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Molecular-genetic identification of chameleon goby *Tridentiger trigonocephalus* (Gill, 1859) in the Black Sea

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Abstract

The article focuses on the molecular-genetic identification of the chameleon goby, which has recently become established in the Black Sea basin. The natural range of the chameleon goby are the seas of the Far-East. Genetic variation was studied for the 16S rRNA mtDNA gene. The obtained genetic data were compared with similar data of chameleon goby from the natural range and other species from the *Tridentiger* genus. We determined that, according to the 16S rRNA gene, the identification of the goby from the mouth of the Chernaya River as a species of *Tridentiger trigonocephalus* was confirmed, and the parameters of the variability indicate an extremely low genetic variability for this gene. According to the authors, the adaptive success of the chameleon goby in the conditions of the Black Sea basin is similar to such of invasive species, namely pumpkinseed (*Lepomis gibbosus*) and stone moroko (*Pseudorasbora parva*).

Key words: chameleon goby, *Tridentiger trigonocephalus*, invasion, genetic diversity, developmental trajectory, adaptive success.

Introduction

Chameleon goby – *Tridentiger trigonocephalus* (Gill, 1859) is a typical inhabitant of the coasts, estuaries and lower reaches of the rivers of the basins of the Sea of Japan, the Yellow, East China, South China Seas and the adjacent areas of the Pacific Ocean in the areas of the coastal territories of Russia, Korea, Japan and Philippines (Berg 1949; Pinchyk 1978; Courtenay et al. 1986; Akihito & Sakamoto 1989; Meng et al. 1994; Annotirovanny catalog..., 1998). This species belongs to the category of small euryhaline fishes which can live both in marine and saline waters (up to fresh water), often inhabits mussel and oyster plantations. Its active expansion outside the natural range is assumed to have started since the mid 1980s as a result of non-

intentional introduction with transportation of oysters (Courtenay et al. 1986) and ballast water (Eschmeyer et al. 1983). It is quite likely that one of the vectors of its distribution is aquarium husbandry, because gobies are undemanding as to the conditions of maintenance and have a bright natural colour. This thesis statement about aquarium husbandry is confirmed by the fact that since the late 1980s, simultaneous expansion has been observed in the distribution of three species of tripletooth gobies *Tridentiger trigonocephalus* (Gill, 1859), *Tridentiger barbatus* (Günther, 1861) and *Tridentiger bifasciatus* (Steindachner, 1881). At the same time, *Tridentiger bifasciatus* was often considered a synonymous species with *Tridentiger trigonocephalus* (Berg 1949; Pinchyk 1978; Akihito & Sakamoto 1989), their status as separate species was recognized only in the mid 1990s (Matern & Fleming 1995). Mass distribution around the world of the representatives of *Tridentiger* genus began in the mid 1980s and is due to the global warming, intensification of marine transport communications and enhancement of mineralization of freshwater ecosystems (Molnar et al. 2008).

Modern data indicate that the chameleon goby – *Tridentiger trigonocephalus* has experienced the greatest success. By the end of the 1990s it was found in marine ecosystems of the West Coast and in many internal water bodies of the USA (Welcomme 1988; Fuller et al. 1999), Oceania and Australia (Welcomme 1988), in 2006 it was found in the Mediterranean Sea and the coast of Israel (Goren et al. 2009) and in the Black Sea – in the estuary part of the Chornaya River and Sevastopol Bay (Boltachev et al. 2007). In all the new areas, chameleon goby has successfully naturalized, especially in the areas with developed oyster and mussel farms (Meng et al. 1994). At the same time, the extent of scientific knowledge of the genetic variability of *T. trigonocephalus* in its newly settled range is obviously insufficient. So far only the mitochondrial and whole-genome sequences (Wei et al. 2016), separate genes of mtDNA: COI, 12S, 16S, cyt *b* (Mukai et al. 1997; Miya et al. 2015; Bingpeng et al. 2018), and also nuclear gene –RyR3 (Gu & Chen 2013) have been studied, usually for the purposes of taxonomic identification and to some extent phylogeny. These studies dealt with areas of the natural range of the chameleon goby (Japanese Islands, Taiwan, coast of mainland China in the area of city of Fuzhou and the mouth of the Yangtze River). Therefore, the main objective of our study was the analysis of population-genetic changes in the chameleon goby (*Tridentiger trigonocephalus*) in the newly colonised water area of the Black Sea basin – estuary of the Chornaya River.

