin of the rostrum and more than ten dorsal teeth on the latter. All native species have a single row of setae along the inferior margin and no more than nine dorsal teeth of the

rostrum (González-Ortegón and Cuesta, 2006). Its easily noticeable distinguishing feature is its long second pereiopods (they exceed the length of the rest of the body in larger adults) that have claws bearing numerous setae.

2.3 DNA extraction and PCR

The collected specimens of *P. virginalis* and *M. nipponense* were transferred to the Laboratory of Biogeography and Invertebrate Ecology, Department of Invertebrate Zoology and Hydrobiology (University of Lodz, Poland) for molecular analysis. A small piece of muscle tissues was taken for each DNA extraction. Genomic DNA was isolated using the standard phenol-chloroform method (Hillis *et al.*, 1996). Air-dried DNA pellets were eluted in 100 μl of TE buffer, pH 8.00, stored at 4 °C until amplification, and subsequently kept at −20 °C for a long-term storage.

The standard animal DNA barcode gene region of mitochondrial cytochrome *c* oxidase subunit one (*cox1*, *COI*) was selected for molecular analyses (Hebert *et al.*, 2003). It was amplified using the following primers (Folmer *et al.*, 1994): HCO2198, 5′- TAAACTTCAGGGTGAC-CAAAAAATCA-3′ and LCO1490, 5′-GGTCAACAAATCA-TAAAGATATTGG-3′. The amplification was conducted under the following PCR conditions: 94 °C for 3 min; 5 cycles of 30 s at 94 °C, 1:30 min at 45 °C, and 1 min at 72 °C; 35 cycles of 30 s at 94 °C, 1:30 min at 51 °C, and 1 min at 72 °C; 5 min of denaturation step at 72 °C (Hou *et al.*, 2007). PCR products (5 μl) were cleaned up using Exonuclease I (20 U/μl; EURx, Poland) and alkaline phosphatase Fast Polar-BAP (1 U/μl, EURx, Poland) treatment, following the manufacturer's guidelines. Cleaned PCR product was then sequenced by Macrogen Inc. (the Netherlands) using the same primers as at the amplification stage. The resulting sequences were manually assembled to a uniform length by removing the remaining parts of the primer regions using FinchTV v. 1.5.0 (Geospiza Inc.). These sequences were then submitted to GenBank (accession numbers: MT881672–MT881675). The length of the resulting *COI* sequences is 635–642 bp for *P. virginalis*, and 630–641 bp for *M. nipponense*.

2.4 Phylogenetic analysis

BLAST searches (blast.ncbi.nlm.nih.gov/Blast.cgi) were performed to check for the amplification of contaminants. The sequences of the *COI* gene region were aligned using MAFFT ver.7 online (Katoh *et al.*, 2017) with the FFT-NS-i strategy selected by the "Auto" option. The *COI* sequences were also checked for mistaken stop codons at the amino acid level using Molecular Evolutionary Genetics Analysis (MEGA) program version X (Kumar *et al.*, 2018).

