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Cryptic Species of Ponto-Caspian Bighead Goby of the Genus *Ponticola* (Gobiidae)

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Abstract—Based on karyological data, the validity of the species *Ponticola iljini* is re-established; its distribution is limited to the coast of the Mangyshlak Peninsula. The morphological characteristics and the basic color pattern of *P. iljini* are very similar to *P. kessleri* from the Black Sea basin and *P. gorlap* which inhabits the northern, western, and southern Caspian Sea. These three allopatric species have ctenoid scales to varying degrees on the crown and nape (100% presence in *P. kessleri* and complete absence in *P. iljini*), and there are small differences in the shape of the head, the thickness of the caudal peduncle, and in the form of otoliths; at the same time, they are clearly differentiated by the number of chromosomes and the presence of marker submetacentric chromosomes in *P. iljini*. In the phylogenetic tree based on the variation of the mitochondrial cytochrome *b* gene in the main species of Ponto-Caspian gobies, *P. iljini, P. gorlap,* and *P. kessleri* form independent phyletic lineages within a clade of the genus *Ponticola*. Redescription of the species *P. iljini* is provided; the possible causes of the identified intraspecific variation in the shape of the otoliths and temporal bounderies of the beginning of divergence of the considered three cryptic species, as well as isolating barriers that led to allopatric speciation, are discussed.

Keywords: *Ponticola gorlap*, *P. kessleri*, *P. iljini*, karyotypes, mtDNA, morphology, taxonomy, phylogeny, origin
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INTRODUCTION

The bighead goby was described by Kessler (Kessler, 1857) as an independent species *Gobius platycephalus* from the Dniester River and its tributaries, and the Southern Bug. Since the name "*platycephalus*" has repeatedly been preoccupied in the genus Gobius (see *Catalog of Fishes...*, 2015), Günther (Günther, 1861) proposed a replacement name "*kessleri*" (based on materials from Kessler) for this species. The bighead goby from the Black Sea basin for the first time referred to as *Gobius platyrostris* (Nordmann, 1840) was subsequently regarded as an endemic Ponto-Caspian species (Kessler, 1874, 1877) and for this reason, among other endemic gobies of the Ponto-Caspian was classified in a separate genus *Neogobius* Iljin 1927, by Berg (1949). Thereafter, the structure of the genus *Neogobius* sensu Berg (1949) has been repeatedly discussed and, at present, the bighead goby and a number of phylogenetically closely related species belong to the genus Ponticola Iljin 1927, based on the data of molecular genetic studies (Neilson and Stepien, 2009a; Medvedev et al., 2013).

The first morphological differences between Caspian and Black Sea bighead gobies have been recognized as early as by Kessler (1874), who pointed out small differences between the two forms in the number of rays in the pectoral fin and distances from the snout to the anus and from the latter to the end of the caudal fin. However, Iljin (1956) only considered the Caspian bighead goby as an independent species and described it as *Gobius gorlap* from the area of the Mangyshlak Peninsula in his unpublished manuscript, which was subsequently lost. Berg (1949, P. 1091), referring to Iljin, published a brief description of the Caspian subspecies *Neogobius kessleri gorlap* (therefore, Iljin in Berg 1949 is the author of the taxon), according to which the Caspian gobies "differ greatly by yellowish coloration, not swollen and almost triangular head, lack of a round-meshy pattern on the cheeks and on the base of *P.*" Independent subspecies status of the Caspian bighead goby was retained in subsequent publications (Pinchuk, 1977; Kazancheev 1981; Miller, 1986), although Pinchuk (1977) showed that the features pointed out by Berg, as well as the external morphological characteristics studied by himself, are not suitable for the identification of the Caspian and Black Sea subspecies.