Materials and methods

In July 2018, in the estuary of the Chornaya River, 7 specimens of chameleon goby were caught. After the catching, pieces of the muscular tissue were taken and fixated in 96% ethanol for the further laboratory analysis. Total DNA was extracted from the muscular tissue using innuPREPDNA Mini Kit (AnalytikJena Company, Germany). Analysis of nucleotide sequences was performed for mitochondrial gene 16S rRNA. Amplification of fragment 16S rRNA of the length of 583 bp was conducted in previously developed primers (Palumbi et al. 1991): forward 16SH 5'–CCGGTCTGAACCTCAGATCACGT–3' and reverse 16SL5'–CGCCTGTTTATCAAAAACAT–3'.

As amplifying mixtures we used ready-to-use lyophilized reaction mixtures (master mixes) made for amplification of DNA in the volume of 20 µl. Mastermixes for separate reaction contained all components necessary for the reaction, including inhibited for “a hot start” Taq DNA polymerase, dNTP and stain for electrophoresis (PLC Genlab Scientific-production firm, Moscow). The PCR products generated were sequenced at Evrogen Ru (Moscow, Russia) in the forward and reverse directions. A phylogenetic tree with the calculation of bootstrap supports of ramification nodes (1,000 replications) was developed in MEGA 7.0 program using the method of “neighbor joining” (Neighbor Joining, NJ) (Saitou, Nei 1987). The parameters of genetic variability were calculated with DNASP 5.10 software pack (Librado, Rozas 2009). During the development of the phylogenetic tree, for comparison, we used data for haplotypes of 16S rRNA taken from the GenBank (NCBI) for 2 individuals of species *Tridentiger bifasciatus* (KF415482, JN244650), *Tridentiger obscurus* (MF663787, KT601092), *Tridentiger trigonocephalus* (KT282115, NC029738) and one specimen of *Tridentiger barbatus* (JX536694). As the external group, we used species *Acanthogobius hasta* (Temminck & Schlegel, 1845) – KM891736, phylogenetically remote from the *Tridentiger* genus of the Gobionellinae subfamily. During the calculations of the parameters of variation, we used the data obtained using 16S rRNA of all individuals of *T. trigonocephalus* from the estuary of the Chornaya River which we examined and the data from NCBI for *T. trigonocephalus* (haplotypes NC029738 and KT282115) (see Table 1).

Table 1. Collection details of the generated and GenBank sequences in the present study.

GenBank Accession No	Collection localities	References
MK691657	Chornaya River, Crimea	This Study
NC029738	China, Shanghai	Wei H. et al., 2016
KT282115	China, Shanghai	Wei H. et al., 2016
KF415482	United Kingdom	Agorreta A. et al., 2013
JN244650	China, Zhejiang	Jin X. et al., 2015
MF663787	China, Zhejiang	Gong L. et al., 2018
KT601092	Republic of Korea	Park C.E. et al., 2015
JX536694	China, Zhejiang	Jin X. et al., 2015
KM891736	China, Guizhou	Wang X.D. & Chen D.X., 2015

Results

Taxonomic identification of chameleon goby from the Chornaya River revealed that all the tested individuals had one haplotype of the 16S gene. We recorded it in the NCBI base (haplotype MK691657). During the development of the phylogenetic tree of gobies of the *Tridentiger* genus of the Gobionellinae subfamily, we determined that the closest to the haplotype we found was the fragment of the 16S rRNA gene of *Tridentiger trigonocephalus* (haplotypes recorded by other researchers in the NCBI by numbers NC029738 and KT282115) and which forms with them one clade (Fig. 1).

At the distance of Nei representatives of other closely related species were placed at considerable distances and form separate clades. This result was also confirmed when calculating the *p*-distance (see Table 2).

Table 2. Matrix of *p*-distances (%) between haplotypes of 16S rRNAmDNA gene of gobies of *Tridentiger* genus. Conventional signs: 1–4, 9, 2C, 7C haplotypes of chameleon gobies from the Chornaya River. The remaining haplotypes of NCBI: KT282115, NC029738 = *Tridentiger trigonocephalus*; KF415482, JN244650 = *Tridentiger bifasciatus*; MF663787, KT601092 = *Tridentiger obscures*; JX536694 = *Tridentiger barbatus*; KM891736 = *Acanthogobius hasta*.