To perform our phylogenetic analysis, previously published GenBank nucleotide sequences (Salman *et al.*, 2006; Feng *et al.*, 2008; Wowor *et al.*, 2009; Martin *et al.*, 2010a, 2010b; Filipová *et al.*, 2011; Liu and Cui, 2011; Aoki *et al.*, 2013; Bohman *et al.*, 2013; Quan *et al.*, 2014; Vojkovská *et al.*, 2014; Vogt *et al.*, 2015; Usio *et al.*, 2017 Cui *et al.*, 2018; Zheng *et al.*, 2019) were combined with our molecular data. Localities and GenBank accession numbers of sequences used in the present study are listed in Supplementary Table S1.
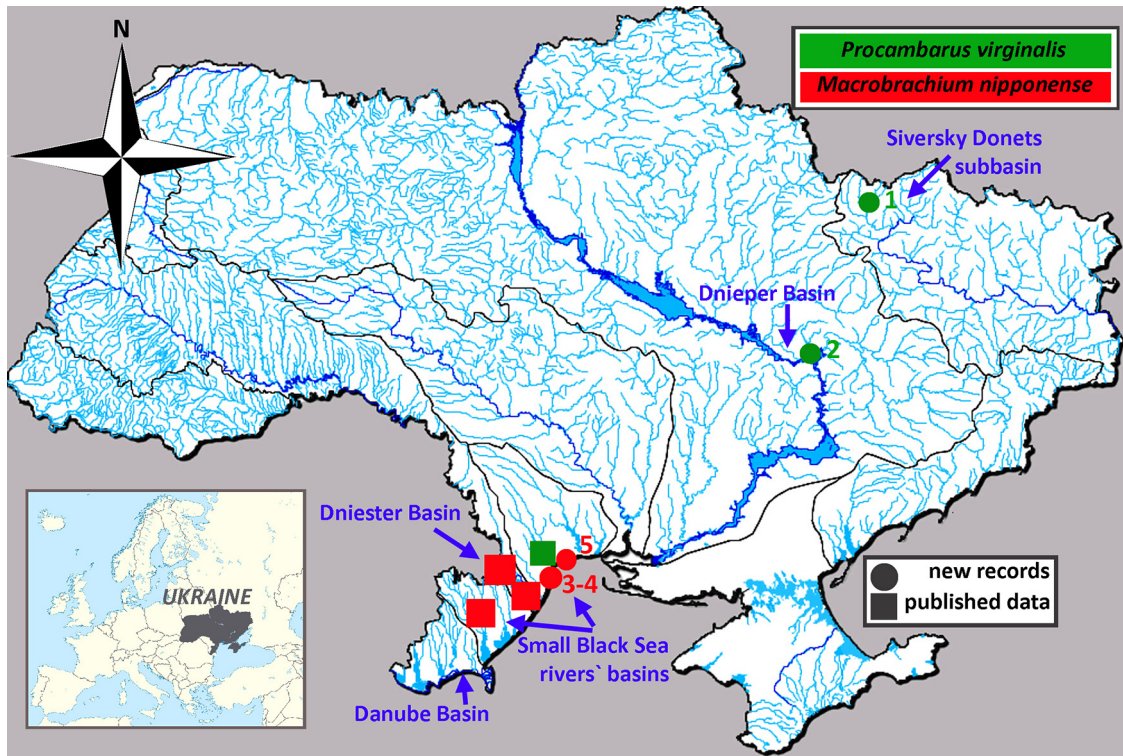
Fig. 2. Distribution of *Procambarus virginalis* and *Macrobrachium nipponense* in the river basins of Ukraine. See Table 1 for numbering.
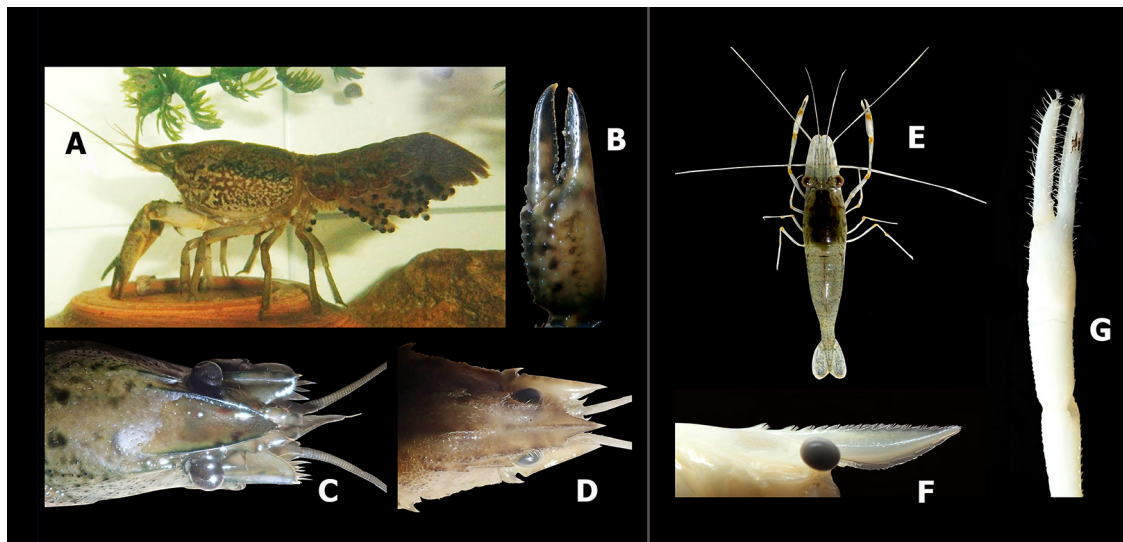

Fig. 3. Diagnostic features of exotic decapodes. *Procambarus virginalis*: A, general view; B, heliped; C, rostrum without median carina. D, rostrum of Astacidae (*Astacus leptodactylus*) with median carina. *Macrobrachium nipponense*: E, general view; F, rostrum; G, claw.

