Turk. J. Fish.& Aquat. Sci. 23(7), TRJFAS20729
https://doi.org/10.4194/TRJFAS20729

Turkish Journal of FISHERIES and AQUATIC SCIENCES

RESEARCH PAPER

Chromosome Complement of *Pomatoschistus marmoratus* and Karyotype Evolution in Sand Gobies (Gobiidae: Gobionellinae)

Denis V. Prazdnikov [1,*]

[1]Severtsov Institute of Ecology and Evolution, Russian Academy of Sciences, Moscow, Russian Federation

How to Cite

Prazdnikov, D.V. (2023). Chromosome Complement of *Pomatoschistus marmoratus* and Karyotype Evolution in Sand Gobies (Gobiidae: Gobionellinae) *Turkish Journal of Fisheries and Aquatic Sciences*, *23(7), TRJFAS20729.* https://doi.org/10.4194/TRJFAS20729

Article History
Received 04 October 2021
Accepted 20 January 2023
First Online 25 January 2023

Corresponding Author
Tel.: +79166330929
E-mail: pdvfish3409@rambler.ru

Keywords
Gobiid fishes
Pomatoschistus marmoratus
Mediterranean basin
Karyotypic diversity
Chromosomal rearrangements

Abstract

The family Gobiidae constitutes one of the most diverse groups among teleost fish; some of its species are objects of aquaculture and fisheries. Despite many years of cytogenetic studies, the chromosome complements of many species of gobies remain unknown, and the trends of karyotypic evolution are poorly understood. Here I describe a previously unstudied karyotype of a widespread species from the group of sand gobies (*Pomatoschistus marmoratus*) and analyze the trends of karyotypic evolution of this group in comparison with other taxonomic groups of gobies from the Atlantic-Mediterranean and Ponto-Caspian regions. For *P. marmoratus*, the diploid number is 2n=46; it consists of 11 pairs of submetacentric chromosomes, 12 pairs of subtelocentric and telocentric chromosomes (NF=68). A comparative karyological analysis showed that the main trend in the karyotypic evolution of sand gobies differs from that of other groups of gobies and is associated with an increase in the number of chromosomal arms as a result of chromosomal rearrangements leading to a change in the centromere position. The results obtained contribute to understanding the role of chromosomal rearrangements in the evolution of Gobiidae and are of interest for further cytogenetic studies of this large family of fish.

Introduction

Gobies (Gobiidae) are one of the largest families of teleost fish that includes, according to the latest data, more than 1,950 species (Eschmeyer *et al*., 2021), most of which inhabit the marine and brackish waters of the tropics and subtropics (Nelson *et al*., 2016). There are also some species of gobies living in freshwater habitats (Freyhof, 2011). Many species of gobies are objects of fisheries and aquaculture (Bell, 1999; Groover *et al*., 2020; La Mesa *et al*., 2005).

Among the Atlantic-Mediterranean and Ponto-Caspian fish species, there is a taxonomic group of sand gobies consisting of five genera. Two largest genera are *Knipowitschia* and *Pomatoschistus* which differ in ecology and distribution (McKay & Miller, 1997; Thacker *et al*., 2019).

The sand gobies are characterized by a number of common cytogenetical features, namely: in most species, the diploid number of chromosomes (2n) is 46; the nucleolar organizer regions are located in the terminal-centromeric zone on the short arm in one submetacentric pair of chromosomes; they have similar values of the DNA amount per cell (Caputo, 1998; Rampin *et al*., 2011). Molecular phylogenetic data calibrated using fossils indicate the origin of the sand goby clade near the Eocene-Oligocene boundary (about 33.0 Mya) (Thacker *et al*., 2019). The relatively long evolutionary history of this group of gobies (Huyse *et al*., 2004; Malavasi *et al*., 2012) was reflected in their karyotypic diversity. Among the karyologically analyzed species of the sand gobies, the number of chromosomes varies from 2n=32 to 2n=46, while the fundamental arm number (NF) varies from NF=50 to NF=70. Nevertheless,

Published by Central Fisheries Research Institute (SUMAE) Trabzon, Türkiye

the chromosome complement of a number of sand goby species remains unknown, and karyotypic evolution remains poorly understood.

