A Comparative Study of Chromosomes of Twelve Species of Gobioid Fishes in Japan

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Abstract Karyotypes of 12 species (in 9 genera, 5 subfamilies, 2 families) of Japanese gobioid fishes were examined. The ranges are 44~50 in diploid number and 44~96 in arm number. The diploid numbers of 33 species and 2 subspecies including those that have already been reported, show no clear relationships at the subfamily level of the current classification, though they are well stabilized at generic level. Among genera examined, *Odontobutis* and *Rhinogobius* have the simplest karyotype, and *Acanthogobius*, *Chasmichthys*, *Tridentiger*, *Chaenogobius*, *Boleophthalmus*, *Periophthalmus* and *Acentrogobius* follow in due order.

Introduction

Fishes of the suborder Gobioidei are a specialized large group in the order Perciformes, and widely distributed in various types of habitats in aquatic environments. In Japan, these fishes are highly successful with 147 species in about 72 genera (Matsubara, 1955; Takagi, 1963). Several ichthyologists have examined their morphological features (Tomiyama, 1936; Takagi, 1950, 1963; Prince Akihito, 1967, 1969, 1971; etc), and proposed their schems for classification. This group is, however, so complex that these opinions have not reached complete agreement. Recently, with the improvement of technique for observing fish chromosome, karyotypes become a tool for fish systematics (Miller, 1972; etc). On fishes of Gobioidei, chromosomes are reported for only 33 species and 2 subspecies (Nogusa, 1950, 1955, 1960; Kaur and Srivas-

Table 1. Material used in this study. Species are arranged after Takagi (1963).

Family, subfamily and species names	Number of specimens	Total length (mm)	Localities
Eleotridae			
Odontobutis obscura	3	53~132	Nagata River, Shimonoseki
Gobiidae			
Periophthalminae			
Periophthalmus cantonensis	3	about 10	Honmyo River, Ariake, Nagasaki
Tridentigerinae			
Tridentiger obscurus obscurus	3	71~78	Yoshimi Coast, Shimonoseki
T. trigonocephalus	5	56~69	Yoshimi Coast, Shimonoseki
Rhinogobiinae			
Rhinogobius giurinus	1	52	Nagata River, Shimonoseki
R. brunneus	3	41~49	Nagata River, Shimonoseki
Chasmichthys gulosus	7	78~87	Yoshimi Coast, Shimonoseki
Acanthogobius flavimanus	8	53~151	Yoshimi Coast, Shimonoseki
Chaenogobius annularis	4	71~113	Nagata River, Shimonoseki
C. castanea	2	45~47	Nagata River, Shimonoseki
Gobiinae			
Acentrogobius pflaumi	14	52~73	Yoshimi Coast, Shimonoseki
Apocrypterinae			
Boleophthalmus pectinirostris	3	about 10	Honmyo River, Ariake, Nagasaki

tava. 1965; Post, 1965; Yamada, 1967; Verma, 1968; Subrahmanyam, 1969; Chen and Ebeling, 1971; Arai and Kobayasi, 1973; Arai and Sawada, 1974).

Karyotypes of 12 species (9 genera, 5 subfamilies, 2 families) belonging to the suborder Gobioidei are described, and their chromosomes are compared with those of species previously reported. On the basis of karyotypes, some relationships of the group are provisionally discussed.

Material and methods

Species names, number of specimens, body length, and localities of specimens examined here are shown in Table 1. Fish were kept in aquaria for a short period. They received abdominal injection of colchicine dissolved in distilled water in the amount of about 5 μg per 1 g body weight. Gill epithelia were removed 4 hours after injection, treated with distilled water, half sea water, 1/50 physiological salt water, 0.5 % sodium citrate hypotonic solution, and 0.075 M KCl, for 2 hours. Then they were fixed with carnoy's solution (ethyl alchol 3: acetic acid 1). Chromosome preparations were made according to the modified air-drying method and stained with Giemsa solution. The basic chromosome number of each species was given by modal count (Table 2). Following Chen and Ebeling (1971), chromosomes are classified into 2 types: metacentric, submetacentric, and subtelocentric chromosomes are considered as biarm, and telocentric and acrocentric chromosomes as uniarm (monoarm).