The phylogeny was reconstructed using Maximum Likelihood (Strimmer and von Haeseler, 1996) with the interior branch tests of 1000 bootstrap replicates (Sitnikova *et al.*, 1995) using MEGA X. Analyses were based on the HKY model with a discrete Gamma distribution [+G, α = 0.0500] for the *P. virginalis* phylogeny and Tamura 3-parameter model (Tamura, 1992) with a discrete Gamma distribution [+G, α = 0.5572] for the *M. nipponense* phylogeny, as they were the best substitution models computed in MEGA X using the Bayesian information criterion. The tree was drawn to scale with branch lengths measured in the number of substitutions per site.

In addition, the number of base differences per site (p-distances) between sequences and their standard errors were calculated. All positions containing gaps and missing data were eliminated. Haplotypes were determined using DnaSP version 6.12.03 (Rozas *et al.*, 2017). There were a total of 614 positions for *P. virginalis* and 551 positions for *M. nipponense* in the final dataset.

3 Results

3.1 Field research

Several sequential attempts were made to find *P. virginalis* based on previous records and anecdotal evidences. This approach revealed some fluctuations in the occurrence of this species. In a previous study (Novitsky and Son, 2016), *P. virginalis* was first recorded for Lake Kotlovan on 23 October 2015. However, according to Alexander Trushlyakov's personal communication, in the summer of 2014, large numbers of *P. virginalis* already occurred in this locality. Later, in November 2015, *P. virginalis* was not found, which could be explained by a low temperature (+6 °C) at that time. In the autumn of 2018 (11 October 2018), the species was again recorded in that locality in the course of night sampling. In the shallow waters of the lake, we counted up to 2–3 individuals per 100 square meters of a diving transect. Later, on 16 November 2018, 34 adult individuals and 2 juveniles of *P. virginalis* were caught by means of night sampling.

One of the first recorded localities of *P. virginalis* − a cascade of ornamental ponds in Odesa, where three live individuals were observed in June 2015 (Novitsky and Son, 2016) − was also surveyed. Shortly after that discovery, those reservoirs underwent multi-stage cleaning and renovation (Fig. 1d). During the winter-spring period of 2017–2018, a consistent complete drying of all ponds was carried out. In the course of those activities, large animals such as fish and turtles, including invasive red-eared sliders *Trachemys scripta* (Schoepff, 1792), were placed in aquariums and released back later. Conversely, soft sediment accumulated at the bottom along with benthic invertebrates was removed from these water bodies. As a result, we can confidently assert that *P. virginalis* was eradicated in that locality.

One individual of *P. virginalis* was found in a man-made pond in Kharkiv on 10 October 2019. There were no previous information on its occurrence in Kharkiv and neighboring areas.

Outside the Dniester Basin, we found *M. nipponense* in the basin of the small river Akkarzhanka, which flows into the estuarine Sukhyi Liman. Several specimens of the species were sampled on 23 July 2018. Later, a density of up to 15 individuals per square meter was estimated in August 2018. In all samplings, young individuals were found.

Five other ponds of the Sukhyi Liman basin were examined, and the shrimp was also found in the Oleksandrivka Reservoir on 2 September 2018 with a density of 3–4 individuals (adults, including females with eggs) per square meter.

Only one specimen was sampled in a reservoir of the Great Adzhalyk Liman Basin on 13 March 2020. This locality and the Oleksandrivka Reservoir represent a rare type of habitat − the upper estuary separated from the rest of the estuary by dams and turned into a freshwater body which is still highly mineralized.

3.2 Molecular studies

The molecular phylogenetic analysis revealed that all examined specimens of *P. virginalis* both from the Dnipropetrovsk and Kharkiv regions were identical in all 614 base pairs to *COI* GenBank sequences from Germany, Japan, Italy, and Sweden (LC228303, KJ690261, KT074364, KF033123, HM35810, HM358011, JF438007) (Martin *et al.*, 2010a, 2010b; Filipová *et al.*, 2011; Bohman *et al.*, 2013; Vojkovská *et al.*, 2014; Usio *et al.*, 2017) (see Supplem. Tab. S2). Remarkably, *COI* sequences for the Ukrainian samples are also identical to the holotype of *P. virginalis* (accession number: KT074364), which was described from Germany (Vogt *et al.*, 2015; Lyko, 2017). The phylogenetic relationships are shown on the tree in Figure 4. See Table 2 for between and within group nucleotide divergence estimation.

