

PRELIMINARY RESULTS REGARDING PHYLOGENY OF THE NOBLE CRAYFISH (DECAPODA, ASTACIDAE, *ASTACUS ASTACUS*) IN SERBIA

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Abstract:

The noble crayfish is one of the three autochthonous species that inhabit the freshwater ecosystems of Serbia, along with stone and Danube crayfish. The noble crayfish has a complex historical and genetic status shaped by geological events, habitat loss, pollution, translocations, and reintroductions of both autochthonous and allochthonous crayfish species. That led to the disruption of the species genetic structure, mixing, and loss of populations across Europe. According to recent data, its populations in the freshwater ecosystems of Serbia are significantly reduced, so it has the status of a "strictly protected species". The genetic structure of the species must be known for endangered species conservation. Unfortunately, there is lack of such data for the territory of Serbia, which due to its position on the Balkan Peninsula, was an important refuge during the glaciation period. In this paper, the genetic structure of seven crayfish populations in freshwater ecosystems of Serbia was examined. Analyzes were performed on the *COI* and *16S rRNA* genes of mitochondrial DNA. The study results showed a significant diversity of *COI* and *16S rRNA* haplotypes compared to already described haplotypes. Three haplotypes were detected, of which Hap26 is the most common and was detected in five studied populations. Haplotypes Hap47 and Hap49 were detected in one and two populations, respectively. The results obtained in this study, together with previously published morphometric data, represent a good starting point for further genetic and population research, which are the basis for the proposal of conservation measures.

Key words: crayfish, phylogeography, Balkan Peninsula, mtDNA

1. Introduction

The freshwater crayfish are significant components of biodiversity, known as key species for the normal functioning of the food chain [1, 2] and with an important ecological role in the proper functioning of a freshwater ecosystem [3, 4].

The noble crayfish (*Astacus astacus*, L. 1758) is a native European species with a large range of distribution. During the last century, its populations diminished or became extinct [5, 6] due to climate change, pollution, habitat loss, but also because of translocations and reintroductions of both autochthonous and allochthonous species [7, 8], but also due to parasites and diseases carried by non-native species [9]. For the territory of Serbia, its area of occurrence decreased more than 65% between

1960 and 2006 [10]. Previous phylogeographic studies reported significant genetic diversity in south-eastern Europe, with most divergent ones in the southern part of the Balkan Peninsula [11, 12, 13, 14, 15]. The objective of this study was to examine the phylogeny of noble crayfish on the territory of Serbia, considering Serbian central position on the Balkan Peninsula, one of the biodiversity hot spot areas and refugium during the Pleistocene [16].

2. Materials and Methods

A total of 53 noble crayfish specimens from seven ecosystems on the territory of Serbia (Table 1) were collected by hand or trapped by baited LiNi traps. From each specimen, one pereopod was taken and preserved in 96% ethanol, while specimens were released after sampling.

Genomic DNA was extracted from the pereopod muscle tissue using the Sigma GenElute Mammalian Genomic DNA Miniprep Kit (Sigma-Aldrich, USA) following the manufacturer's protocol. Mitochondrial *COI* and *16S rRNA* were amplified as in Gross et al. [15]. Purification of PCR products and sequencing were performed by Macrogen Service Centre (Amsterdam, Nederland).

Sequences were manually edited in SEQUENCHER 5.4.6 (Gene Codes Corp., USA) and aligned with AliView 1.27 [17]. The genealogical relationships among haplotypes were presented as a Median-Joining network [18] created in PopArt (<http://popart.otago.ac.nz/>). For the construction of the network, in addition to the ones from this work, 75 reference sequences from Schrimpf et al. [12] and Laggis et al. [13] were included in the analysis. The genetic variability of the sample set was estimated by the haplotype (*Hd*) and the nucleotide (*Pi*) diversity [19] using the program DnaSP v6 [20].

Table 1. Sample locations with a summary of *COI* and *16S rRNA* haplotype frequencies and number of samples (N)

Population	Drainage	Coordinates	N	Hap26	Hap47	Hap49
Kačer River	Sava/Danube	44.222N 20.280E	9	-	-	9
Kozeljjska River	Sava/Danube	44.211N 20.366E	2	-	-	2
Resnički stream	Great Morava/Danube	44.090N 20.937E	5	5	-	-
Petrovačka River	Great Morava/Danube	44.052N 20.878E	12	11	1	-
Korenica Reservoir	Great Morava/Danube	44.228N 21.413E	7	7	-	-
Gazivode Reservoir	Great Morava/Danube	42.942N 20.648E	9	9	-	-
Grište Reservoir	Timok/Danube	43.812N 22.232E	9	9	-	-
Total			53	41	1	11

3. Results and discussion

Sequencing of concatenated *COI* and *16S rRNA* provided readable sequences of 768 bp.