Specimens used in this study are preserved in Shimonoseki University of Fisheries.

Results

Family Eleotridae

1. *Odontobutis obscura* (Temminck and Schlegel), "Donko". Fig. 1A.

This species is a large eleotrid freshwater fish. The diploid number is 44 in both sexes. The karyotype consists of subequal uniarmed chromosomes.

Remarks: Although Nogusa (1955, 1960) reported that the present species has 2n=62r chromosomes, the present result clearly indicates that this species has 2n=44. Among

Table 2. Frequency distribution of chromosome numbers and percentages of mode in species examined. Mode is represented by boldface figure.

Species	Number of diploid chromosomes																		Total cell counts	Percentage of modal numbers
	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52		
Odontobutis obscura			3			1	2	8	18	**107**	2	3							144	74.3
Periophthalmus cantonensis									1	1	1	**18**	1						22	81.8
Tridentiger obscurus obscurus				1	1	2	3	5	5	**74**									91	81.3
T. trigonocephalus	1					1	1			**21**									24	87.5
Rhinogobius giurinus							1			**8**	1								10	80.0
R. brunneus					1		1		4	**21**									27	77.8
Chasmichthys gulosus		1			1	3	1	4	6	**70**	5	5	1						97	72.2
Acanthogobius flavimanus						2	3	4	12	**38**	2								61	62.3
Chaenogobius annularis				3	2	1	1	1	1	**35**	1	1							46	76.1
C. castanea					1	2		2	4	**36**		3	1						49	73.5
Acentrogobius pflaumi							1		2	1	3	2	1	13	4	**60**	1	3	91	65.9
Boleophthalmus pectinirostris									1	3	8	**33**							45	73.3