The phylogenetic analysis of *COI* sequences of *M. nipponense* did not reveal novel haplotypes. The whole alignment consisted of 551 base pairs with 29 variable sites. The haplotypes and their affiliation to all sequences obtained are shown in Supplementary Table S3.

Our sequences were identical to those of *M. nipponense* from Gaoming Disrict, Foshan, Guangdong Province, China (MK412772) and *M. nipponense* obtained during a survey of biodiversity in the Yangtze River, China, especially from the largest freshwater lakes (JN874532) (Feng *et al.*, 2008; Zheng *et al.*, 2019). The Maximum Likelihood tree based on *COI* sequences is shown in Figure 5. The between and within group nucleotide divergence estimation is shown in Table 3.

4 Discussion

4.1 Identification and phylogenetic relationships

Morphological characters and molecular data suggest assigning the specimens examined to two decapod species, *M. nipponense* and *P. virginalis*. The sequences of the latter were identical to GenBank samples from Germany, Sweden, Italy and Japan in its *COI* genes, thus sharing the same haplotype. This can be explained by a recent invasion, which has not provided sufficient time for the divergence of their mitochondrial genes. *Macrobrachium nipponense* was found to have low genetic diversity as well; Ukrainian samples share the same haplotype with two individuals from China. The shallow phylogenetic structure revealed in both the species is in agreement with the founder effect hypothesis, which is commonly used to explain the recent evolutionary history of a number of invasive species (Estoup *et al.*, 2016).

Procambarus virginalis is also known under its former name *Procambarus fallax* forma *virginalis* (Martin *et al.*, 2010a, 2010b). In 2017, the systematic rank of this taxon was elevated to the level of species (Lyko, 2017). It occurs in Germany and elsewhere, while the native range of its parental species *P. fallax* is restricted to southern Georgia and Florida, USA (Hobbs, 1989). In our study, we compared the *COI* gene region of our samples to the complete genome of *P. virginalis*, which was published alongside with its formal taxonomic
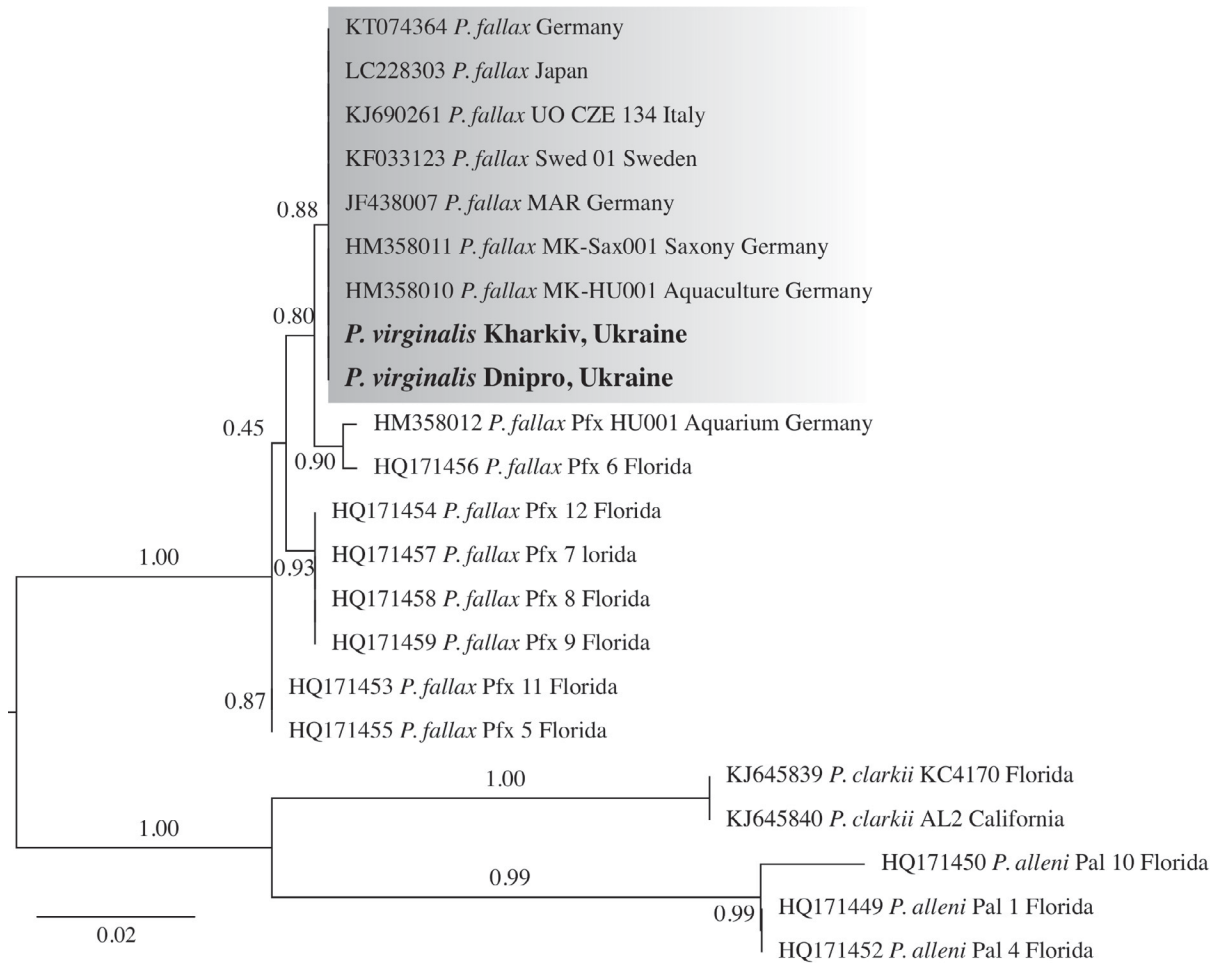
Fig. 4. Maximum Likelihood phylogenetic tree of *Procambarus virginalis* and related species based on *COI* gene sequences (highest log likelihood-1285.73). Samples from the current research are highlighted in bold, grey box showing sequences with the same haplotype. Bootstrap values are shown for clades. See Table S1 for details of the analyzed specimens and their GenBank accession numbers.

