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Diversity, morphology and karyotypes of species from genus *Neogobius* (*Perciformes*; *Gobiidae*) at coastal zone of the Caspian Sea (Aktau city)

Abstract: The paper presents the results on the diversity, morphology and karyotypes of species from the genus *Neogobius* collected during the summer seasons 2012-2016 in the shallow coastal zone of the Caspian Sea (Aktau city). A total of 6 species of goby fish belonging to two genera were found: *Neogobius* Iljin, 1927 and *Ponticola* Iljin, 1927. Most species are widespread in the Northeast and Middle Caspian. The results of the morphological analysis did not reveal any significant differences between the populations of goby fish of the Northern and Middle Caspian, and also in comparison with the literature data. Chromosome kits and karyotypes detected in the observed gobies *N. fluviatilis pallasii*, *N. melanostomus affinis*, *N. caspius*, *P. gorlap* and *P. syrman eurystomus* are represented in the diploid set mainly by 46 acrocentric chromosomes, and in one species previously identified by us as *N.sp (Piljini)* $2n = 40$ chromosomes with a number of chromosome arms (NF) 46.

Key words: diversity, morphology, karyotypes, goby fish, Caspian.

Introduction

The biological diversity of animals in the Caspian Sea basin is characterized by the greatest number of endemics of a diverse taxonomic rank – from subspecies to families [1]. A significant number of endemics from all taxa were found in the Middle Caspian. According to Kazanchev E.N., the number of endemics at the genus level is 8.2%, species – 43.6%, subspecies – 100% [2].

The fauna of the fish of the Caspian Sea and adjacent areas of deltas and freshened bays and lagoons (continuously occurring species, migrating or occasionally setting) include 119 species and subspecies (including controversial status) belonging to 52 genera from 15 families, of which 35 are from 12 genera belong to the family *Gobiidae* order *Perciformes* (*Perciformes*) [1; 2].

The fish of the *Gobiidae* family are one of the most complex in the taxonomic group of the Ponto-Caspian region [3]. Representatives of this family are not fully studied, and the status of some species is subject to discuss. This is mainly due to the large variability and in some cases to the difficulty of analyzing the external morphological features used in the taxonomy of gobies. The greatest difficulties are

usually encountered in determining the species, some groups and genera contain many closely related and hardly distinguishable forms [4].

According to the literature, at present the family of gobies in the Caspian Sea includes the following genera: *Anatirostrum* Iljin, 1930, *Babka* Iljin, 1927, *Asra* Iljin, 1941, *Benthophilus* Eichwald, 1831, *Caspiosoma* Iljin, 1927, *Chasar* Vasilieva, 1996, *Hyrsanogobius* Iljin, 1928, *Knipowitschia* Iljin, 1927, *Mesogobius* Bleeker, 1874, *Neogobius* Iljin, 1927, *Ponticola* Iljin, 1927, *Proterorhinus* Smitt, 1899 [1]. The largest discussions about the taxonomic composition arose around the genus *Neogobius*. For the genus the Black Sea-Caspian gobies (*Neogobius* Iljin, 1927) are the senior synonym for the simultaneously published names of the generic group (*Neogobius* and *Apollonia*), *Neogobius* chosen by Berg L.S. [5]. The genus *Neogobius* now includes four species, three of which live in the Caspian Sea: *N.caspicus* (Eichwald, 1831) – caspian goby, *N.melanostomus affinis* (Eichwald, 1831) – caspian round goby, *N.pallasii* (Berg, 1916) – caspian monkey goby. In the genus *Ponticola* Iljin, in 1927 there are at least 10 species, in the Caspian Sea there are three: *P. goebelii* (Kessler, 1874) – caspian ratan goby, *P.gorlap* (Iljin, 1949) – caspian big-head

goby, *P.syrman eury stomus* (Kessler, 1877) – caspian shirman goby [6; 7; 8].

The ichthyofauna of the coastal zone of the Kazakhstan part of the Middle Caspian is poorly understood. This applies to almost all taxa of fish fauna. However, several publications have recently appeared on the distribution, morphology, biology and karyology of the gobies of the coastal zone of Aktau [8, 9, 10]. In the present work, in addition to these studies, the results are presented for the species diversity of the gobies, as well as morpho-biological and karyological characteristics for 4 species.

Materials and methods

The material was collected in summer seasons of 2012-2016, in the coastal zone of the Caspian Sea near the city of Aktau (Mangistau region of the Caspian Sea), on Figure 1 the collection points are marked with the sign (▲).