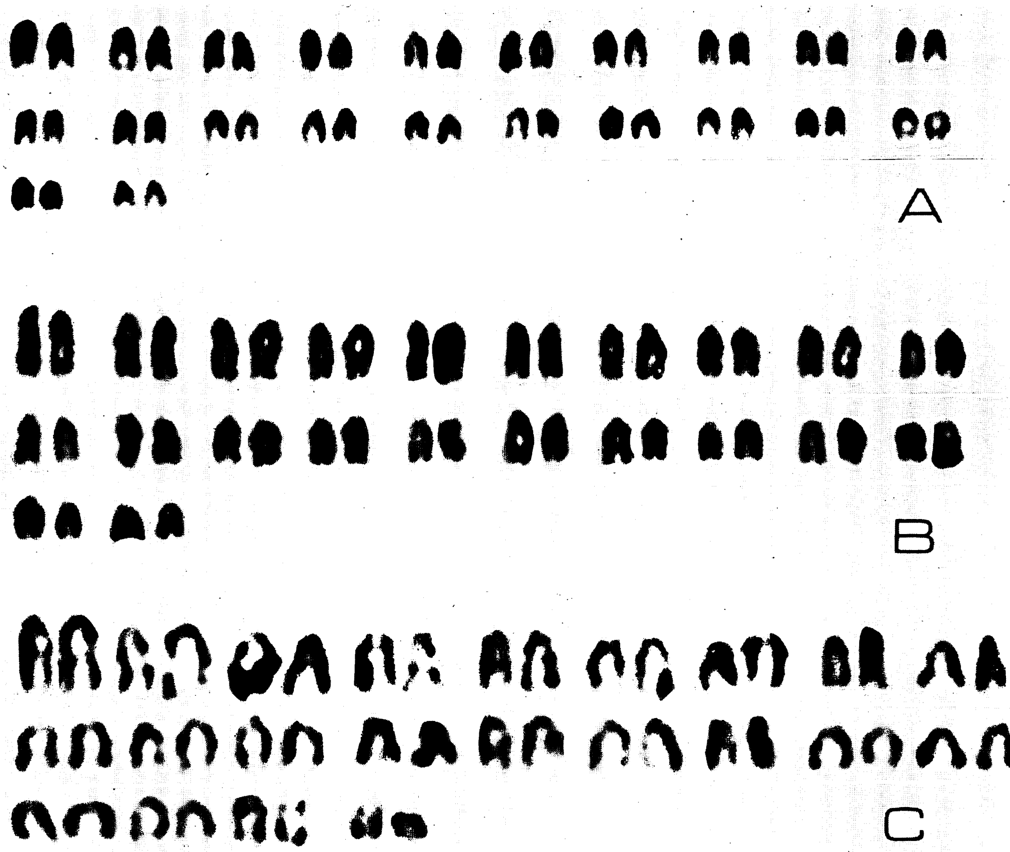


Fig. 1. Karyotypes of three species of the Gobioidei. A, *Odontobutis obscura*; B, *Rhinogobius brunneus*; C, *Rhinogobius giurinus*.

chromosome counts previously reported, data presented by Nogusa (1955, 1960) was obtained by a method of sectioning gonads. His results mentioned above do not always agree with those obtained by recent methods.

Family Gobiidae

Subfamily Rhinogobiinae

2. *Rhinogobius brunneus* (Temminck and Schlegel), "Yoshinobori". Fig. 1B.

The diploid number is 44, and the karyotype consists of uniarmed chromosomes which include a rather large pair.

Remarks: Nogusa (1957, 1960) reported that *Gobius similis* (synonym of the present species) has 2n=44r chromosomes. Recently, Arai and Kobayasi (1973) reported that both of two forms of the species have the same number of diploid chromosomes, 2n=44. The present result agrees well with those of the previous investigators in number and shape.

3. *Rhinogobius giurinus* (Rutter), "Gokuraku-haze". Fig. 1C.

The diploid number is 44, and the karyotype consists of uniarms including a somewhat large pair.

Remarks: The karyotype of this species is similar in number and element to that in *R. brunneus*, and also that in *R. flumineus* (2n=44) of the same genus reported by Arai and Kabayasi (1973). The three species of *Rhinogobius* apparently show close relationships.

4. *Acanthogobius flavimanus* (Temminck and Schlegel), "Mahaze". Fig. 2A.

The diploid number is 44. The karyotype consists of 5 pairs of biarms and 17 pairs of uniarms.

Remarks: The choromosomes of the present species were reported as 2n=44r by Nogusa (1950, 1960) and as 2n=44 consisting of all