Table 2. Estimates of average evolutionary divergence over sequence pairs of the *P. virginalis* dataset. The number of base differences per site (based on p-distances) between groups and within each group are shown.

Group		Between groups				Within group	
		[1]	[2]	[3]	[4]	Mean p-dist	SE
Procambarus virginalis	[1]		±0.002	±0.019	±0.019	0.000	±0.000
P. fallax	[2]	004		±0.018	±0.018	0.003	±0.002
P. alleni	[3]	0.040	0.040		±0.018	0.004	±0.003
P. clarkii	[4]	0.040	0.039	0.037		0.000	±0.000

Note: standard error estimates are highlighted in bold.

description (Lyko, 2017), and sequences of *P. fallax* from Florida (Martin *et al.*, 2010b), which is usually considered as its native range. The phylogenetic analysis revealed that the Ukrainian marmorkrebs shared the same haplotype with the type specimen of *P. virginalis*, whereas sequences of *P. fallax* from the USA formed separate clades. Remarkably, the genetic distance observed between these two species is low and just slightly exceeds the within group mean p-distances observed in them. *Procambarus fallax*, which is a parental species in relation to *P. virginalis*, was found to be paraphyletic. Although species are usually expected to be monophyletic groups, this topology can be viewed as a consequence of analyzing only one mtDNA