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Ecological Truth

Editors
Radoje V. Pantovic
Zoran S. Marković

EcoIst '16

12 – 15 June 2016
Hotel "BREZA" Vrnjacka Banja,
SERBIA

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TOWARDS ASSESSING GENETIC DIVERSITY OF *Theodoxusdanubialis* (C. PFEIFFER, 1828)(GASTROPODA; NERITIDAE) FROM CENTRAL BALKAN

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ABSTRACT

Specimens from three populations of native neritid snail *Theodoxus danubialis* (C. Pfeiffer, 1828) from the Balkan (the Una, Lepenica and Nišava Rivers) were processed. Obtained 16s rRNA sequences, along with additional sequences taken from GenBank were analysed. Maximum likelihood consensus tree showing relations of plotted sequenceswas discussed. Sequences from the Nišava River sample differ from the other *T. danubialis* sequences, pointing to specific16s rRNA haplotype. In order to better access intraspecific variability and diversity of *T. danubilis*, an use of less conservative markers should be implemented.

Key words:16s rRNA, haplotype, Danube nerite, Balkan.

INTRODUCTION

Theodoxus danubialis, Danube nerite, as the name suggests, is native to the Danube and its tributaries. Although not on the IUCN red list of threatened species, this species is critically endangered in the northern part of its range: Germany [1], Austria [2] and Czech Republic (Red List of the molluscs (Mollusca) of the Czech Republic). Danube nerit, once common in large rivers, today mainly has been observed in the Danube tributaries and smaller watercourses [3].

The 16s RNA is one of the most popular and widely used mitochondrial markers in phylogeography studies. The *Theodoxus* genus, although being one of the oldest and the most interesting snail lineages, and despite efforts of a few authors is yet rather understudied. This is particularly true in the case of the Balkan, which although considered as one of the most important regions of Europe, regarding biodiversity, is till date poorly studied. Hence, we aim to assess genetic variability and diversity of

members of this snail genus in the Balkan. Here we present results of preliminary analysis regarding 16s rRNA variability in the case of *T. danubialis* from this region.

MATERIAL AND METHODS

For our analysis we used snail specimens from the samples from the Una, Nišava and Lepenica Rivers. All samples were fixed in 95% ethanol and stored in the zoological collection of Institute for Biological Research Siniša Stanković (IBISS), Belgrade, Serbia.

An identification of snails was performed using appropriate determination keys [4, 5].

In total, 10 individuals from three populations of *T. danubialis* were processed. The DNA was extracted from the snail material (the foot and the head of snail specimens) by using the kit for the isolation of genomic DNA from eukaryotes tissues (AccuPrep® Genomic DNA Extraction Ki, Bioneer Inc. Alameda, CA, USA). Universal primers 16Sar and 16Sbr [6] were used, and PCR products were obtained according to the protocol given in [7]. An automatic sequencing of amplified 16s rRNA fragments, were done bidirectional, on an automatic sequencer by chain-termination method (ABI 310, AppliedBiosystems, Foster, CA, USA). Obtained amplicons were sequenced using BigDye Terminator Cycle Sequencing v.3.1 kit (PE Applied Biosystems, Foster City, CA, USA), and the sequences were read using an appropriate software (ABI software v.5.1 and Sequencing Analysis SeqScape software, v.2.5). In order to assess the quality of acquired forward and reverse 16s rRNA sequences (chromatograms), and to eliminate ambiguities in these sequences, the software Finch TV, v.1.4.0 (http://www.geospiza.com) was used.

For analysis purposes, an additional 16s r RNA sequences of *Theodoxus* and *Nerita polita* (as outgroup) were taken from the GenBank (http://www.ncbi.nlm.nih.gov/genbank). Assess numbers and basic information of these sequences are provided in the Table 1.

An alignment of allanalysed sequences was done by Clastal W method [8] in the software MEGA, ver. 5.2 [9]. Phylogenetic analysis and cladograms were performed by Maximum likelihood method (ML) [10]in the same software. In order to assess the most suitable ML model, the lowest BIC (Bayesian Information Criterion) scores were tested [11] by phylogenetic Bootstrap analysis, a popular statistical method for estimating the mean values in phylogenetic analyses [12].