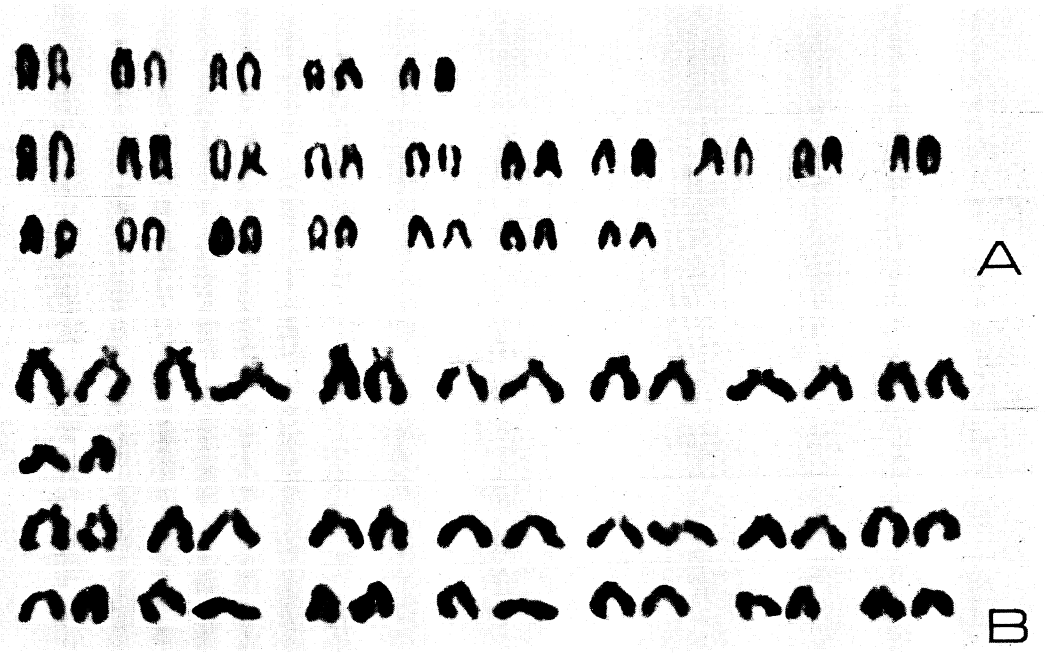


Fig. 2. Karyotypes of two species of the Gobioidei. A, *Acanthogobius flavimanus*; B, *Chasmichthys gulosus*.

uniarms by Arai and Kobayasi (1973). However, some subtelocentric chromosomes, which Arai and Kobayasi (1973) described as uniarms, are seen in their somatic metaphase figure. If these chromosomes are regarded as biarms, the present result closely agrees with theirr result.

5. *Chasmichthys gulosus* (Sauvage), "Dorome". Fig. 2B.

The diploid number is 44. The karyotype contains 8 pairs of biarms and 14 pairs of uniarms.

Remarks: This species occurs in rocky tide pools, where it is sympatric with *C. dolichognathus*. The chromosomes of the present species agree well in number with *C. dolichognathus* reported by Arai and Kobayasi (1973) as 2n=44. They described that all chromosomes of the species are uniarms consisting of subtelocentric or acrocentric ones. If some subtelocentric chromosomes found in their somatic metaphase figure are regarded as biamrs, the chromosomes of the both species are alike, and they can be considered as close relatives.

6. *Chaenogobius castanea* (O'shaghnessy), "Biringo". Fig. 3A.

The diploid number is 44. The karyotype contains 18 pairs of biarms and 4 pairs of uniarms.

Remarks: The chromosomes of the present species, which are shown as 2n=44(36V+8r), resemble those of *C. annularis* belonging to the same genus in number and element. But they disagree with *C. isaza* (2n=46) reported by Nogusa (1957, 1960) in number. If Nogusa's count is correct, it is unusual, for intraspecific variation of chromosome number in the same genus has not been found among the gobioid fishes.

7. *Chaenogobius annularis* (Gill), "Ukigori" Fig. 3B.

The diploid number is 44. The karyotype contains 19 pairs of biarms and 3 pairs of uniarms. The karyotype is characterized by having a pair of large biarmed and a pair of small uniarmed chromosomes.

Remarks: The present result shown as 2n=44(38V+6r) disagrees with 2n=42(14V+28r) described by Yamada (1967) under the name as *C. urotaenia* (synonym of the present species) in number and element, and also with 2n=44(44r) of Nogusa (1957, 1960) in element.