gene (Coyne and Orr, 2004). The paraphyly can be explained by incomplete lineage sorting due to the recency of the emergence of *P. virginalis*, which still retains its relationships with certain ancestral populations of the parental species. Currently, the overall genetic structure of *P. virginalis* cannot be estimated with certainty due to the lack
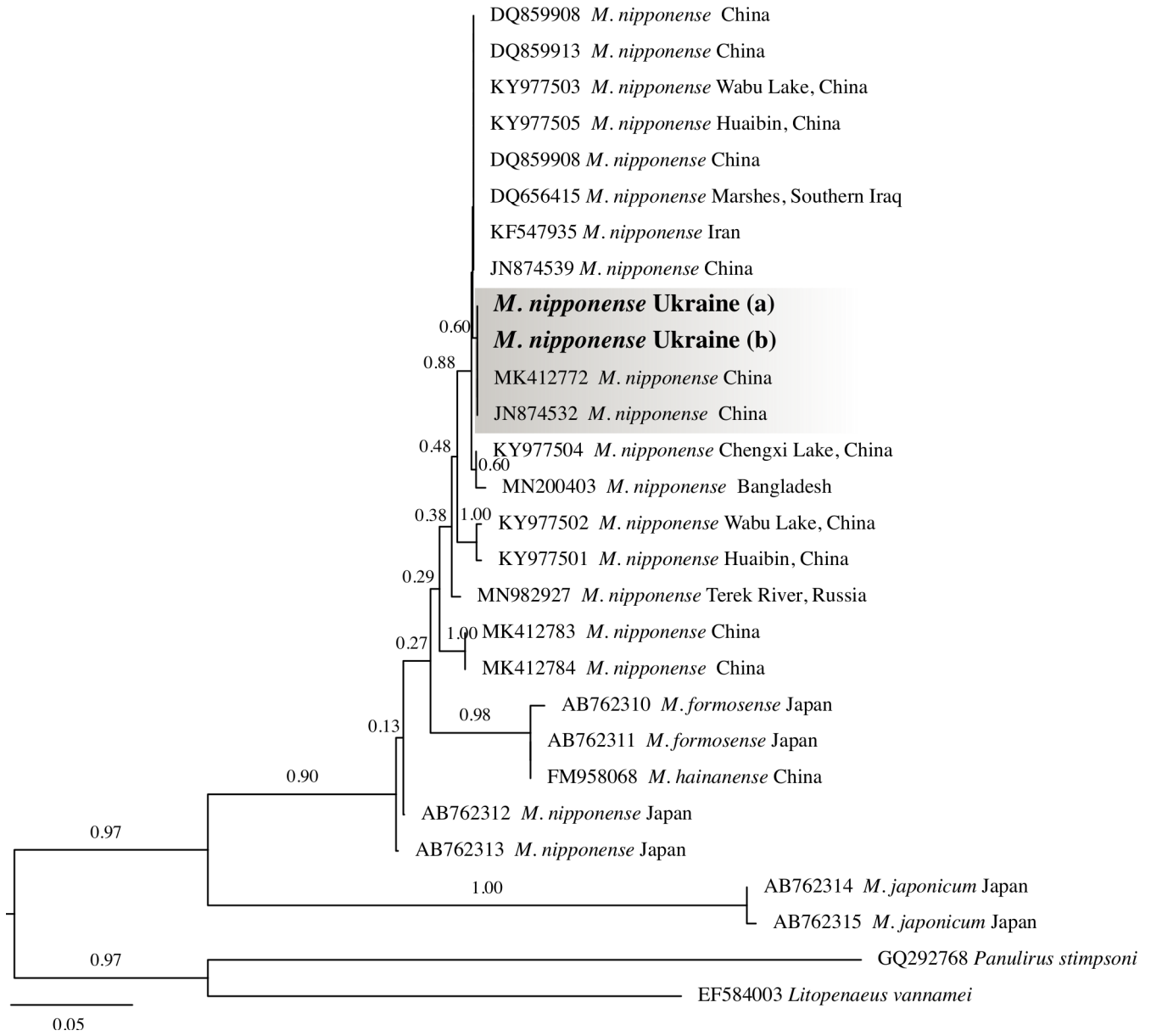
Fig. 5. Maximum Likelihood phylogenetic tree of *Macrobrachium nipponense* based on *COI* gene sequences (highest log likelihood-2143.56). Samples from the current research are highlighted in bold, grey box showing sequences with the same haplotype. See Table S1 for the details of the analyzed specimens and their GenBank accession numbers.

Table 3. Estimates of average evolutionary divergence over sequence pairs of the *M. nipponense* dataset. The number of base differences per site (based on p-distances) between groups and within each group are shown.

Group		Between groups					Within group	
		[1]	[2]	[3]	[4]	[5]	Mean p-dist	SE
Macrobrachium nipponense	[1]		±0.01	±0.02	±0.02	±0.02	0.01	±0.00
Macrobrachium sp.	[2]	0.05		±0.02	±0.02	±0.02	0.00	±0.00
M. japonica	[3]	0.19	0.20		±0.02	±0.02	0.00	±0.00
P. stimpsoni	[4]	0.23	0.23	0.25		±0.02	n/a	n/a
L. vannamei	[5]	0.22	0.22	0.26	0.23		n/a	n/a