Table 1. Basic data on 16Sr RNA sequences taken from GenBank

Species	Code	Access Number (GenBank)	Locality
T.danubialis	TD005	AY771236	Garda, Italija
T.danubialis	TD137	AY771238	Kustány, Mađarska
T.danubialis	TD093	AY771236	Pischelsdorf, Austrija
T.prevostianus	TP001	AY771254	Bad Vöslau, Austrija
T.prevostianus	TP032	AY771255	Kàcs, Mađarska
T. euxinus	TE001	AY771239	Bilhorod-Dnistrovs'kyi, Ukrajina
T. fluviatilis	TF396	AY771245	Rugia, Nemačka
T. fluviatilis	TF225	AY771241	Rheinsberg, Nemačka
T. fluviatilis	TD121	AY771237	Esztergom, Mađarska
T. transversalis	TT001	AY771259	Edelény, Mađarska
Neritapolita	/	KJ458472	

*sequences coded TD005 and TD093, belong to the same haplotype (assess number AY771236), so for the analysis was used only one of them (TD005)

RESULTS AND DISCUSSION

The chromatograms of forward and reverse 16S rRNA sequences of 10 specimens from three snail populations (Una (code "U"), Lepenica (L) and Nišava (N)) have been reviewed by software FinchTV (ver. 1.4.0) and from further analysis were excluded three sequences (L1, N3 and U5) due to insufficient quality (i.e. high ambiguity). The minimum length of remaining sequences selected for further analysis was 296bp (base pairs). A substitution of purine bases (adenine (A) instead of guanine (G)) at 154th nucleotide site in sequences from the Nišava was spotted, pointing to a separate haplotype. Moreover, registered pyrimidine ambiguity (cytosine (C)/thymine (T)) at the 159th nucleotide sitein case of one "Nišava sequence" (N1) could indicate another potential base substitution, i.e. another new haplotype. Testing of most suitable ML models for analysis, was carried out in accordance with the lowest BIC scores, and as the most suitable model singled out "Tamura 3-parameter" substitution model with gamma-distributed substitution pattern, which then was applied to perform phylogenetic analysis. The obtained bootstrap consensus tree based on 500 replicates was shown in Figure 1.

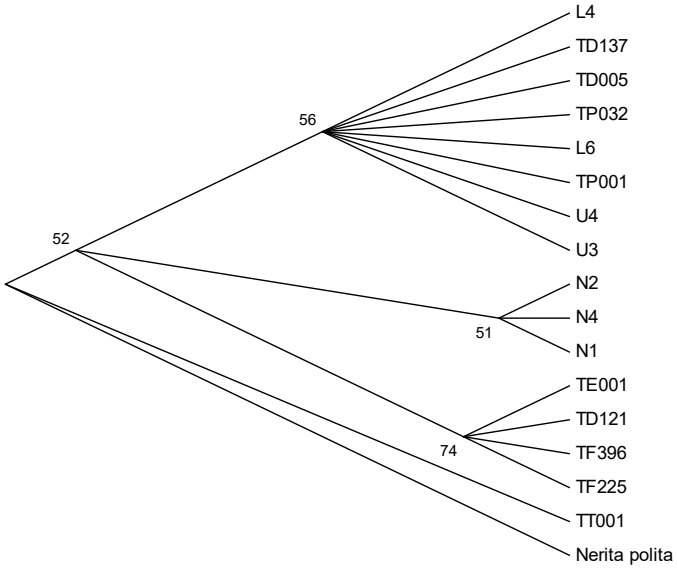

Figure 1. Consensus ML (Maximum likelihood) tree based on 16SrRNA sequences. Bootstrap values are shown for branches with more than 50% support. The tree is rooted with outgroup *Nerita polita*. The sequences taken from GenBank are coded as follows: species are labelled as TF for *T. fluviatilis*, TD for *T. danubialis*, and TT for *T. transversalis,* with three-digit numbers as suffix; our sequences of *T. danubialis* are labelled as N (Nišava), L (Lepenica) and U (Una), with single-digit numbers.

Observing this result a few points should be made. Firstly, used 16Sr RNA marker is not so suitable for resolution at finer taxonomic scale(intraspecific analysis) in

case of these snails, given its pronounced phylogenetic conservatism and relative youth especially close-related species of snails. This is consistent with the literature, so besides Bunje [7,13], 16Sr RNA was rarely had been used for analyses of this genus. In GenBank only two 16Sr RNA haplotypes of *T. danubialis* (Table 1) could be found till date. Our analysis thus gains importance, particularly by indicating presence of a new, separate haplotypes from the Nišava sample. These "Nišava haplotypes" were separated from the others by only one (or two, in case of N1 specimen) base/nucleotide substitution. Similar result with low degree of divergence for this snails was obtained by [14] in his comprehensive analysis of *T. danubialis*/*T. prevostianus* based on another conservative mitochondrial marker COI.