In the present work, I describe the chromosome complement of a previously unstudied species (*Pomatoschistus marmoratus*) and a comparative karyological analysis of a group of sand gobies. The results obtained showed that the main trend of karyotypic evolution of this group differs from that of other taxonomic groups of gobies and is associated with an increase in the proportion of two-armed chromosomes (metacentric and submetacentric) as a result of rearrangements leading to a change in the centromere position. This work contributes to the expansion of knowledge of the mechanisms for the formation of karyotypic diversity and creates opportunities for further cytogenetic studies of the family Gobiidae.

Materials and Methods

The material for karyological analysis was collected in the Black Sea, near the coast of the Bugaz Spit (45°04'15.5"N, 37°00'48.0"E). A total of 12 specimens of *Pomatoschistus marmoratus* (Risso, 1810) were caught. Most of the specimens were adult males with a total length (*TL*) of 53–58 mm (Figure 1). Mitotic activity sufficient for chromosome analysis was observed in 5 specimens (4 males and 1 female). A total of 67 metaphase plates were studied.

Chromosome preparations were obtained from the anterior part of the kidney according to previously published methods (Blanco *et al*., 2012; Ojima & Kurishita, 1980) with the initial treatment of live fish with colchicine (injection of 0.03% solution into the spinal muscle). The anterior kidney tissue was incubated in 75 mM KCl (hypotonic solution) for 22 min at 27°C and fixed in 96% ethanol mixed with glacial acetic acid (3:1 ratio). Chromosome preparations made using standard air-drying techniques were stained with 5% Giemsa solution in phosphate buffer at pH 6.8 for 7 min.

Analysis of mitotic chromosomes was performed using the KaryoType software (Altinordu *et al*., 2016). Chromosomes were classified according to Levan et al. (1964): metacentric (m), submetacentric (sm), subtelocentric (st) and telocentric (t). To determine the fundamental arm number (NF), chromosomes of the m and sm groups were considered two-armed and those of the st/t group one-armed. The regression between the proportion of two-armed chromosomes and diploid chromosome number and the Spearman correlation were calculated. For statistical analysis of the results, the Excel 2016 program was used.

Results and Discussion

Karyological studies of *Pomatoschistus marmoratus* from the Black Sea near the coast of the Bugaz Spit showed the chromosome complement containing 2n=46. The karyotype consists of 11 pairs of submetacentric and 12 pairs of subtelocentric and telocentric chromosomes; the fundamental arm number NF=68 (Figure 2). No sex chromosomes were found in the studied chromosome complements. A characteristic feature of the *P. marmoratus* karyotype is the presence of pairs of large submetacentric and

Figure 1. A male of *Pomatoschistus marmoratus*. Scale bar – 5 mm.

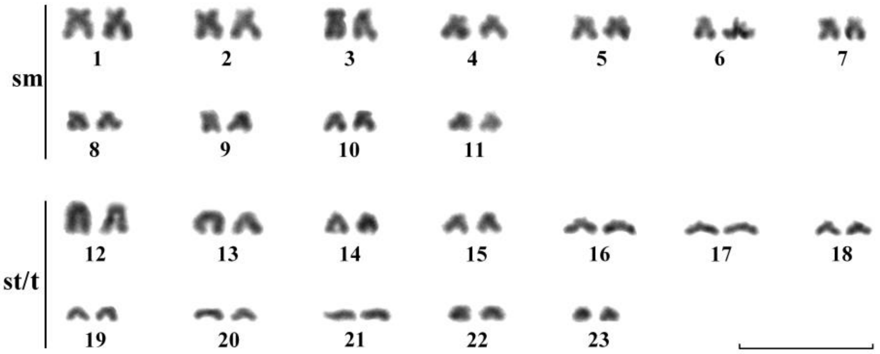

Figure 2. Karyotype of *Pomatoschistus marmoratus* from the Black Sea (2n=46). Scale bar – 10 µm.

telocentric chromosomes (Figures 2, 3). A marker pair of large telocentric chromosomes has also been previously found in the karyotypes of other sand goby species: *Pomatoschistus bathi*, *P. lozanoi*, *P. minutus* and *P. norvegicus* (Boltachev *et al*., 2016; Webb, 1986).