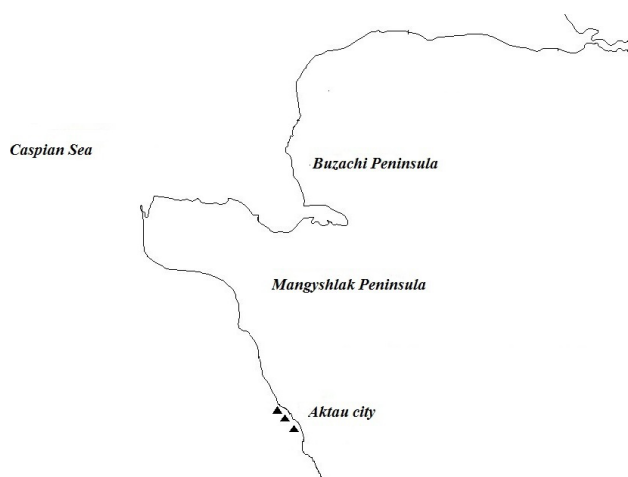


Figure 1 – Collection points within Aktau city.

During the period of research, 200 specimens of goby fish of various species were collected, of which 30% had a bridal detail. In determining the fish of the genus *Neogobius* and *Ponticola*, standard sources, descriptions and determinants were used [1; 2; 11; 12].

Fish catching was done with a net (mesh 8 mm, width 55 cm). The captured material was fixed in a 4% formalin solution. Methods of morphological and cytogenetic analysis of fish were applied. In the collection and analysis of the material, the methods commonly used in ichthyological studies were used [13]. The analysis included 4 biological indexes, 4 meristic and 22 plastic signs. Statistical processing

of primary data was carried out according to standard methods of variation statistics and included: *max* – maximum, *min* – minimum, calculation of mean values of the indicator for each indicator (*M*), their error (*m*) [14]. Chromosome preparations were prepared from fixed pieces of tissue according to *Kligerman A., Bloom S.* [15]. Chromosomes were classified according to the Levan system, distinguishing four main types of chromosomes [16]. The analysis of the preparations was carried out with the help of the Microscope *MicroOptix MX 300 T*, metaphase photographs were taken with a digital camera *MicroOptix C600*. The search for metaphase plates was carried out at low magnification; the best metaphases were photographed at 100x under the immersion oil. The layout of karyotypes was made using the *Adobe Photoshop 12.0.3* program.

Results and their discussion

During the research period near the city of Aktau, we found 6 species of goby fish: caspian monkey goby, caspian round goby, caspian goby, caspian big-head goby, caspian shirman goby and *N.sp (P.Iljini)*. The most numerous for the whole period of observations were two species of gobies – eastern-caspian big-head goby and the caspian round goby. At the same time, their ratio over the years was the same (Table 1). The catches dominated the above species, which occur with a wider range of salinity than, for example, caspian monkey goby and caspian goby.

Morphological analysis of meristic and plastic signs revealed no significant differences between the genera *Ponticola* and *Neogobius*. Of the 22 plastic signs calculated for the length of the body, significant differences were noted only for 7. At the same time, of the 10 plastic signs of the head part, significant differences were observed in 6 indices. The percentage of differences in meristic characters from the total number of indicators studied was 15%. This indicates that the external morphology of gobies belonging to different genera is sufficiently similar, the latter circumstance complicates their species identification.

Biological characteristics, morphological and karyological analysis were carried out on 4 species of gobies, of which 3 species belong to the genus *Neogobius*, and one, previously described by us as *N.sp.*, is referred to the genus *Ponticola* Ilijn, 1927. Morphological and biological features and karyology of gobies we described earlier [10]. It is shown that the caspian round goby is characterized by high morphological variability depending on the conditions and habitat [17]. According to several au-

thors [18; 19] in the populations of caspian round goby, males predominate. Analyzing the sex structure of goby fish in our catches, the ratio of males and females goby close to 1:0, juvenile specimens also was not found. This is due to the biology of breeding gobies, males in a bridal detail guard the oviposition with caviar, without leaving it even for nutrition, while females after oviposition leave for a longer distance.

There is no complete morphological and karyological data on the caspian goby, which is poorly studied in the Kazakhstan part of the sea. Probably,

this is due to laborious and incorrect definition of a species or morphological similarity with a caspian round goby.

For all the goby species of the Mangistau region of the Caspian Sea, we conducted cytogenetic analysis for the first time, with the subsequent determination of karyotypes and formulas. The chromosome data on six species of goby fishes are presented, from which more than 300 metaphases have been studied and a “modal” number, i.e., the number of chromosomes most common for a given fish species was esteemed (Table 1).

Table 1 – Species diversity of goby fish (*Gobiidae*) in different years near Aktau, the number of chromosomes and the fundamental number of arms

Species, subspecies	Frequency of occurrence by years					2n	NF
	2012	2013	2014	2015	2016		
<i>P. Iljini</i>	+	+	+	+	+	38-40	46
<i>N. fluviatilis pallasii</i>	-	-	-	-	+	46	46
<i>N. melanostomus affinis</i>	+	+	+	+	+	46	46
<i>N. caspius</i>	-	-	+	+	+	46	46
<i>P. gorlap</i>	+	+	-	-	-	46	46
<i>P. syrman eurystomus</i>	+	-	-	-	-	38	46
Total	16	30	42	31	81		

Note: 2n is a diploid set of chromosomes; NF is the fundamental number of chromosomal arms

A karyotype of 46 acrocentric chromosomes is considered to be generic or basic for many gobies, including the genus *Neogobius* [4]. According to the results of conducted studies on goby fish of some authors, including ours, the chromosome set of the caspian round goby consists of 46 acrocentric chromosomes, with a fundamental number of chromosomal arms 46 [4; 9; 10; 20; 21].

