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The first discovery of the invasive marbled crayfish *Procambarus fallax* (Hagen, 1870) in Crimea

Первая находка инвазивного мраморного рака *Procambarus fallax* (Hagen, 1870) в Крыму

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KEY WORDS: invasion, Decapoda, Crustacea, crayfish, COI mtDNA, Crimean Peninsula.
КЛЮЧЕВЫЕ СЛОВА: инвазия, Decapoda, ракообразные, раки, COI мтДНК, Крымский полуостров.

ABSTRACT. Data on the discovery of the invasive marbled crayfish *Procambarus fallax* (Hagen, 1870) (Decapoda: Cambaridae) in the western Crimea, in the lower streams of the Alma and Belbek rivers, is presented. This is the first report of its presence on the peninsula, adding another invasive species to the list in the Russian Federation. Phylogenetic analysis reveals that the crayfish found in Crimea share the same genetic haplotype as other invasive subpopulations from Europe, Sweden, Japan, and parts of Florida. The European subpopulation has a single haplotype, suggesting a relatively recent introduction, likely from a single parent within its native range in Florida. In the case of the lower reaches of the Alma and Belbek rivers, we propose a hypothesis based on the possible involvement of large migratory birds, such as hissing swans and ducks, and plants attached to their paws in the spread of juvenile crayfish to nearby water reservoirs.

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РЕЗЮМЕ. Представлены данные об обнаружении инвазивного мраморного рака *Procambarus fallax* (Hagen, 1870) (Decapoda: Cambaridae) в западном Крыму, в нижнем течении рек Альма и Бельбек. Это первое свидетельство его присутствия на полуострове и находка еще одного инвазивного вида в Российской Федерации. Филогенетический анализ показал, что раки, обитающие в Крыму, имеют тот же гаплотип, что и инвазивные субпопуляции из Европы, Швеции, Японии, и некоторых районов Флориды. Европейская субпопуляция имеет единственный гаплотип, что указывает на относительно недавнюю интродукцию этого вида, возможно, от одного родителя из естественного ареала во Флориде. В случае с низовьями рек Альма и Бельбек мы предлагаем гипотезу, основанную на возможном участии крупных перелетных птиц, таких как лебеди-шипуны и утки, и растений, прикрепленных к их лапам, в распространении молоди раков в близлежащие водоемы.

Introduction

The marbled crayfish *Procambarus fallax* (Hagen, 1870) (Decapoda: Cambaridae) is a species native to fresh water habitats in southern Georgia and Florida, USA [Hagen, 1870; Hobbs, Hobbs, 1989]. It was introduced into Germany in 1995 as an ornamental aquarium pet from the USA, and since then it has spread rapidly throughout Europe as an invasive species. In 2017, based on the specimens from Germany, the European subpopulation was described as a separate species, *Procambarus virginalis* Lyko, 2017 [Lyko, 2017], which is represented by exclusively triploid parthenogenetic specimens, the only known case of decapod crustacean that obligatory reproduces by apomictic parthenogenesis [Scholtz *et al.*, 2003; Martin *et al.*, 2007; Vogt *et al.*, 2008]. Early genetic studies suggested that the clonal population of marbled crayfish descended descended from a single individual of *P. fallax* about 25 years ago [Martin *et al.*, 2010a]. Lately, it was proven that both parental haplotypes of *P. virginalis* were inherited from the sexually reproducing parent subpopulation of *P. fallax* found in the Everglades of Florida, where triploid individuals are also present [Gutekunst *et al.*, 2021].