The diploid karyotype of most of the studied sand gobies consists of 46 chromosomes (Table 1). The modal number of chromosomes is 2n=46 both for the goby groups of Atlantic-Mediterranean, Ponto-Caspian and sand gobies (Figure 4) and, apparently, for the entire suborder Gobioidei (da Silva *et al*., 2021; Prazdnikov, 2013). Moreover, from a cytogenetic point of view, the diploid karyotype consisting of 46 one-armed chromosomes is the most primitive and should be considered as the basic/ancestral karyotype of goby fish.

An analysis of karyological data (Arai, 2011; Galvão *et al*., 2011; Prazdnikov, 2013) indicated that the evolution of the karyotype in goby fish occurred at different rates and in different directions, which led to a wide chromosomal divergence (2n=29–56, NF=38–96).

For the Atlantic-Mediterranean and Ponto-Caspian groups of goby fish, the proportion of two-armed chromosomes in the karyotype varies widely from 0% to 59%. The regression between the proportion of two-armed chromosomes in the karyotype and the diploid number is $y=-2.135x+103.77$ ($R^2=0.63$), and the Spearman correlation is $R_s=-0.604$ (Figure 5a). For the group of sand gobies, the proportion of two-armed chromosomes in the karyotype varies from 13% to 65%. The regression between the proportion of two-armed chromosomes and the diploid number is $y=-1.1509x+90.689$ ($R^2=0.08$); $R_s=0$ (Figure 5b). The absence of a statistically significant association between the two variables (2n and proportion of m/sm chromosomes) in sand gobies is apparently due to chromosomal rearrangements that affected the trends of karyotypic evolution in this group.

The main trend in the karyotypic evolution of most groups of gobies from the Atlantic-Mediterranean and Ponto-Caspian regions is the result of the rearrangements of the centric fusion type (Robertsonian translocations) leading to a decrease in the number of chromosomes (without changing the fundamental arm number) (Figure 6a). Another direction of karyotype transformation associated with an increase in the number of chromosomes (as a result of centric fission) and chromosomal arms (mainly as a result of pericentric inversion) is less pronounced.

In contrast, the main trend of karyotypic evolution for the group of sand gobies is associated with an increase in the number of chromosomal arms (without changing the number of chromosomes) as a result of rearrangements leading to a change in the centromere position, mainly due to pericentric inversion (Figure 6b). As a result, the karyotypes of *Economidichthys pygmaeus* and some populations of *Pomatoschistus*

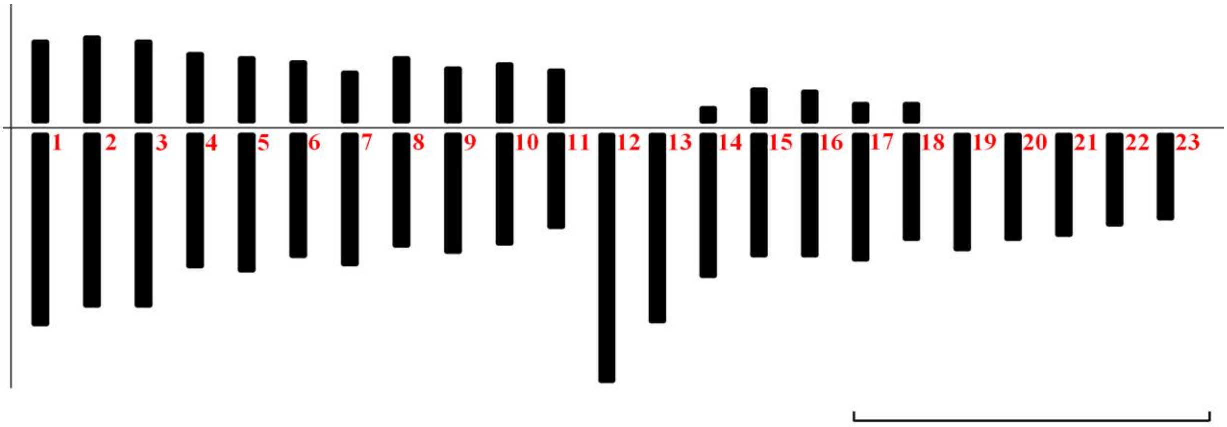

Figure 3. Ideogram of *Pomatoschistus marmoratus* (n=23). Scale bar – 3 µm.