Among 53 individuals originating from seven sampling sites, a total of three haplotypes were revealed (Table 1). Haplotype Hap26 was the most frequent, occurring on five out of seven localities, followed by the Hap49, recorded on the two localities. On each locality, one haplotype was recorded, except the Petrovačka River, which harbors, besides specimens characterized with Hap26, one specimen with Hap47.

The haplotype diversity within the whole sample set was 0.365, while the recorded value of the nucleotide diversity was 0.00181. Haplotype diversity values can be characterized as moderate comparing to populations from the Black Sea basin ($Hd = 0.851$), Adriatic Sea basin ($Hd = 0.576$), and the North and Baltic Seas ($Hd = 0.316$, $Hd = 0.276$) [12].

In the MJ network (Fig. 1), haplotypes were grouped within four already described lineages [12] and two groups [13]. Haplotypes Hap26 and Hap47, from this work, were within Lineage 3, with two

mutational steps separating them. The third recorded haplotype from the territory of Serbia, Hap49, was within Lineage 2. It was separated with eight mutational steps from the Hap26 and ten mutational steps from Hap47.

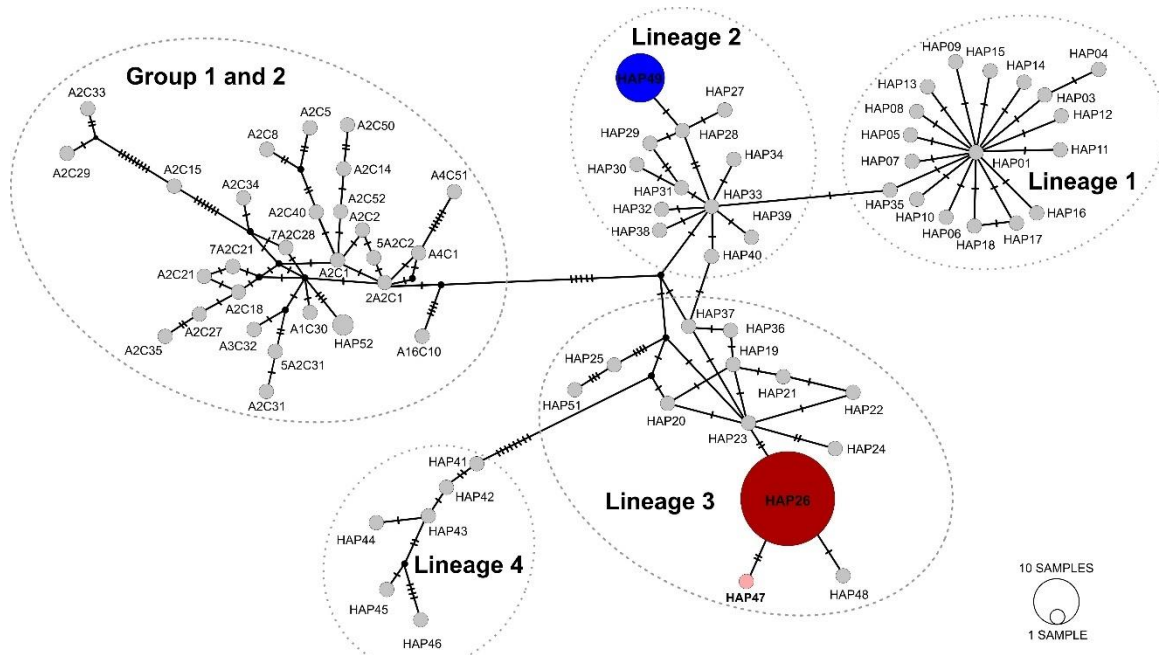


Fig. 1. Median-Joining network based on *COI* and *16S rRNA* sequences. Detected haplotypes are represented by colored circles, which size is proportional to haplotype frequencies in the overall sample. The numbers of variable sites are indicated with hatch marks

4. Conclusions

The present study's results could aim in maintaining the initial step for future effective conservation plans and management strategies for protecting the genetic diversity of *A. astacus* populations on the territory of Serbia.

Acknowledgments

This work was supported by the Serbian Ministry of Education, Science and Technological Development (Agreement No. 451-03-9/2021-14/200122).

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