The parthenogenetic subpopulation of the European origin may even differ from the native population in the USA in its ability to tolerate a wide range of environmental conditions, including low oxygen levels and temporary exposure to temperatures lower than 8 °C [Seitz *et al.*,

2005; Souty-Grosset *et al.*, 2006; Feria, Faulkes, 2011] and even to survive under direct ice cover [Pfeiffer, 2005]. Other authors argue that the optimal temperature for these crayfish is significantly higher (18–25 °C) than in winter in many temperate water bodies in Europe, and, accordingly, they may not have the potential for widespread distribution [Martin *et al.*, 2010a, b]. Probably due to new biological features and anthropogenic releases, parthenogenetic individuals of *P. fallax* (as *P. virginalis*) have colonized various freshwater habitats across Europe since the second half of the 20th century, and has recently even expanded to Madagascar, China, Taiwan and Japan, and some northern regions of North America, such as Saskatchewan and Ontario in Canada (e.g., Sánchez *et al.* [2024]). The findings of this species in Dnepropetrovsk and the middle reaches of the Dnieper River (e.g., Novitsky, Son [2016]) indicate that these crayfish can form stable and breeding populations in the temperate zones [Chucholl, Pfeiffer, 2010]. The species is a nocturnal and omnivorous feeding on a variety of food sources, including algae, aquatic plants, insects, and small invertebrates (e.g., Lipták *et al.* [2019]).

Currently, there is no proven conclusive evidence of harm from the introduction of *P. fallax* from North America to European waters. However, there is concern that the introduction of crayfish from North America could threaten local crayfish species due to competition for resources, predation, and the potential transmission of pathogens. The most well-known and serious pathogen affecting Eurasian crayfish populations is *Aphanomyces astaci* Schikora, 1906, the causative agent of plague among non-North American crayfish [Chucholl, Schrimpf, 2016; Jussila, Edsman, 2020]. Although other crayfish pathogens [Grandjean *et al.*, 2019; Stratton *et al.*, 2023; Zingre *et al.*, 2023] have not been extensively studied due to their less severe effects on crayfish, they still pose a potential risk [Longshaw, 2011].

In 2015, individuals of *P. fallax* (reported as *P. virginalis*) were firstly discovered in several locations in Ukraine: an old flooded quarry in Dnepropetrovsk City (48°30’19.15” N; 35°06’08.56” E) (now Dnipro City), a cascade of ornamental ponds (46°26’53.85” N; 30°45’12.54” E) in Odessa City [Novitsky, Son, 2016]; and lately in Kharkiv Area [Son *et al*., 2020]. These were the easternmost known locations for the species in Europe at the time, but it is clear that the species is spreading eastwards. Here, we present the first discovery of the marbled crayfish *P. fallax* in Crimea. The individuals of this species were found in the lower reaches of two rivers, the Alma and Belbek, which flow into the Black Sea on the western coast of the peninsula.

Materials and Methods

Crayfish were caught using a hand net and then fixed in a 90% ethanol solution. The crayfish collected in the Belbek River were genotyped and their morphological features were examined using an Olympus SZX10 light stereomicroscope. The crayfish collected in the Alma River were raised to adulthood in an aquarium, and then their live coloration (color pattern) was photographed. The total body length (tl., mm), measured from the distal margin of the rostrum to the posterior margin of the telson, was used as a standard measurement. The studied material is deposited in the author’s personal collection at the A.N. Severtsov Institute of Ecology and Evolution of the Russian Academy of Sciences, Moscow, and A.O. Kovalevsky Institute of Biology of the Southern Seas of RAS, Sevastopol, Russia.

Total genomic DNA was extracted from muscle tissue using the innuPREP DNA Micro Kit (AnalitikJena, Germany). The COI mtDNA gene marker was amplified using universal primers LCO1490 (5′–GGTCAACAAATCATAAAGATATTGG–3′) and HC02198 (5′–TAAACTTCAGGGTGACCAAAAAATCA–3′) under standard protocol conditions [Folmer *et al.*, 1994]. PCR products were then sequenced using Genetic Analyzer ABI 3500 (Applied Biosystems, Waltham, Massachusetts, USA) and BigDye 3.1 (Applied Biosystems, Waltham, Massachusetts, USA) with forward and reverse primers. The dataset of aligned sequences of COI mtDNA gene markers of *Procambarus* spp. were obtained from the GenBank (NCBI) database (see Fig. 1D).