Table 1. Cytogenetic data for species of sand gobies

Species	2n	Karyotype formula	NF	References
Economidichthys pygmaeus	46	24sm+22st	70	Rampin et al., 2011
Pomatoschistus bathi	44	6sm+38st/t	50	Boltachev et al., 2016
Pomatoschistus flavescens	46	6m/sm+40t	52	Klinkhardt, 1992
Pomatoschistus lozanoi	37	3m+12sm+10st+12t	52	Webb, 1980
Pomatoschistus marmoratus	46	22sm+24st/t	68	Present study
Pomatoschistus microps	46	30sm+16st	76	Webb, 1986
	46	4m+16sm+20st+6t	66	Klinkhardt, 1989
Pomatoschistus minutus	46	4m+16sm+16st+10t	66	Klinkhardt, 1989
	46	18sm+18st+10t	64	Klinkhardt, 1992
	46	6sm+24st+16t	52	Webb, 1980
Pomatoschistus norvegicus	32	10m+10sm+8st+4t	52	Webb, 1980
Pomatoschistus pictus	46	22m/sm+12st+12t	68	Klinkhardt, 1992

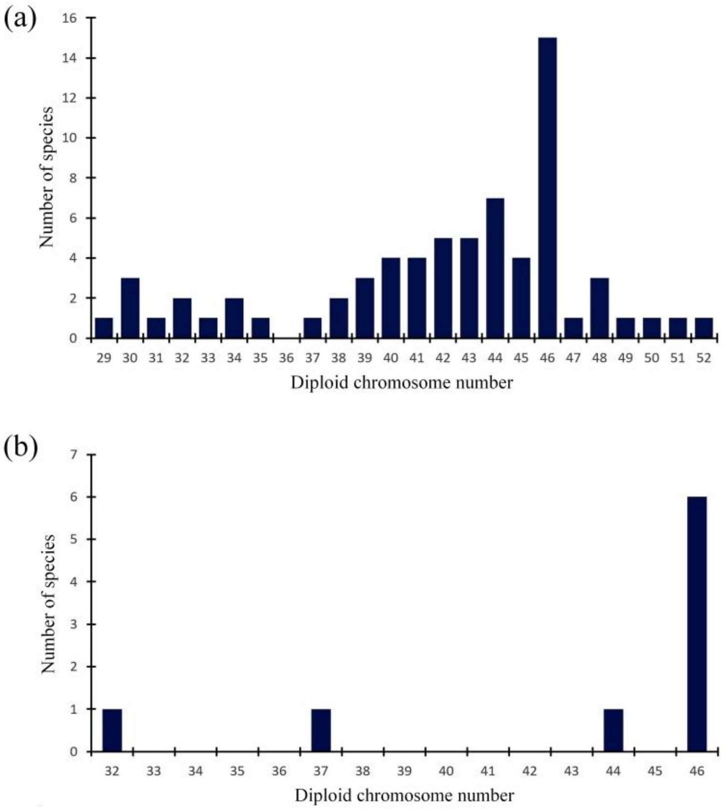

Figure 4. Histogram of the distribution of the diploid chromosome number: (a) for the Atlantic-Mediterranean and Ponto-Caspian groups of gobies, (b) for the group of sand gobies. An odd number of chromosomes is associated with either heterozygous karyotype variants or Y-autosomal translocation.