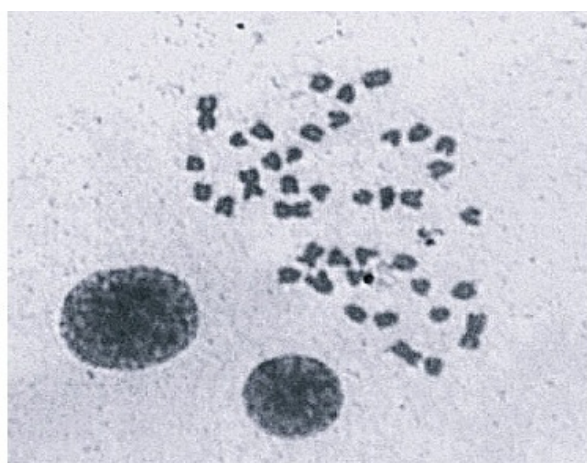
Our research has shown that in the studied gobies karyotype is represented mainly by $2n = 46$ acrocentric chromosomes, with the exception of *N.sp.* (*P. iljini*) in which the karyotype is represented by 38-40 chromosomes, at the same time $NF = 46$ (Fig. 2).

Previously obtained long-term data also allows us to separate the goby which was originally identified as the caspian big-head goby or *N. kessleri gorlap* because of similar morphological features, however, cytogenetic analysis showed that it has characteristic differences in the chromosome set. So, according to the literary sources *N. kessleri gorlap* in the diploid set has from 43 to 46 with $NF = 46$ [9]. In the population of *N.sp.* from the coastal zone of Aktau

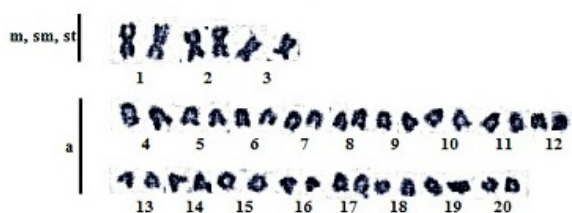
we found three cariomorphs with $2n = 38$, $2n = 39$; $2n = 40$; with the number of arms $NF = 46$. Thus, in all the studied cariomorphs, from 1 to 4 large metacentric and submetacentric chromosomes are present. The discrepancy in the number and morphology of the chromosomes is explained by the large number of Robertsonian translocations that significantly reduced the number of chromosomes in its karyotype. Thus, the goby *N.sp.* has in the diploid set no more than 40 chromosomes, which significantly differs from the caspian big-head goby, which implies its species independence [22].

According to molecular genetic studies conducted abroad, the data obtained make it possible to classify Black Sea-Caspian gobies on a fundamentally new and higher taxonomic level and contribute to the solution of some issues at the level of genera. Thus, a certain morphological similarity between the species of the modern genera *Gobius*, *Neogobius*, *Babka*, *Ponticola* and *Proterorhinus* caused them to be considered within the same genus *Gobius*, i.e. to unite representatives of two different subfamilies into

one genus [23]. However, many authors [24; 25] returned to the scheme of Ilyin B.S. [26], which singled out subgenera in the genus *Neogobius* in 1927. At the same time, molecular data made it possible to impart a phylogenetic context to Ilyin's classification and substantiated the genus status of the subgenus *Ponticola*.



A



B

Figure 2 – A is the metaphase plate of *P. iljini*; karyotype formula: $2n = 40; 3m + 3sm + 34a$; $NF = 46$, B – karyogram.

Recent results of the karyological and molecular genetic analysis of Russian scientists allowed separating into an individual genus, giving validity to the species *P. iljini*, or the so-called «eastern-caspian big-head goby», whose range is limited by the coast of the Mangyshlak Peninsula. On a phylogenetic tree constructed from the variability of the mitochondrial cytochrome b gene in the main species of Ponto-Caspian gobies, *P. iljini*, *P. gorlap* and *P. kessleri* form independent phyletic lines within the clade of the genus *Ponticola* [8].

At the same time, our long-term studies confirm the above thesis [22]. It should be noted that this species was previously registered by us as *N.sp.*

Thus, according to our latest and literary data in the Caspian Sea there are not three but four species

of gobies of the genus *Ponticola* Ilijin, 1927 of the 10 existing species.

The existence of the ecosystem of the sea sections under study is explained by the vast area of well-warmed habitats of representatives of the goby family. There are still many new species and subspecies waiting to be described.

The endemism of species and intraspecies forms of the family of goby species attests to a particularly energetic process of speciation [1; 27; 28].

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