

## Karyology of *Mullus barbatus* (Pisces, Perciformes) from the Mediterranean basin

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**Abstract:** Data on karyotypes of *Mullus barbatus* from the Black Sea (Taman Peninsula, Russia) are presented. Chromosome preparations were made using cells of the anterior parts of the kidney and thymus. The karyotype of the population under study consisted of 44 diploid chromosomes with fundamental arm number 52. The chromosomal divergences in fishes with similar ecology and geographical distribution to that of *M. barbatus* are discussed.

**Key words:** Karyotype, chromosomal divergence, *Mullus barbatus*, Mullidae, Mediterranean basin

*Mullus barbatus* Linnaeus, 1758 belongs to the family Mullidae (goatfishes). This fish species is widely distributed in the eastern Atlantic (from the British Isles to Senegal) and in the Mediterranean Sea, the Black Sea, and the Sea of Azov (<http://www.fishbase.org/>).

Previously, based on some morphological traits, *M. barbatus* from the Black Sea was described as a separate subspecies, *M. barbatus ponticus* (Esipov, 1927). Comparative morphological analysis of traits used to identify subspecies from the Black Sea demonstrated that the morphological divergence between the populations from the Mediterranean and the Black Sea does not reach formal subspecies level (Vasil'eva, 2012). Phylogenetic analysis of different populations of *M. barbatus* from the Mediterranean basin based on three mitochondrial DNA genes showed a lack of genetic distance for allocation of the Black Sea subspecies *M. barbatus ponticus* (Keskin and Can, 2009).

Due to the complicated geological history of the Mediterranean basin (associated with significant geomorphological changes as a result of tectonic activity), and the extensive geographical distribution of *M. barbatus*, we have accomplished a karyological study of the species from an unstudied locality. The main goal of this study was to analyze karyotypes of *M. barbatus* from the Black Sea and from different parts of its range of the Mediterranean basin.

The material was collected in the Black Sea (Bugazskaya sand spit, Taman Peninsula). For karyotype analysis of *M. barbatus*, cells were used from the front part of the kidney and from the thymus. Chromosome preparations

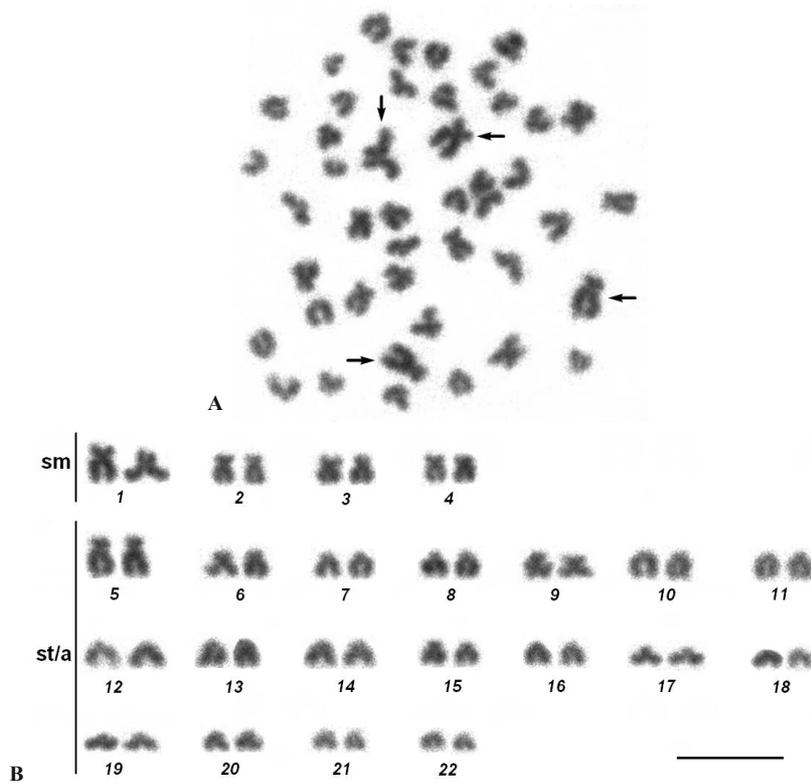
made using standard air-drying techniques and were stained with 5% Giemsa solution (concentrate, Merck). In total 56 metaphase plates from two individuals were studied. Chromosomes were classified according to the system of Levan (Levan et al., 1964) with calculation of the centromeric index using Leica Application Suite V3.3.0.

Chromosome preparations were studied using a Leica DM 1000 microscope. Photos of mitotic chromosomes (at metaphase) were taken with a Leica DFC 295 digital camera.

Karyological studies of *M. barbatus* from the Black Sea (close to the Bugazskaya sand spit) revealed a chromosomal complement containing  $2n = 44$ ,  $NF = 52$  (Figure 1A). The karyotype consists of 4 pairs of submeta- and 18 pairs of subtelo- and acrocentric chromosomes (Figure 1B). The largest chromosomes in the *M. barbatus* karyotype are presented by pairs of submeta- and subtelocentric chromosomes (Figure 1A).