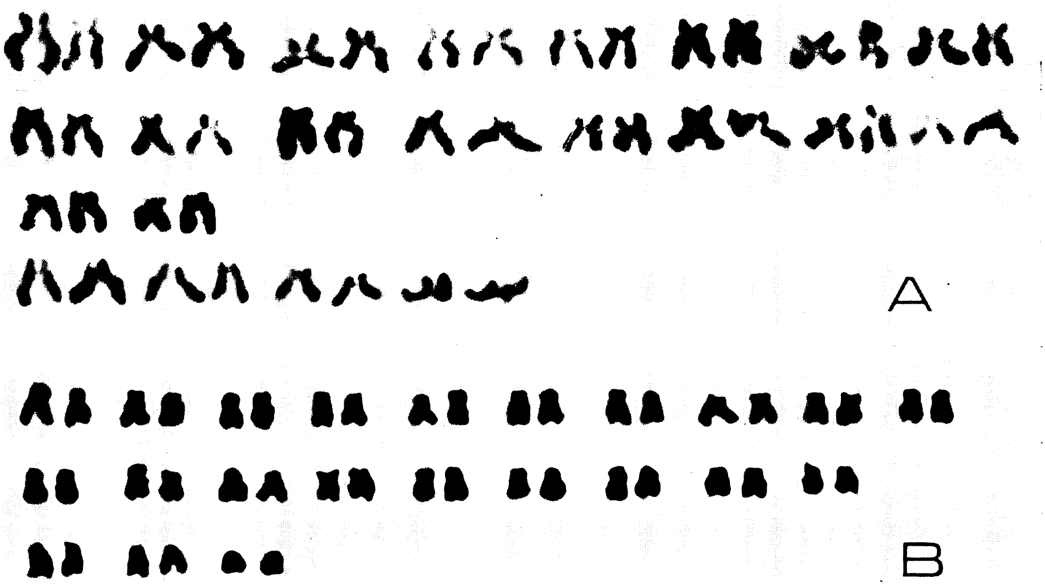


Fig. 3. Karyotypes of two species of the Gobioidei. A, *Chaenogobius castanea*; B, *Chaenogobius annularis*.

Recently, Arai and Kobayasi (1973) reported that in this species the chromosome is 2n=44 consisting of biarms and uniarms, though each number of elements was not stated.

Subfamily Tridentigerinae

8. *Tridentiger obscurus obscurus* (Temminck and Schlegel), "Chichibu". Fig. 4A.

The diploid number is 44. The karyotype contains 13 pairs of biarms and 9 pairs of uniarms. The subspecies is remarkable by having large pairs of biarms and uniarms.

Remarks: Katsuyama et al. (1972) reported a new subspecies under a name of *T. obscurus brevispinis* for inhabitants in fresh water. Since the present specimens were collected from sea water or blackish water, they are regarded as *T. obscurus obscurus*. The present data agrees in number with 2n=44r reported by Nogusa (1950, 1960) for *T. obscurus*, but disagrees in element. Moreover, the two pairs of small-sized (micro) chromosomes observed by him were not found. Arai and Kobayasi (1973) reported that *T. o. brevispinis* has 2n=44 chromosomes, and recognized uni- and biarms. The chromosomes of the two subspecies well resemble each other in elements, when compared in their somatic metaphase figures, though each count is not shown by them.

9. *Tridentiger trigonocephalus* (Gill), "Shima-haze". Fig. 4B.

The diploid number is 44. The karyotype contains 14 pairs of biarms and 8 pairs of uniarms.

Remarks: The chromosome number accords with 2n=44 reported by Arai and Kobayasi (1973), and the both results resemble each other in elements consisting of biarms and uniarms, though these numbers are not shown by them. The present species seems to show also close relationships in element to 2 subspecies within the same genus, as discussed above.

Subfamily Apocrypterinae

10. *Boleophthalmus pectinirostris* (Linnaeus), "Mutsugoro". Fig. 5A.

The diploid number is 46. The karyotype consists of uniarmed chromosomes. It is characterized by a pair of somewhat small sized chromosomes, and others gradually decrease in size.

Remarks: The result agrees well in number and elements with that of Nogusa (1957, 1960). But two pairs of small-sized chromosomes reported by him were not found, though there is a pair of somewhat small ones.