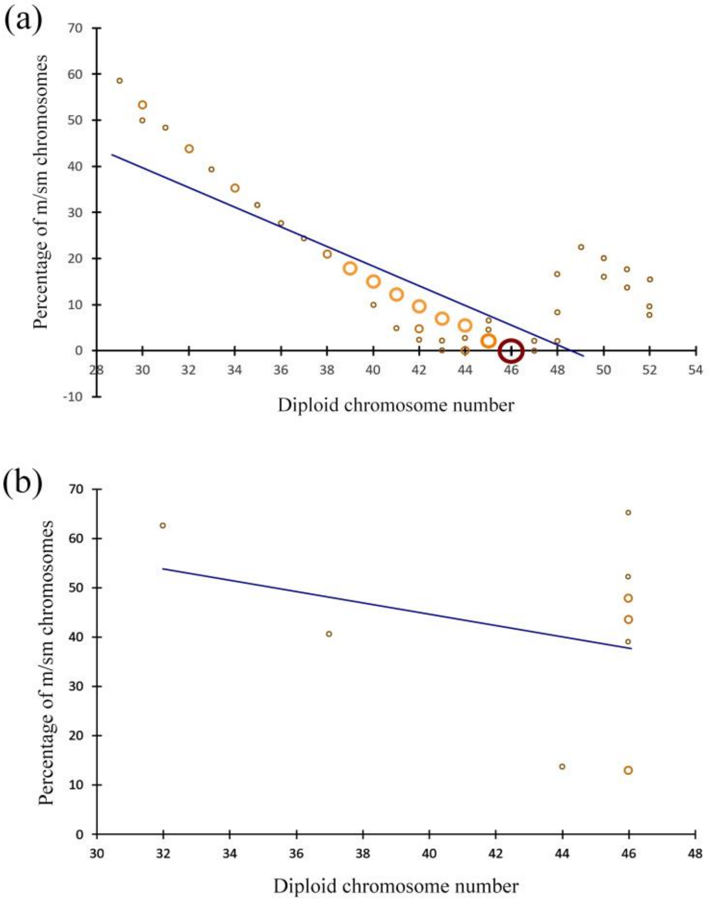

Figure 5. Scatter-plot of a diploid chromosome number and proportion of metacentric/submetacentric chromosomes (m/sm) with overall regression line: (a) for the Atlantic-Mediterranean and Ponto-Caspian groups of gobies, (b) for the group of sand gobies. The diameter and color of a circle indicate the number of species from 1 to 15.

microps consist exclusively of submetacentric and subtelocentric chromosomes (Table 1) (Rampin *et al*., 2011; Webb, 1986). An additional direction of karyotype transformations of sand gobies is associated with a decrease in the number of chromosomes due to Robertsonian translocations and an increase in the number of chromosome arms due to pericentric inversions relative to the basic chromosome complement (Figure 6b). This direction is typical for the karyotypes of *Pomatoschistus lozanoi* and *P. norvegicus* (Table 1). Interestingly, the *P. lozanoi* karyotype is heterozygous for the Robertsonian translocation (2n=37), and the appearance of an unpaired metacentric element is not associated with sex chromosomes (Webb, 1980). It should be noted that changes in the position of the centromere in the chromosome complements of sand gobies are probably caused not only by pericentric inversions, but also by the formation of neocentromeres which can be observed in the karyotypes of various groups of animals (Schubert, 2018; Sobita & Bhagirath, 2006).

Among the karyologically studied species of sand gobies, two species, *Pomatoschistus microps* and *P. minutus*, are characterized by the interpopulation differentiation of karyotypes (Table 1). It is possible that the karyotypic diversity of sand gobies may be increasing as a result of hybridization. To date, there are known cases of hybridization in sympatric zones between *P. microps* and *P. marmoratus* (Berrebi & Trébuchon, 2020), as well as between *P. lozanoi* and *P. norvegicus*, and *P. lozanoi* and *P. minutus* (Wallis & Beardmore, 1980; Webb, 1980). Thus, it can be expected that in the course of further cytogenetic studies both homozygotes and heterozygotes for various chromosomal rearrangements that affect the fertility of sand goby hybrids shall be found in sympatric zones.

Analysis of the karyotypic evolution of different taxa of fish shows that the probability of a purely random fixation of chromosomal rearrangements without the participation of natural selection is very small, which is evidence in favor of the adaptive significance of such rearrangements (Cayuela *et al*., 2020; Kirpichnikov, 1987; Kirubakaran *et al*., 2016; Martinez *et al*., 2015). It has been earlier suggested that the karyological differentiation in sand gobies of the genus *Pomatoschistus* may have an adaptive value (Webb, 1980). The relatively ancient origin of *Pomatoschistus* about 18.3 Mya and major tectonic events in the Mediterranean basin contributed to the diversification of sand gobies (Thacker *et al*., 2019). A series of complex hydrographic and geological events associated with the isolation of the Tethys and Paratethys seas in the Miocene with subsequent epochs of regression and elevation of the water levels, as well as fluctuations in salinity, led first to the isolation of the goby lineages and then to their adaptive radiation (Huyse *et al*., 2004; Malavasi *et al*., 2012; Thacker, 2015). Probably, all these events influenced the ecology and distribution of sand gobies and led to the formation of karyotypes composed of a large number of two-armed chromosomes.