A consensus dataset of aligned sequences (~606 base pairs) was obtained using MEGA 7.0 [Kumar *et al.*, 2016]. A phylogenetic analysis was conducted with PhyML 3.0 [Guindon *et al.*, 2010] with several models based on BIC (Bayesian Information Criterion) and AIC (Akaike Information Criterion) showing the congruent results.

Results

Order Decapoda Latreille, 1802
Family Cambaridae Hobbs, 1942
Genus *Procambarus* Ortmann, 1905

Procambarus fallax (Hagen, 1870)

Fig. 1A, B.

Material examined. Russian Federation, Crimean Peninsula: 2 immature spcms (tl. 25–30 mm), Sevastopol area, in a small canal at the mouth of the Belbek River near Lubimovka village, 44°39′41.6″N 33°32′47.7″E, hand net sampling, coll. I. Marin, 15.12.2022; 2 spcms (tl. 61–69 mm), the lower current of Alma River near Peschannoe village, 44°50′30.6″N 33°36′47.7″E, hand net sampling, coll. S. Statkevich, 19.04–31.05.2024.

Taxonomic features. *Procambarus fallax* differs from the rest of alien cambarids of Europe in its specific marble coloring and very small chelipeds, which had palm about half of the length of carapace. In all European crayfish, the palm of the claw is also either equal to the length of the carapace in females, or significantly exceeds it in males. The other distinctive feature from European crayfishes is a rostrum with subdistal marginal spine and lacking median carina.

This species can be reliably distinguished from congeners within the genus *Procambarus* by the following features: lateral half of ventral surface of ischium of third maxilliped lacking conspicuous mat of long plumose setae; annulus ventralis bell-shaped with an S-shaped sinus, ventrally elevated cephalic region bisected by narrow trough leading caudally into median depression or s-shaped sinus, and post-annular sclerite slightly narrower than annulus [Kawai *et al*., 2009; Lyko, 2017; Sun *et al*., 2020].

Genetic features. Phylogenetic analysis has revealed that the crayfish found in Crimea share the same haplotype as invasive subpopulation in Europe, Sweden, Japan, and certain areas of Florida (e.g., Son *et al.* [2020]). A single haplotype observed in the European subpopulation suggests a very recent introduction and spreading of this species, possibly from a single parthenogenetic parent (e.g., Gutekunst *et al.* [2021]).