Similar research to [14] was conducted by [15], but samples from the eastern part of *T. danubialis* range (the Drina, Nišava and Crni Timok Rivers) were included as well. According to these authors *T. danubialis* populations from the area we investigated here, belong to so-called "central group", which includes the "Danube clade" (D2; the Nišava sample), and the "Sava clade" (S; The Kupa River and the Drina, as the samples geographically nearest to our samples). In the same research a segregation of haplotypes from the Nišava (and the Crni Timok) relative to western haplotypes from this "central group" was observed. These COI data are in accordance with our findings (based on 16s rRNA), and point to distinction of the eastern (the Nišava) populations. Moreover, a more than one "Nišava haplotype" could be present, according to our 16s r RNA analysis. Observed genetically differences of the Nišava populations were supported by morphological differences, registered, as well [3]. In order to better assess genetically variability of *T. danubialis*, an additional samples from this area should be analysed, and additional and less conservative molecular markers used. As there is general lack of data from the eastern part of *T. danubialis* range (and the Balkan) our result could be considered as a small complement to knowledge regarding genetic diversity of these snails.

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REFERENCES

1. Jungbluth J. H., Von Knorre D. (2009). Rote Liste der Binnenmollusken [Schnecken (Gastropoda) und Muscheln (Bivalvia)] in Deutschland. 6. revidierte und erweiterteFassung 2008. *Mitteilungen der Deutschen Malakozoologischen Gesellschaft*, **81**: 1-28.
2. Reischütz A., Reischütz P. L. (2007). Rote Liste der Weichtiere (Mollusca) Österreichs, 150 pp.
3. Marković V. (2014). Morfološkavarijabilnost i distribucija rodaTheodoxus Montfort, 1810 (Neritomorpha, Gastropoda) u centralnom delu Balkanskog

poluostrva i na južnom obodu Panonske nizije. Disertacija, Univerzitet u Beogradu, 142.str (eng. Morphological variability and distribution of Theodoxus Montfort, 1810 (Neritomorpha, Gastropoda) in the central Balkan and southern edge of Pannonian plain)

4. Pfleger V. (1999). A field guide in colour to Molluscs. Blitz Editions, Leicester, United Kingdom, 216 pp.
5. Glöer P. (2002). Süsswassergastropoden Nord-und Mitteleuropas: Bestimmungsschlüssel, Lebensweise, Verbreitung, ConchBooks, Hackenheim, **73**: 327 pp.
6. Hillis D. M., Mable B. K., Larson A., Davis S .K., Zimmer E.A. (1996). Nucleic acids IV: sequencing and cloning. In: Hillis, D.M., Moritz, C., Mable, B.K. (Eds.), Molecular Systematics. Sinauer, Sunderland, Mass, pp. 321–381.
7. Bunje P. M., Lindberg D. R. (2007). Lineage divergence of a freshwater snail clade associated with post-Tethys marine basin development. *Molecular Phylogenetics and Evolution*, **42**: 373-387.
8. Thompson B. (2004). Exploratory and confirmatory factor analysis: Understanding Concepts and Applications. American Psychological Association, Washington, 195 pp.
9. Tamura K., Peterson D., Peterson N., Stecher G., Nei M., Kumar S. (2011). MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular biology and evolution*, **28**: 2731-2739.
10. Tamura K. (1992). Estimation of the number of nucleotide substitutions when there are strong transition-transversion and G + C-content biases. *Molecular Biology and Evolution*, **9**: 678-687.
11. Nei M., Kumar S. (2000). Molecular Evolution and Phylogenetics. Oxford University Press, New York, 333 pp.
12. Felsenstein J. (1985). Confidence limits on phylogenies: An approach using the bootstrap. *Evolution*, **39**:783-791.
13. Bunje P. M. (2004). Diversification and comparative phylogeography of neritid gastropods. *Unpublished PhD Dissertation*, University of California, Berkeley, pp. 411.
14. Bunje P. M. (2007). Fluvial range expansion, allopatry, and parallel evolution in a Danubian snail lineage (Neritidae*: Theodoxus*). *Biological Journal of the Linnean Society*, **90**: 603–617.
15. Fehér Z., Zettler M., Szabo K. (2009). An attempt to reveal the systematic relationship between *Theodoxus prevostianus* (C. Pfeiffer, 1828) and *Theodoxus danubialis* (C. Pfeiffer, 1828) (Mollusca, Gastropoda, Neritidae). *Mollusca*, **27**: 95-107