№	Haplotypes	1	2	3	4	5	6	7	8	9	10	11	12	13	14
p/p															
1	1	X													
2	2	0	X												
3	KT282115	0,6	0,6	X											
4	3	0	0	0,6	X										
5	2C	0	0	0,6	0	X									
6	4	0	0	0,6	0	0	X								
7	7C	0	0	0,6	0	0	0	X							
8	9	0	0	0,6	0	0	0	0	X						
9	KF415482	65	65	65	65	65	65	65	65	X					
10	JN244650	65	65	65	65	65	65	65	65	0,6	X				
11	MF663787	44	44	44	44	44	44	44	44	71	71	X			
12	NC029738	0,6	0,6	0,6	0,6	0,6	0,6	0,6	0,6	65	65	43	X		
13	KT601092	44	44	43	44	44	44	44	44	71	71	0	43	X	
14	JX536694	44	44	43	44	44	44	44	44	68	68	8,9	44	8,9	X
15	KM891736	68	68	68	68	68	68	68	68	69	68	68	68	68	69

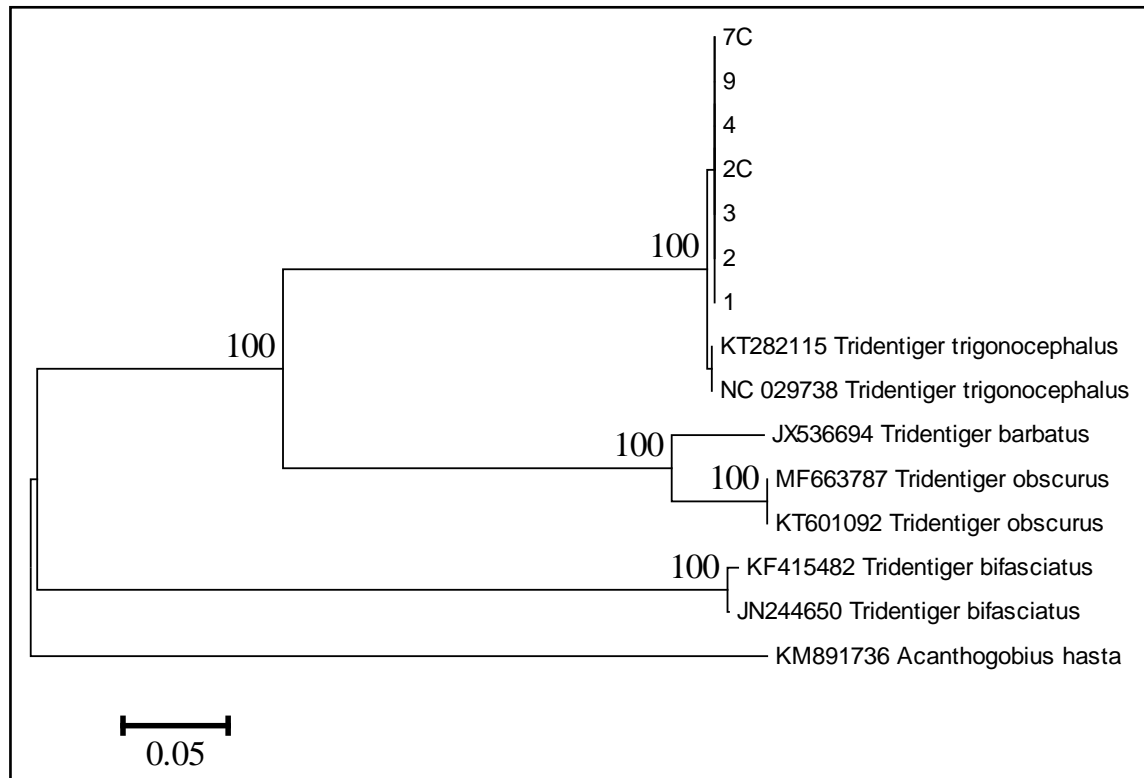


Figure 1. The Neighbor Joining tree reflects phylogenetic relations between found variants of nucleotide sequence (haplotypes) of the 16S rRNA gene in gobies of the *Tridentiger* genus. In ramification nodes, their bootstrap supports are indicated. Below the figure, the unit of measurement of the lengths of the branches is indicated = 0.05 nucleotides. *Conventional signs*: 1–4, 9, 2C, 7C = haplotype MK691657 of 16S rRNA of *Tridentiger trigonocephalus* from the estuary of the Chornaya River (water area of the Black Sea). KM891736 of *Acanthogobius hasta* – representative of the external group.

