ABSTRACT

Morhun H.M. Features of microevolution and adaptation in alien invertebrate species due to invasion into the Azov-Black Sea basin. – Qualification scholarly paper: a manuscript.

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This dissertation studies the invasions of invertebrate animals in water bodies of the Azov-Black Sea basin and specifies the features of microevolution in these populations as well as their phenotypic diversity.

Exotic animals are believed to be useful objects to study the processes of geographical isolation, hybridization with closely related species, and adaptation to new natural environments. Such studies help to better understand microevolutionary processes occurring during invasion, since genetic diversity of non-native animals is determined by the founder effect (random genotypes from the native habitat). New information on non-native populations contributes to the knowledge of their ecology, systematics, distribution and the variability of these species that can be shaped by new ecological conditions that may be other than their native ones.

The Azov-Black Sea basin is characterized by great anthropogenic pressure and high abundance of biotopes, which makes it a donor, recipient, and transit region for many non-native species. Taxonomic identification of a number of alien species of this region (cnidaria, molluscs, crustaceans, and ringworms) using modern molecular methods, comparison of their genetic diversity with the native region and studies of morphological diversity are helpful to answer several regional questions, in particular add to the understanding of evolutionary pathways (e. g. singular and multiple of invasions), which became the aim of our study. For this aim, we studied the morphological variability of non-native

populations, identified taxa by molecular markers, and investigated the phylogenetic structure of several model species. We also identified a number of native taxa of the Azov-Black Sea basin.

This study was undertaken using materials from the collection of the Department of Zoology and Animal Ecology of V. N. Karazin Kharkiv National University, specimens from the collection of the Institute of Marine Biology of the NAS of Ukraine, and specimens collected by the author personally. In total, there were about 83 samples analyzed. First, the external morphology of the animal was examined, then molecular tests were performed (genomic DNA was isolated and *cox1* gene sequences were obtained), and then phylogenetic analyses were performed using newly generated sequences and those taken from GenBank.

Our BLAST analysis of invertebrates of the Azov-Black Sea basin confirmed the presence of 47 native and 19 foreign taxa belonging to four phyla of animals. After taxonomic revision by morphological and molecular methods, the genetic structure of a few model species was revealed (leech *Helobdella europaea*, crustaceans *Procambarus virginalis* and *Macrobrachium nipponense*, mollusks *Arcuatuala senhousia*, *Rapana venosa* and *Corbicula* sp.). For two species (*R. venosa* and *Corbicula* sp.), we additionally applied multivariate statistical analysis of morphometric data using traditional morphometric methods and geometric morphometry to reveal morphological variability. A total of five molecular phylogenetic analyses and two morphometric analyses were performed. For 13 non-native species, DNA sequences were obtained for the first time for populations of these animals from the Azov-Black Sea basin. *Helobdella europaea* and *A. senhousia* were recorded in the region for the first time in the course of the field survey in the study area. *Procambarus virginalis* and *M. nipponense* were recorded in new localities, indicating the range expansion throughout the Azov-Black Sea region.

The phylogenetic analysis revealed an extremely low genetic diversity in sequences of *H. europaea* both in the type locality (Germany) and in other regions of invasion. This low genetic diversity of *H. europaea* in all invasion regions is interpreted as a corroboration of the hypothesis of the introduction of this species from Central or South America (founder effect). However, the low diversity can also be explained by selective pressure in respiratory conditions of aquaculture. The variability of the coloration pattern of longitudinal stripes was documented, which probably may be an adaptation to the color of substrates, since pigmentation affects the visibility of leeches.

Phylogenetic analysis of the rapa whelk *R. venosa* demonstrated that the population of Odesa Bay shared one haplotype. It is known from previous publications as the only one that occurs both in all invasion regions and the native region. The high conchological variability of the Odesa population was analyzed using traditional and geometric morphometry and differences in shell elongation were found. These tendencies in shape changes were attributed to the mollusk's adaptation to the substrate, in particular the need to hunt burrowing mollusks in the sand. Consequently, it is likely that the high diversity of prey in Odesa Bay is a driver for the high conchological heterogeneity of the rapa whelks. No significant differences were found in the shape of shells between males and females, but males had larger sizes than females, which may be a result of the need of the latter to spend additional energy to produce offspring. Also, it has been demonstrated that the Black Sea population of the mollusks is smaller in size than the native ones and has a contrasting sculpture and massiveness of the shells.

The phylogenetic analysis of the nucleotide sequences of the parthenogenetic crayfish *P. virginalis* revealed one haplotype in Dnipropetrovsk and Kharkiv regions identical to the sequences of crayfish from other invasion regions throughout the world, suggesting the founder effect. This species originated from the parental gonochronic *P. fallax* from the USA by autotriploidy during invasion. Compared to the parental species, *P. virginalis,* after invasion in Europe, increased in size, became more fertile, and doubled its average lifespan.

This is attributed to epigenetic mechanisms, in particular to intensive DNA methylation.