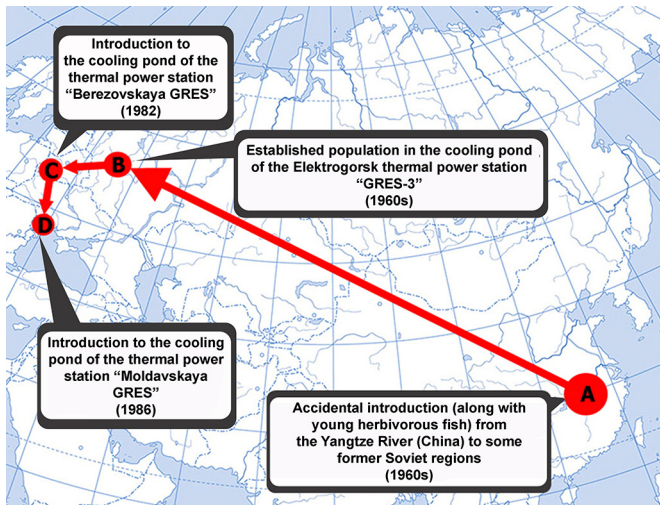Note: standard error estimates are highlighted in bold.

Fig. 6. Invasion history of Ukrainian populations of *Macrobrachium nipponense*. A, China; B, Russia; C, Belarus; D, Moldova and Ukraine.

of reliable distinguishing between *P. virginalis* and *P. fallax* sequences in GenBank. One or very few nearly identical *COI* haplotypes of *P. virginalis* are expected to exist.

The phylogenetic relationships of the Ukrainian *M. nipponense* corroborate the known invasive history of the species (see below), confirming the Chinese origin of the populations studied.

4.2 Invasion history

Previous sources have provided contradictory information on the origin of the Dniester population of *M. nipponense*. Both a Chinese (Alekhnovich and Kulesh, 2001) and Japanese (Stepanok, 2014) origin of the shrimp has been supposed. Based on available information, we traced all stages of this invasion (Fig. 6).

In the 1960s, this species was accidentally introduced (along with young herbivorous fish) from the Yangtze River (China) to some former Soviet regions. As a result of this, stable populations were reported from water bodies of Uzbekistan and Kazakhstan (Alekhnovich and Kulesh, 2001) and from the cooling pond of the Elektrogorsk thermal power station "GRES-3" on outskirts of Moscow (Ivanov and Starobogatov, 1974). The latter population was a source for subsequent successful intentional introductions to the cooling ponds of the thermal power stations "Berezovskaya GRES" (Belarus) and "Moldavskaya GRES" (Moldova) (Vladimirov *et al.*, 1989; Khmeleva *et al.*, 1997; V.A. Kulesh, personal communication).

Before the 2000s, *M. nipponense* had not been reported from the Dniester Basin, except the Kuchurhan Liman (an estuary on the border of Ukraine and Moldova associated with the Dniester Delta and impacted by the thermal power station "Moldavskaya GRES"). Records from the Dniester Delta started to occur in the 2000s (Son *et al.*, 2013). In 2013–2016, the next expansion of the range was revealed. *Macrobrachium nipponense* was recorded in two separate

areas of the Dniester Liman (Stepanok, 2014), which is the estuary of the Dniester River; in the Moldovan Dniester near Tiraspol (Filipenko, 2014); and in fishponds in the Ukrainian Dniester Delta (Shekk and Astafurov, 2017), which became a key event for its further expansion. These fishponds are associated with the largest local fish farms in the area, where fishes are bred for subsequent stocking in other fishponds in the Lower Dniester, Lower Danube and neighboring small river basins. Our findings of this species in small river basins in 2018–2020 are most likely explained by this vector.

Moreover, since 2017, freshwater shrimps were observed in waterbodies near the town of Sarata, Odesa Region. Based on photographs from local Internet sources (*e.g.*, https://bessarabiainform.com/2018/09/vozle-saraty-rybaki-nahodyat-gigantskih-krevetok-fotofakt/), they can be provisionally identified as *M. nipponense*. In addition, the shrimp from the fishponds in the Dniester Delta were repeatedly caught in the abovementioned farms for keeping in small decorative ponds in private gardens in Odesa (personal communications from local fishermen). Thus, its range may be wider than this research suggests.