The specifics of the complex history of the Mediterranean basin also influenced the karyotypic diversification of other fish taxa with the formation of intrapopulational and interpopulational chromosomal polymorphism (Prazdnikov, 2016; Vitturi *et al*., 1986; Vitturi & Lafargue, 1992).

Conclusion

The diploid karyotype of *Pomatoschistus marmoratus*, one of the widespread sand goby species, consists of 46 chromosomes with a high proportion of submetacentric elements. A comparative karyological analysis showed that the trends of chromosomal evolution in the sand goby group differ from those of other taxonomic groups of gobies. In the karyotypes of sand gobies, the proportion of two-armed chromosomes increases without changes in the basic number of chromosomes (2n=46), mainly due to pericentric inversions. The ancient origin of the sand goby group together with a series of hydrographic and geological events in the region of their habitat, as well as differences in ecology and distribution, provide evidence in favor of the potentially adaptive value of

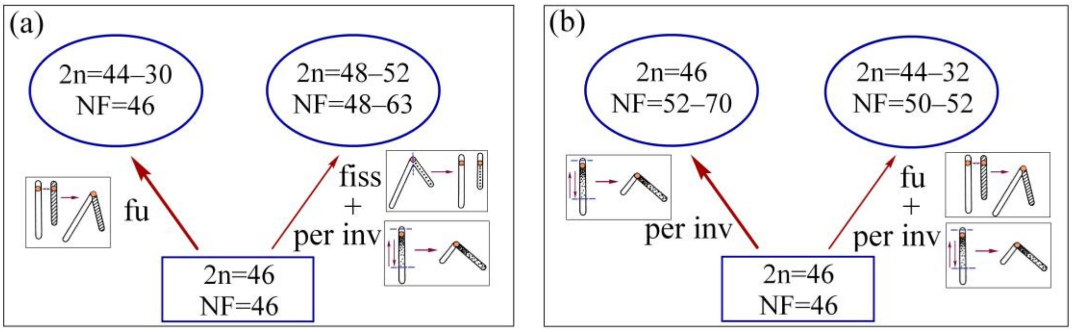

Figure 6. The trends of karyotypic evolution (a) for the Atlantic-Mediterranean and Ponto-Caspian groups of gobies, (b) for the group of sand gobies. The red thick arrow shows the most probable main trend in karyotypic evolution. The lower rectangle shows the ancestral karyotype. 2n – number of chromosomes in a diploid complement, NF – fundamental arm number, fu – centric fusions, fiss – centric fissions, per inv – pericentric inversions.

karyological differentiation. Taking into account that some sand gobies are characterized by a disjunct distribution with overlapping geographic ranges between species, further cytogenetic studies are likely to reveal an even greater karyotypic diversity in this group of fish.

Ethical Statement

Not applicable

Funding Information

Not applicable

Conflict of Interest

The author declares no conflict of interest.

References

Altınordu, F., Peruzzi, L., Yu, Y., & He, X. (2016). A tool for the analysis of chromosomes: KaryoType. *Taxon*, 65(3), 586–592. https://doi.org/10.12705/653.9

Arai, R. (2011). *Fish karyotypes: a check list*. Springer, Japan.

Bell, K.N. (1999). An overview of goby-fry fisheries. *Naga, the ICLARM quarterly*, 22(4), 30–36.

Berrebi, P., & Trébuchon, M. (2020). Distribution and hybridization of two sedentary gobies (*Pomatoschistus microps* and *Pomatoschistus marmoratus*) in the lagoons of southern France. *Scientia Marina*, 84(4), 355–367. https://doi.org/10.3989/scimar.05029.15A

Blanco, D.R., Bertollo, L.A.C., Lui, R.L., Vicari, M.R., Margarido, V.P., Artoni, R.F., & Moreira-Filho, O. (2012). A new technique for obtaining mitotic chromosome spreads from fishes in the field. *Journal of fish biology*, 81(1), 351–357. https://doi.org/10.1111/j.1095-8649.2012.03325.x

Boltachev, A., Karpova, E., & Vdodovich, I. (2016). Distribution, biological and ecological characteristics of alien species *Pomatoschistus bathi* Miller, 1982 (Gobiidae) in the Black Sea. *Turkish Journal of Fisheries and Aquatic Sciences*, 16(1), 113–122. https://doi.org/10.4194/1303-2712-v16_1_12