The diploid chromosome number in the

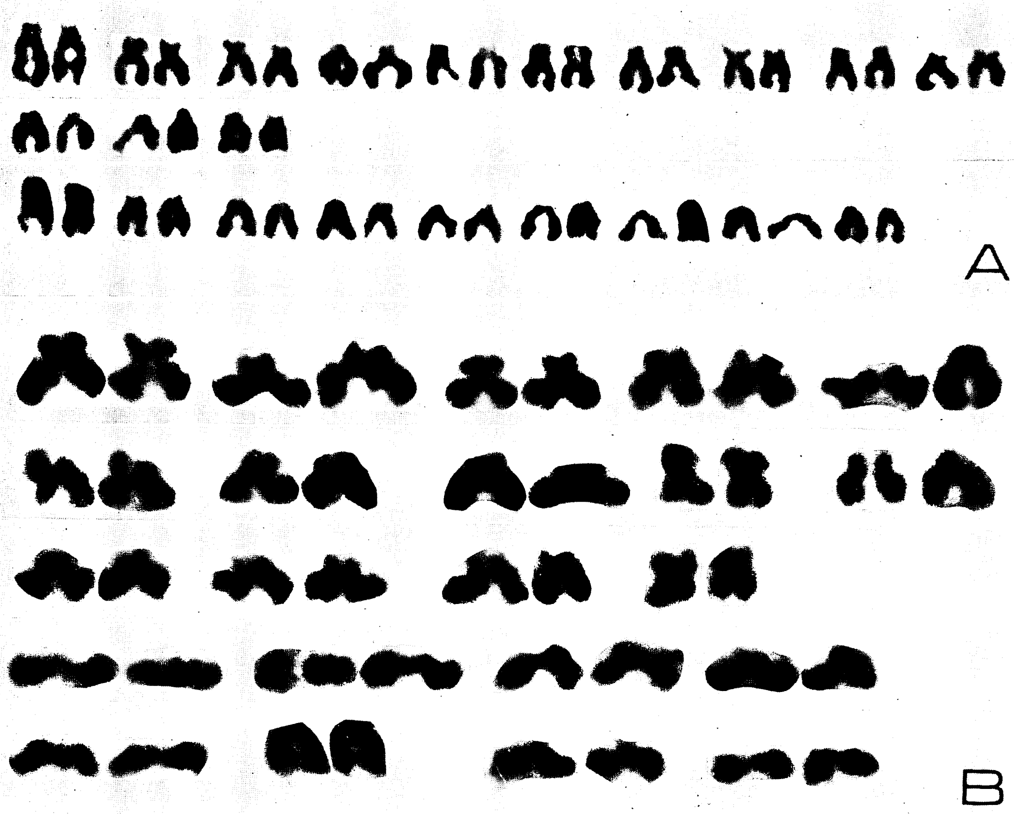


Fig. 4. Karyotypes of two species of the Gobioidei. A, *Tridentiger obscurus obscurus*; B, *Tridentiger trigonocephalus*.

present species agrees well in number with those in the two Indian mud skipper, *B. boddaerti* and *B. dussumieri* of the same genus reported by Verma (1968) and Subrahmanyan (1969). But the karyotype in the present species differs markedly from that of *B. boddaerti* reported as having all metacentric chromosomes by the latter investigator.

Subfamily Periophthalminae

11. *Periophthalmus cantonensis* (Osbeck), "Tobi-haze". Fig. 5B.

The diploid number is 46. The karyotype contains 17 pairs of biarms and 6 pairs of uniarms. The species is characterized by having two pairs of more or less larger biarm and a pair of small uniarm chromosomes.

Remarks: The present result agrees in number with those of Nogusa (1957, 1960) and Arai and Kobayasi (1973). In elements, Nogusa recognized three pairs of small chromosomes, but the authors found only a pair of relatively small ones. Arai and Kobayasi (1973) reported that the species consists of biarmed and uniarmed chromosomes, though the counts are not shown. The present result accords with their somatic metaphase figure. On the other hand, the present species agrees with *Boleophthalmus pectinirostris* in number of chromosomes, as shown above, but these karyotypes are distinguishable.