Previously, the karyotype of *M. barbatus* was studied in only three localities of the Mediterranean basin (Table). In all populations the same diploid number of chromosomes, 44, was found. However, between some populations differences in the structure of the karyotype were discovered. This could be due to interpopulation differentiation as well as differences in the classification of chromosomes as described by various researchers (classification error can be associated with varying degrees of spiralization of chromosomes in studied metaphases). Data in the Table show that chromosomal polymorphism probably exists in the population of the Ligurian Sea. Chromosomal variability may be associated

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**Figure 1.** A) Mitotic chromosomes (at metaphase) of *M. barbatus* from the Black Sea. Arrows show the large submeta- and subtelocentric chromosomes. B) Karyotype of *M. barbatus*. Numerals indicate the paired chromosomes; sm: submetacentric chromosomes, st/a: subtelocentric and acrocentric chromosomes. Bar = 5  $\mu$ m.

with rearrangements, leading to a change of the position of the centromere, resulting in an increase in the number of two-armed chromosomes in the karyotype of *M. barbatus*. Such rearrangements (presumably pericentric inversions) are due to interpopulation differentiation of karyotypes between the two populations from the Black Sea (Table; Figure 2). Nevertheless, chromosomal divergence between *M. barbatus* populations from the Mediterranean basin is negligible in comparison with populations of *Uranoscopus scaber* (Figure 2). Geographical distribution and some ecological characteristics (pelagic eggs; adults are coastal

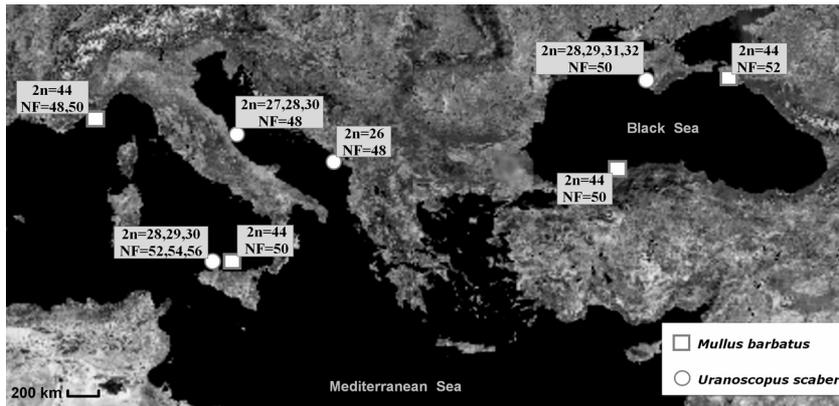
demersal species; pelagic larvae and juveniles) in this species and in *M. barbatus* are similar.

Polymorphism and interpopulation variability of the karyotype are characteristic for *U. scaber* from the Mediterranean basin. In general, the diploid number of chromosomes in all studied populations of the species varies from  $2n = 26$  to  $2n = 32$ , and the fundamental arm number varies from  $NF = 48$  to  $NF = 56$  (Figure 2). The polymorphism in each separate population is the result of different numbers and types of chromosomal rearrangements (Vasil'ev et al., 2012). Besides genetic

**Table.** Karyotype characteristics in *Mullus barbatus* from the Mediterranean basin.

Locality	2n	NF	Karyotype formula	Information source
Ligurian Sea (Monaco)	44	48	4m/sm+40a	Laliberte et al., 1979
	44	50	6m/sm+38a	
Tyrrhenian Sea (Gulf of Palermo, Italy)	44	50	6m/sm+16st+22a	Vitturi et al., 1992
Black Sea (Zonguldak, Turkey)	44	50	6m/sm+16st+22a	Saygun et al., 2006
Black Sea (Taman Peninsula, Russia)	44	52	8sm+36st/a	Present paper

2n: number of chromosomes in a diploid set; NF: fundamental arm number; m, sm, st, and a: meta-, submeta-, subtelocentric, and acrocentric chromosomes, respectively.



**Figure 2.** Karyotype distributions of *Mullus barbatus* and *Uranoscopus scaber* in the Mediterranean basin. In the rectangles are shown the diploid number of chromosomes ( $2n$ ) and the fundamental arm number (NF).

causes, the high level of chromosomal divergence in *U. scaber* is also caused by the geological history of the Mediterranean basin. Geomorphological changes in the Mediterranean basin likely led to multiple isolations of populations and subsequently to secondary contact between them, which resulted in an increase in chromosome variability. However, in populations of *M. barbatus* from the Mediterranean, there is not a high level of chromosomal divergence:  $2n = 44$ ,  $NF = 48-52$  (Table; Figure 2). This may be related to features of functional elements of chromosomes of *M. barbatus*, including the satellite DNA in the centromeric region of chromosomes.

At present, the chromosomal complements of seven Mullidae species are known. The ancestral karyotype for Mullidae probably contained 48 acrocentric chromosomes. Such a structure is typical for the karyotype of *Mulloidichthys flavolineatus*:  $2n = 48a$ ,  $NF = 48$  (Ojima and Yamamoto, 1990). The appearance of the

44-chromosomal complement in *M. barbatus* is apparently a result of Robertsonian rearrangements (chromosomal fusions) of the base karyotype ( $2n = 48a$ ); the evidence for this is probably the large marker pairs of submeta- and subtelocentric chromosomes (Figure 1A).

Thus, the karyotypes of all studied *M. barbatus* populations from the Mediterranean basin have stable numbers of chromosomes. Further karyological study of other *M. barbatus* populations will probably allow us to reveal chromosomal variability associated with the rearrangements that change the structure of separate chromosomes without changing their number.

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