New localities and biotopes for the shrimp *M. nipponense* were found, from which this species had not previously been reported. The regional question about the origin of the Dniester population (Chinese versus Japanese) was also resolved. It was identified that the Ukrainian shrimps share the same haplotype with those from China. The work also revealed an invasive history of the species: shrimp was gradually recorded first in Uzbekistan and Kazakhstan, then Russia, Belarus, Moldova, and only after that appeared in the Dniester basin. The phylogenetic analysis of the shrimp found that there are different haplotypes in invasion regions, for instance, in the neighboring Caspian basin other haplotypes are present. This may indicate a multiple invasion of the species rather than a single invasion (founder effect).

A new non-native bivalve in the region, *A. senhousia*, was recorded. The molecular analysis revealed two distinct haplotypes unknown from previous studies and, in particular, from the native range. The large genetic distance between these haplotypes is the result of the phenomenon of double uniparental mtDNA inheritance, which is specific to this species: two different mitogenomes occur simultaneously: F- (females) and M-type (males). The phylogenetic analysis involving sequences from previous studies showed that our M-type sequence belongs to the cold-water clade, revealed by Asif & Krug (2012), together with other sequences from Europe. The formation of two clades (warm-water and cold-water) is thought to be associated with temperature preadaptation of these molluscs. Our field survey of this species for the period of 2018-2021 years revealed its successful establishment in the local community. The phenotypic variability of *A. senhousia* in the coloration of the clams, the expression of sinuous bands and the presence of light and dark radial areas was observed. The pigmentation pattern and color of clams depend on heredity, so that the phenotypic variability is probably explained by adaptation to different environments where mollusks occur.

Cytonuclear mismatches in *Corbicula* were found that resulted in the wrong morphotypes identification by means of DNA barcoding. The Ukrainian sample of *Corbicula* (morphotype R) from the Danube River has the same haplotype *cox1* as morphotypes Rlc and S. The *Corbicula* clams in the Danube consisted of two different morphotypes. A study of the shape variations of this Ukrainian Danube clams with some closely related species (included material from many different regions of the world) identified four morphospecies: *C. fluminalis, C. fluminea, C. leana* and an unknown species *Corbicula* sp., which is most probably of a hybrid origin between *C. fluminalis* and *C. leana,* since it has intermediate characters: sculpture of *C. leana* and hinge of *C. fluminalis*. In addition, the variability of *C. leana* clams in the native region was studied and two "geographic" morphotypes ("Japanese" and "Korean") were distinguished.

Based on the molecular diversity and variability, the model species (leech *H. europaea*, crustaceans *P. virginalis* and *M. nipponense,* molluscs *A. senhousia*, *R. venosa* and *Corbicula* sp.) were classified in the context of the concept of Genetic Paradox of Biological Invasions (Estoup et al. 2016), where the paradox is that, during invasion, exotic organisms that are not initially adapted to their novel environment and exhausted by bottleneck effect during invasion, are able to establish and thrive in the new environment, often to such an extent that they can even outcompete native, presumably locally adapted, species. According to our classification results, the “genuine” genetic paradox of biological invasions is characteristic to *P. virginalis* and *Corbicula* sp. which, although having extremely low genetic diversity, nevertheless demonstrate significant phenotypic variability. For *H. europaea* and *R. venosa*, it may be both a “genuine” and “spurious” paradox, since their *cox1* haplotypes could have been selected due to selective pressure or preadaptation. There is no genetic paradox in *M. nipponense* invasions, as phylogenetic analysis indicates that several haplotypes occuring in different invasion regions. For *A. senhousia*, we also assume the absence of a paradox since there is a significant genetic diversity in the invasion regions and even the haplotypes which are now known only from the Ukrainian population.

The scientific novelty of this work is that we molecularly determined the taxons of a number of non-native and native invertebrates. Two new non-native taxa not previously reported for the region were discovered. Features of the microevolution of model non-native species were identified. For the first time, significant phenotypic variability was found for these species in the region.

The theoretical value of the work includes the fact that microevolutionary processes in populations of alien species in the Azov-Black Sea basin were investigated on the basis of molecular phylogenetic data. The morphological variability of these animals in the region was analyzed. A classification of model species in the context of the Genetic Paradox of Biological Invasions was proposed when the invasion is characterized by a "genuine" paradox, a "spurious" paradox, or when no paradox is present. The process of establishment of a new exotic mollusk *A. senhousia* in a new community is examined. Morphological analysis of *Corbicula* clams, based on shell characters and geometric morphometrics, was effective for distinguishing the morphospecies within the genus.

The practical value of the work is confirmed by the fact that the results of the study will contribute to the development of population control strategies for non-native species, which will reduce the harmful effect to native species and ecosystems in the water bodies of the Azov-Black Sea basin. The materials of the study can be used in the courses of "Zoology of Invertebrate", "Educational practice in the zoology of invertebrates", "Zoology" and "Molecular evolution and phylogenetics". Moreover, a special manipulator for photographing *R. venosa* was constructed.

Key words: invasion, morphology, phylogenetics, aquaculture, adaptation, fauna, variability, aquatic invertebrates.