Subfamily Gobiinae

12. *Acentrogobius pflaumi* (Bleeker), "Suji-haze". Fig. 5C.

The diploid number is 50. The karyotype contains 24 pairs of biarms and a pair of uniarms. The karyotype has no markedly larger or smaller chromosomes.

Remarks: The species is remarkable in having both large number of chromosomes and largest number of biarms among the suborder Gobioidei. Recently, Arai and Sawada (1974) reported that a species belong-

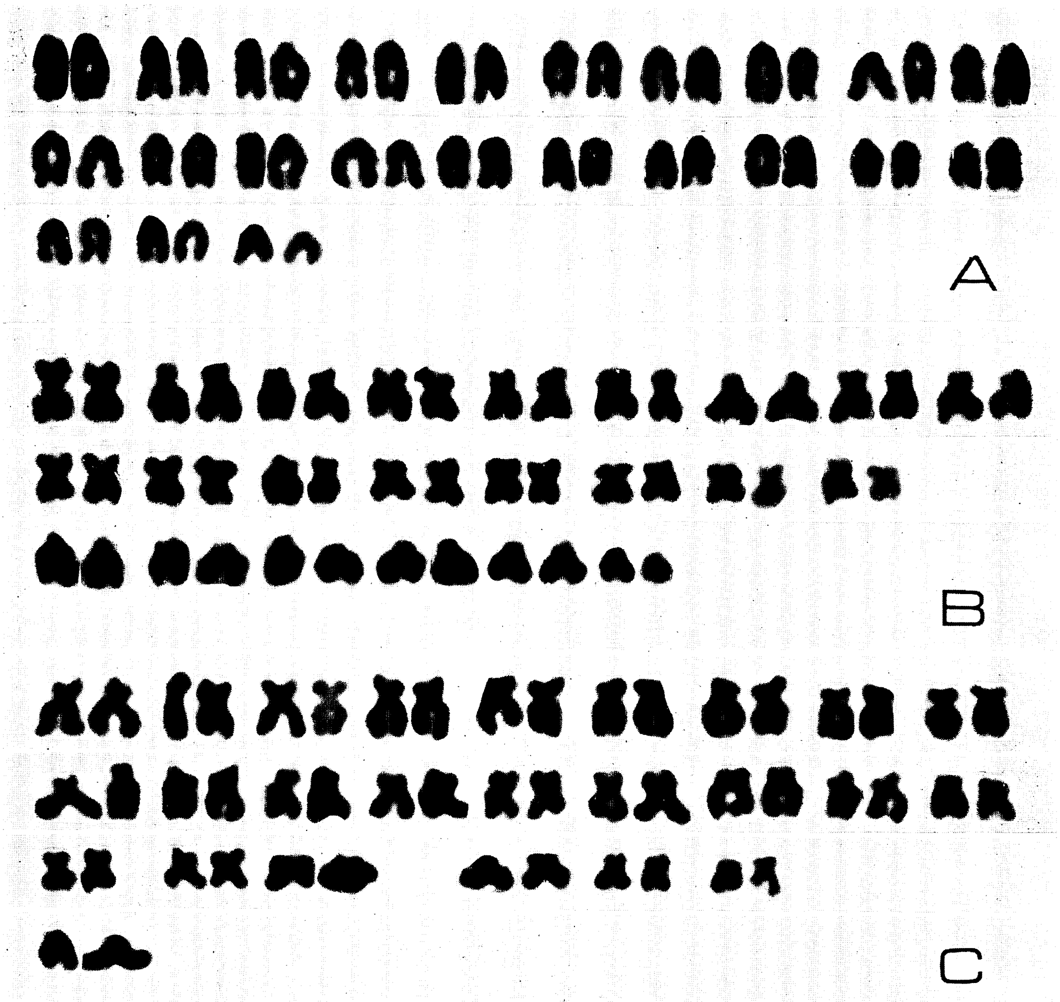


Fig. 5. Karyotypes of three species of the Gobioidei. A, *Boleophthalmus pectinirostris*; B, *Periophthalmus cantonensis*; C, *Acentrogobius pflaumi*.

ing to same subfamily, *Ctenogobius criniger* has 2n=50 and 90 arm numbers (though they reported that the arm number is 84).