Caputo, V. (1998). Nucleolar organizer (NOR) location and cytotaxonomic implications in six species of gobiid fishes (Perciformes, Gobiidae). *Italian Journal of Zoology*, 65(1), 93–99. https://doi.org/10.1080/11250009809386729

Cayuela, H., Rougemont, Q., Laporte, M., Mérot, C., Normandeau, E., Dorant, Y., ... & Bernatchez, L. (2020). Shared ancestral polymorphisms and chromosomal rearrangements as potential drivers of local adaptation in a marine fish. *Molecular Ecology*, 29(13), 2379–2398. https://doi.org/10.1111/mec.15499

da Silva, S.A.S., de Lima-Filho, P.A., da Motta-Neto, C.C., da Costa, G.W.W.F., de Bello Cioffi, M., Bertollo, L.A.C., & Molina, W.F. (2021). High chromosomal evolutionary dynamics in sleeper gobies (Eleotridae) and notes on disruptive biological factors in Gobiiformes karyotypes (Osteichthyes, Teleostei). *Marine Life Science & Technology*, 1–10. https://doi.org/10.1007/s42995-020-00084-6

Eschmeyer, W.N., Fricke, R., & Fong, J.D. (2021) *Catalog of Fishes: Genera, Species, References.* https://researcharchive.calacademy.org/research/ichthyology/catalog/SpeciesByFamily.asp

Freyhof, J. (2011). Diversity and distribution of freshwater gobies from the Mediterranean, the Black and Caspian seas. *The biology of gobies*, 279–288.

Galvão, T.B., Bertollo, L.A.C., & Molina, W.F. (2011). Chromosomal complements of some Atlantic Blennioidei and Gobioidei species (Perciformes). *Comparative cytogenetics*, 5(4), 259–275. https://doi.org/10.3897/CompCytogen5i4.1834

Groover, E.M., DiMaggio, M., & Cassiano, E.J. (2020). Overview of Commonly Cultured Marine Ornamental Fish. *EDIS*, 2020(3), 1–7.

Huyse, T., Van Houdt, J., & Volckaert, F.A. (2004). Paleoclimatic history and vicariant speciation in the "sand goby" group (Gobiidae, Teleostei). *Molecular Phylogenetics and Evolution*, 32(1), 324–336. https://doi.org/10.1016/j.ympev.2003.11.007

Kirpichnikov, V.S. (1987). *Fish genetics and breeding*. Leningrad, Nauka Press.

Kirubakaran, T.G., Grove, H., Kent, M.P., Sandve, S.R., Baranski, M., Nome, T., De Rosa, M.C., Righino, B., Johansen, T., Otterå, H., Sonesson, A., Lien, S., & Andersen, Ø. (2016). Two adjacent inversions maintain genomic differentiation between migratory and stationary ecotypes of Atlantic cod. *Molecular ecology*, 25(10), 2130–2143. https://doi.org/10.1111/mec.13592

Klinkhardt, M.B. (1989) Investigations into karyology and fecundity of *Pomatoschistus microps* (Krøyer) and *P. minutus* (Pallas) (Teleostei, Gobiidae) living in an inner coastal water of the South-West Baltic Sea. *Zoologischer Anzeiger*, 222(3–4), 177–190.

Klinkhardt, M.B. (1992). Chromosome structures of four Norwegian gobies (Gobiidae, Teleostei) and a hypothetical model of their karyo-evolution. *Chromatin*, 3(1), 169–183.

La Mesa, M., Arneri, E., Caputo, V., & Iglesias, M. (2005). The transparent goby, *Aphia minuta* review of biology and fisheries of a paedomorphic European fish. *Reviews in Fish Biology and Fisheries*, 15(1), 89–109. https://doi.org/10.1007/s11160-005-1613-4

Levan, A., Fredg, A., & Sandberg, A. (1964). Nomenclature for centromeric position on chromosomes. *Hereditas*, 52, 201–220.