Comparative karyological study of bighead goby populations from the Dniester, the Dnieper, and the

from each other. Thus, it can be taken as a base for all the Sarmatian gobies, including the genus *Ponticola* (Vasil'ev and Grigoryan, 1992). The most evolutionarily advanced and sufficiently close karyotypes in the genus *Ponticola* are karyotypes of *P. kessleri* with $2n$ = 29–30 and NF = 46 and *P. eurycephalus* with $2n$ = 32–35 and NF = 46 (Table 6) (not including the species which retained the original karyotype with $2n$ = 46 and NF = 46). A second pair of karyologically similar species is formed by *P. gorlap* and *P. iljini* (Tables 5, 6). This observation corresponds to a pairwise separation of species on the phylogenetic tree.

By morphological characters, *P. eurycephalus* stands against the other three species differing by deeper caudal peduncle, a relatively deep head, whose depth is slightly less than or equal to its width, narrow interorbital distance and noticeably swollen upper lip (Pinchuk et al., 2003). In contrast to *P. kessleri,* morphologically similar forms of *P. eurycephalus* have never been mentioned in the Caspian basin. Thus, this species is the most morphologically divergent within the group of species *P. eurycephalus*–*P. kessleri*–*P. gorlap*–*P. iljini*. In karyological respect, the Black Sea bighead goby is the most divergent from the ancestral form. The absence of clear external differences between the three species of bighead gobies suggests a model of allopatric speciation without forming of prezygotic isolating mechanisms. In this case, the appearance in the Ponto-Caspian region of three cryptic species of gobies with deep genetic differences and a great external morphological similarity becomes obvious.

The beginning of the divergence of *P. kessleri* from the Caspian species can be dated not earlier than end of Miocene (5.8–5.0 million years ago) when the Pontian Sea-Lake was divided into two separate brackish pools: the late Pontian Sea-Lake in the Euxine sink and Babadzhan Sea-Lake in the southern part of the Caspian depression (Reid and Orlova, 2002), but not later than the Pleistocene (2.0–0.7 million years ago), when, after the retreat of the Apsheron Sea, the last link of the Caspian Sea with the Black Sea through the Manych depression disappeared. During the period of the largest transgressions, the Akchagyl (2.5–2.0 million years ago) or Apsheron stages (2.0–0.7 million years ago) (Reid and Orlova, 2002), the ancestors of *P. iljini* could have penetrated to the east coast of the Caspian Sea. Their isolation in the coastal area of the Mangyshlak Peninsula was provided by the existence of two hypersaline bays limiting the peninsula to the north and south and by the lack of a network of rivers on the peninsula.

The average salinity of the Caspian Sea is 12.85‰; in the northern Caspian Sea, it is 5–10‰; in the waters adjacent to the deltas of the Volga, Ural, and Terek, it is 2–4 ‰; in the Middle Caspian, it is 12.7 (decreases only in the area of the Sulak River delta); in the southern Caspian Sea, it is 13 ‰ (it is lower before Kura, Sefidrud, and Atrek river deltas) (Plotnikov and Aladin, 2010). *P. gorlap* inhabits the coastal areas of the northern, middle (by west coast), and south of the Caspian Sea and in the inflowing rivers; successfully spread in the reservoirs of the Volga; penetrated into the Tsimlyanskoe reservoir through the Volga-Don Canal; and migrated to the delta of the Don (Vasil'eva and Luzhnyak, 2013). The highest salinity at the places of distribution of *P. iljini* was observed in the lake Karakol: 13.43–13.92‰. At the same time, at the northern border of the Mangyshlak Peninsula in the shallow bays Mertvyi Kultuk and Kaidak, the water salinity is 30‰ and above, while it is up to 300–350‰ and above at the southern border of the peninsula in the Kara-Bogaz-Gol bay (Plotnikov and Aladin, 2010). According to data by Leont'ev (1961), the depression of the Kara-Bogaz-Gol was formed in the Late Pliocene–Early Pleistocene (approximately 2 million years ago), at almost the same time when the ancestors of *P. iljini* came to the coast of Mangyshlak and were isolated from *P. gorlap,* which was widespread along the Caspian coasts.

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