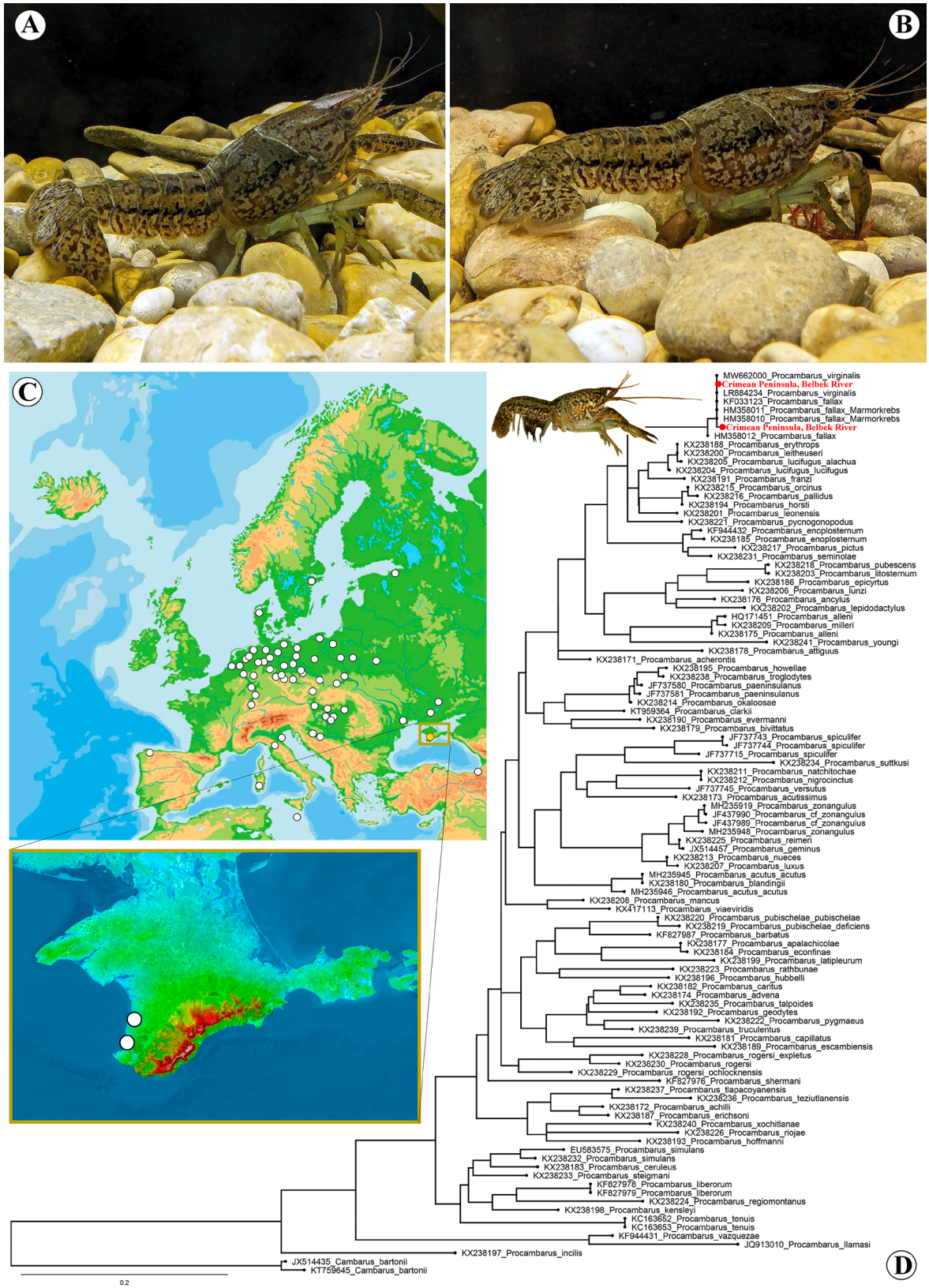

Fig. 1. A, B — live coloration of the specimens of *Procambarus fallax* (Hagen, 1870) from the lower stream of the Alma River, Crimea; C — distribution of *P. fallax* in the Europe and Crimea; D — phylogenetic relationships of the discovered specimens of *Procambarus* from the Belbek River, Crimea.

Рис. 1. А, Б — прижизненная окраска особей *Procambarus fallax* (Hagen, 1870) из нижнего течения реки Альма, Крым; В — распространение *P. fallax* в Европе и Крыму; Г — филогенетические связи обнаруженных особей *Procambarus* из реки Бельбек, Крым.

Coloration. The body, especially the carapace, and the telson, the uropods and the limbs are marbled, which is why it gets its name, especially this coloration is noticeable on the sides of the carapace, which is usually dark brown (see Fig. 1A, B).

GenBank accession numbers. PV544386, PV544387.

Distribution in Europe. Currently, the invasive range of the species in Europe extends from the north to the Gulf of Finland, Estonia and Sweden, south to Sicily and Sardinia, west to the Iberian Peninsula, and east to the Dnieper River (Ukraine), and now the Crimean Peninsula (Russia) (see Fig. 1C).

Discussion

The discovery of *P. fallax* in western Crimea is the first indication of its presence on the territory of the Peninsula, adding another invasive species to the list in the Russian Federation. Interestingly, invasive decapod crustaceans are not abundant, especially among freshwater species that can inhabit cooler temperate regions. However, North American freshwater crayfish *P. fallax*, *Procambarus clarkii* (Girard, 1852), *Faxonius limosus* (Rafinesque, 1817) (Cambaridae), and Signal crayfish *Pacifastacus leniusculus* (Dana, 1852) (Astacidae) have now established stable populations in neighboring countries to Russia, including Poland, the Czech Republic, Ukraine, Belarus (e.g., Soto *et al.* [2023]; Haubrock *et al.* [2024]; Ion *et al.* [2024]). Moreover, *F. limosus* has been found in freshwater reservoirs in the Kaliningrad region [Burba, 2010; Begunova, Sudnik, 2023; Alekhnovich, 2024], while *P. leniusculus* has been already found in lakes in the Pskov [Borisov *et al.*, 2011], Kaliningrad [Gusev *et al.*, 2012] and Leningrad regions [Tamulyonis *et al*., 2023].