According to the values of *p*-distance, the distance between haplotypes from the estuary of the Chornaya River and three specimens of *T. trigonocephalus* from the Sea of Japan did not exceed 0.6%. The species *A. hasta* was distanced from the species of the *Tridentiger* genus by 68%, and distances from *T. barbatus*, *T. obscurus* reached 44%, and 65% with *T. bifasciatus*. Because among individuals of the population of the Chornaya River only one haplotype was present – MK691657, the variability of chameleon goby from this new region according to all parameters was practically zero. This clearly differed from a small selection of chameleon gobies from their native range (the Sea of Japan and the East China Sea) (see Table 3).

Table 3. Parameters of variability of the 16S rRNA gene in *T. trigonocephalus* in the natural part of the range and the population of the Chornaya River. *Conventional signs*: N = number of individuals, h = number of haplotypes, Hd = haplotypic diversity, Pi = nucleotide diversity, S = share of polymorphic sites, Eta = total share of mutations.

Population	N	h	Hd	Pi	S	Eta
Chornaya River	7	1	0.0	0.0	0	0
Sea of Japan	3	2	0.667	0.450	363	363

Selection was small because in NCBI in total 3 haplotypes were deposited for the 16S rRNA gene of this species (Mukai et al. 1997; Wei et al. 2016; Huang et al. 2016).

Discussion

The obtained results for nucleotide sequences of the fragment of the 16S rRNA gene confirm the earlier morphologically determined identification (Boltachev et al. 2007) of the goby from the estuary of the Chornaya River to species *Tridentiger trignocephalus*. The extremely low level of both haplotype and nucleotide diversity can indicate realization of any of the known evolutionary effects which underlie significant impoverishment of genetic diversity – edge of range population effect, founder effect or bottleneck effect (Dobzhansky 1951; Müller 2001; Altuhov 2003). We assume that since the chameleon goby successfully naturalized in the easternmost part of the Mediterranean Sea (Katsanevakis et al., 2012) and there are data on finds near the coast of Israel (Goren et al., 2009), we expect that in the near future we should observe its expansion into the western parts of the Mediterranean Sea. However, one must take into account the fact that the introduction of a chameleon goby into the Black Sea basin is random. This is an unintentional introduction of the species. Therefore, the version of the spread of a chameleon goby to the entire Mediterranean from the Black Sea basin is most likely unlikely. The argument about the unintentional introduction of a chameleon bull in connection with the importation of a giant oyster from the seas of the Pacific Ocean basin (Courtenay et al., 1986) has more arguments. Similarly, as was the case with *Rapana venosa* (Slynko, E.E. et al., 2020). The situation with genetic variation of *T. trignocephalus* is similar to such of invasive populations of pumpkinseed – *Lepomis gibosus* (Slynko et al. 2015). In new populations in the Black Sea basin, we found only one original haplotype in each species – for the gene cytb mtDNA in *Lepomis gibosus* and 16S rRNA in *Tridentiger trignocephalus*. Also, a practically complete absence of variation was seen. It worth to note that molecular study on other Black Sea organisms such as a scallop showed also significant lack of genetic variability when compared to the Mediterranean form (Slynko, Y.V et al., 2020).

As previously determined, especially with the example of Black Sea sprat (Slynko et al. 2015), pumpkin seeds (Slynko et al. 2015) and moroko stone (*Pseudorasbora parva*) (Slynko et al. 2017), in situations involving a significant decrease of genetic diversity in new populations, adaptive success is achieved through polymorphism of development programs or ontogenetic trajectories. Most likely, the adaptive success is achieved in a similar way by *Tridentiger trignocephalus* in the populations of the estuary of the Chornaya River.

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