4.3 Pathways and risks of secondary distribution

Both *P. virginalis* and *M. nipponense* are sold in ornamental animal markets. The ornamental vector of introduction for *P. virginalis* has been discussed many times and looks to be highly likely for Ukrainian records. The marbled crayfish is popular in all large pet markets of Ukraine and in online trade, where they are cheaper than other exotic cambarid species.

Urban water reservoirs are a very common target for introduction of ornamental species by aquarium hobbyists as a result of market saturation, which promotes illegal releases of commercially unprofitable and unwanted juveniles to urban waters (Novitsky and Son, 2016). With regard to *P. virginalis*, this is true because both its parthenogenetic reproduction mode and rapid growth (Martin *et al.*, 2010a) make its population difficult to control even in aquaria.

In recent years *P. virginalis* has become one of the most popular ornamental decapods, while *Macrobrachium* species which were the most popular decapods kept by local aquarists 20 years ago, are now sold much less frequently than other shrimp species. However, the combination of the intentional introduction of *M. nipponense* and its random distribution with stocking individuals poses a high risk of invasion into new river basins. Apparently, climate changes are making the Ponto-Caspian region increasingly suitable for *Macro-brachium* species. For example, these species have been recorded in the Iranian Caspian Sea basin and in the Don Delta (De Grave and Ghane, 2006; Shokhin, 2018). The invasion to Sarata's waterbodies can be a starting point for the further penetration of *Macrobrachium* into local freshwater ecosystems, as it is connected with the Danube Delta through the Sarata River channel, Lake Sasyk and the Danube-Sasyk Canal.

With regard to *P. virginalis*, the localities in Dnipro and Kharkiv promote the possibility of its expansion into the Dnieper and Don basins. Both of these localities are situated in areas close to the Dnieper-Donbas Canal, which connects the Siversky Donets sub-basin with the Dnieper and promotes

exchange of alien species (Semenchenko *et al.*, 2015; Son *et al.*, 2014).

4.4 Harmful effects of invasive decapods

The crayfish plague, caused by the fungus-like pathogen *Aphanomyces astaci* Schikora, 1906, has been identified as key threat to European crayfish from non-indigenous crayfish species (Holdich *et al.*, 2009). Recently the presence of the plague pathogen has been confirmed in a wild population of the marbled crayfish in Germany (Keller *et al.*, 2014). *Aphanomyces astaci* was long considered a specialist pathogen of crayfish; however, freshwater shrimps and crabs have now also been found to be vectors of this parasite (Svoboda *et al.*, 2014; Schrimpf *et al.*, 2014; Mrugała *et al.*, 2019). Recent studies have revealed that this pathogen is present in the Dnieper (Ukraine) and Dniester (Moldova) basins (Panteleit *et al.*, 2018; Ungureanu *et al.*, 2020).

Nutritional studies of *M. nipponense* in eutrophic freshwater wetlands of the Caspian coast, which are very close in terms of environmental conditions to southern Ukraine, show that this species can be a significant additional predator of invertebrates. Its diet also includes other components of food webs: detritus, algae, macrophytes, etc. (Mirzajani *et al.*, 2020).

4.5 Economic use

Giant shrimps of the genus *Macrobrachium* play a key role in modern freshwater aquaculture. Beginning in Soviet times, experimental cultivation of *M. nipponense* and *M. rosenbergii* was established in some natural and warm-water reservoirs (Suprunovich and Makarov, 1990). *Macrobrachium rosenbergii* is cultivated in reservoirs on the Crimean Peninsula, but no information is known about its escape from aquaculture or acclimatization in Ukraine's natural ecosystems. The invasive populations of both *P. virginalis* and *M. nipponense* instantly began to be used by local people as a food resource. According to Alexander Trushlyakov's personal communication, in Dnipro, *P. virginalis* was caught by local people in large numbers in 2014 and sold at small local markets for food. Similarly, in fish ponds of the Dniester Delta, immediately after the invasion of *M. nipponense*, pond owners began to catch them specifically to sell as food or for stocking (according communications from local fishermen).

Supplementary Material

Supplementary Table S1: List of localities and GenBank accession numbers of sequences used in the present study.
Supplementary Table S2: Variable sites in the alignment of *COI* sequences of the *P. fallax* complex with 5 different haplotypes of 17 sequences used in this study.
Supplementary Table S3: Variable sites in the alignment of *COI* sequences of *M. nipponense* with 9 different haplotypes of 20 sequences used in this study.

The Supplementary Material is available at https://doi.org/10.1051/kmae/2020032.

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