Malavasi, S., Gkenas, C., Leonardos, I., Torricelli, P., & Mclennan, D.A. (2012). The phylogeny of a reduced 'sand goby'group based on behavioural and life history characters. *Zoological Journal of the Linnean Society*, 165(4), 916–924. https://doi.org/10.1111/j.1096-3642.2012.00832.x

Martinez, P.A., Zurano, J.P., Amado, T.F., Penone, C., Betancur-R, R., Bidau, C.J., & Jacobina, U.P. (2015). Chromosomal diversity in tropical reef fishes is related to body size and depth range. *Molecular phylogenetics and evolution*, 93, 1–4. https://doi.org/10.1016/j.ympev.2015.07.002

McKay, S.I., & Miller, P.J. (1997). The affinities of European sand gobies (Teleostei: Gobiidae). *Journal of Natural History*, 31, 1457–1482. https://doi.org/10.1080/00222939700770791

Nelson, J.S., Grande, T.C., & Wilson, M.V. (2016). *Fishes of the World*. John Wiley & Sons.

Ojima, Y., & Kurishita, A. (1980). A new method to increase the number of mitotic cells in the kidney tissue for fish chromosome studies. *Proceedings of the Japan Academy, Series B*, 56(10), 610–615.

Prazdnikov, D.V. (2013). Chromosomal evolution of goby fishes of the family Gobiidae (Pisces, Perciformes) from the Ponto-Caspian Basin. *Bulletin of the Tambov University. Series: Natural and Technical Sciences*, 18(6), 3064–3067.

Prazdnikov, D.V. (2016). Karyology of *Mullus barbatus* (Pisces, Perciformes) from the Mediterranean basin. *Turkish Journal of Zoology*, 40(2), 279–281. https://doi.org/10.3906/zoo-1503-15

Rampin, M., Gkenas, C., Malavasi, S., & Libertini, A. (2011). A cytogenetical study on *Economidichthys pygmaeus* Holly, 1929 (Pisces, Gobiidae), an endemic freshwater goby from Western Greece. *Comparative cytogenetics*, 5(5), 391–396. https://doi.org/10.3897/CompCytogen.v5i5.2015

Schubert, I. (2018). What is behind “centromere repositioning”?. *Chromosoma*, 127(2), 229–234. https://doi.org/10.1007/s00412-018-0672-y

Sobita, N., & Bhagirath, T. (2006). Chromosomal differentiations in the evolution of channid fishes—molecular genetic perspective. *Caryologia*, 59(3), 235–240. https://doi.org/10.1080/00087114.2006.10797920

Thacker, C.E. (2015). Biogeography of goby lineages (Gobiiformes: Gobioidei): origin, invasions and extinction throughout the Cenozoic. *Journal of Biogeography*, 42(9), 1615–1625. https://doi.org/10.1111/jbi.12545

Thacker, C.E., Gkenas, C., Triantafyllidis, A., Malavasi, S., & Leonardos, I. (2019). Phylogeny, systematics and biogeography of the European sand gobies (Gobiiformes: Gobionellidae). *Zoological Journal of the Linnean Society*, 185(1), 212–225. https://doi.org/10.1093/zoolinnean/zly026

Vitturi, R., Mazzola, A., Macaluso, M., & Catalano, E. (1986). Chromosomal polymorphism associated with Robertsonian fusion in *Seriola dumerili* (Risso, 1810) (Pisces: Carangidae). *Journal of fish biology*, 29(5), 529–534. https://doi.org/10.1111/j.1095-8649.1986.tb04969.x

Vitturi, R., & Lafargue, F. (1992). Karyotype analyses reveal inter-individual polymorphism and association of nucleolus-organizer-carrying chromosomes in *Capros aper* (Pisces: Zeiformes). *Marine Biology*, 112(1), 37–41. https://doi.org/10.1007/BF00349725

Wallis, G.P., & Beardmore, J.A. (1980). *Pomatoschistus minutus* and *P. lozanoi* (Pisces, Gobiidae). *Marine Ecology Progress Series*, 3, 309–315.

Webb, C.J. (1980). Systematics of the *Pomatoschistus minutus* complex (Teleostei: Gobioidei). *Philosophical Transactions of the Royal Society of London. B, Biological Sciences*, 291(1049), 201–241. https://doi.org/10.1098/rstb.1980.0132

Webb, C.J. (1986). Karyology of *Pomatoschistus microps* (Teleostei: Gobioidei). *Journal of the Marine Biological Association of the United Kingdom*, 66(1), 259–266. https://doi.org/10.1017/S0025315400039771