This finding also suggests that crayfish *P. fallax* from the European parthenogenetic subpopulation can tolerate lower temperatures than previously thought (for example, Seitz *et al*. [2005]; Souty-Grosset *et al.* [2006]; Feria, Faulkes [2011]). These crayfish are likely to establish stable populations in areas where winter air temperatures fall close to 0 °C. For example, on the day when crayfish were discovered in the lower reaches of the Belbek River, the daytime air temperature was +2 °C, while the nighttime air temperature was around 0 °C; the water temperatures in the river was about 6–8 °C. This may explain why crayfish have only been found in rivers, as lentic (stagnant) water bodies (ponds, lakes and others) in this part of Crimea often freeze for short periods during the winter, while rivers do not completely cover with ice. However, even in rivers, water temperatures can reach very low values during winter. These conditions are probably far from optimal for this species, but the parthenogenetic method of reproduction allows even one surviving individual to restore or create a population in a new location [Chucholl *et al.*, 2012].

Phylogenetic analysis has shown that the crayfish collected in Crimea share the same haplotype with invasive subpopulations from all over Europe, Southern Scandinavia, Japan, Madagascar and certain areas of Florida (e.g., Son *et al.* [2020]). A single haplotype has been observed in the European subpopulation, confirming the very recent introduction of this species, likely from several or even a single parent from its native range. Additionally, this single haplotype supports the parthenogenetic reproduction mode for the entire European subpopulation [Martin *et al*., 2007], including Crimea. The distribution of crayfish is thought to follow the course of major rivers (e.g., Sanchez *et al.* [2024]), although some known locations, such as Odessa, are situated at a considerable distance from the freshwater sections of major rivers [Novitsky, Son, 2016]. In addition, even in areas where crayfish have been known for a long time, such as in Germany, their distribution is not continuous but rather highly fragmented [Sánchez *et al.*, 2024], in a contrast to the passive distribution over the course of a river, which implies a continuous range. In the case of the lower streams of the Alma and Belbek rivers, we propose a hypothesis based on the assistance of large migratory birds, such as hissing swans (*Cygnus olor* (Gmelin, 1789)) or ducks, and plants adhering to their paws, in the spread of juvenile crayfish. These birds usually use the studied localities where crayfish are found as resting places in Crimea. It is unlikely that crayfish arrived in these habitats in Crimea from aquariums, as there are only few small rural villages located near these discovery sites. Furthermore, crayfish have not been still found in areas with larger cities that have many aquarium owners (pers. observ.). Secondly, a single haplotype (see above) indicates that all crayfish originate from the same parthenogenetic subpopulation, while crayfish that still enter aquariums are imported from different locations, including native habitats in the United States. It is worth noting that the marbled crayfish coexists with the epigean *Niphargus potamophilus* Birštein, 1954 (Amphipoda: Niphargidae) in the lower Belbek River. This is still the only one known location of this niphargid species in the Crimea, and it has also been suggested that the species could be transported from one water body to another by migratory birds [Palatov, Marin, 2021; Marin, Palatov, 2023].

In conclusion, the new research suggests that the invasive species has expanded its range, including into colder regions in the east, and may continue to spread to other areas of Russia. Despite its small size of approximately 50–60 mm in adulthood, this crayfish provides excellent food for larger fish in the ecosystem. We believe that it faces significant predation pressures, so its overall impact on the ecosystem may not be substantial, except for the potential transmission of certain diseases.

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