The present species was formerly included by Matsubara (1955) in the genus *Rhinogobius* of the subfamily Rhinogobiinae, but it is placed under the genus *Acentrogobius* of the subfamily Gobiinae by Takagi (1963). Judging by the number of chromosomes and its elements, the species does not appear to belong to the genus *Rhinogobius*, because the present species differs from 3 species of the *Rhinogobius* with 2n=44r chromosomes. The chromosome data appear to support the tentative arrangement* by Takagi (1963).

Discussion

Chromosome numbers of 33 species and 2 subspecies of the suborder Gobioidei have already been reported by some investigators including the present result (Table 3).

Their diploid number ranges from 40 to 50, and in most species the number is 44. On the relation of the chromosome number to Takagi's classification (1963) which is mainly based on morphological characters, there seems to be no correlation at the family or subfamily level. The diploid number is 44~46 in the

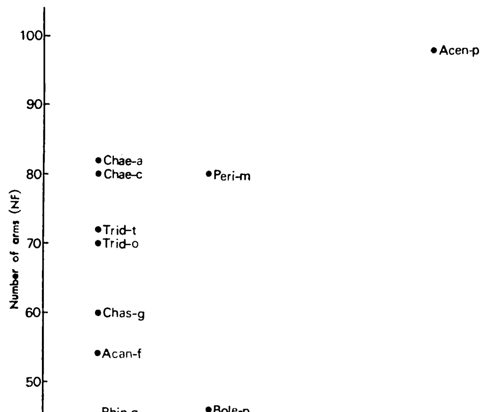
* The classification, which is unpublished, was used here with the permission of the author.

Table 3. Diploid chromosome numbers and elements of the suborder Gobioidei. Species accompanied with asterisks cannot be assigned to subfamilies. V: metacentirc, submetacentric, and subtelocentric chromosomes. r: telocentric and acrocentric chromosomes. Arai and Kobayasi (1973) and Arai and Sawada (1974) treated the metacentric and submetacentric chromosomes as biarm and subtelocentric and acrocentric ones as uniarm.

Family, subfamily, species	2n	Forms	NF	Sources
Eleotridae				
Eleotris acanthopomus	46	46r	46	Arai and Sawada (1974)
Odontobutis obscura	62	62r		Nogusa (1955, 1960)
	44	**44r**	**44**	Present report
Gobiidae				
Amameleotrinae				
Eleotriodes strigatus	44	2V+42r	46	Arai and Sawada (1974)
Rhinogobiinae				
Aboma lactipes	40	uniarm		Arai and Kobayasi (1973)
	40	40r	40	Arai and Sawada (1974)
Rhinogobius brunneus	44	44r		Nogusa (1957, 1960)
	44	uniarm		Arai and Kobayasi (1973)
	44	**44r**	**44**	Present report
R. giurinus	**44**	**44r**	**44**	Present report
R. flumineus	44	uniarm		Arai and Kobayasi (1973)
Acanthogobius flavimanus	44	44r		Nogusa (1950, 1960)
	44	uniarm		Arai and Kobayasi (1973)
	44	**10V+34r**	**54**	Present report
Chasmichthys gulosus	**44**	**16V+28r**	**60**	Present report
C. dolichognatus	44	uniarm		Arai and Kobayasi (1973)
Pterogobius elapoides	44	uni-+biarm		Arai and Kobayasi (1973)
Chaenogobius castanea	**44**	**36V+8r**	**80**	Present report
C. annularis	44	44r		Nogusa (1957, 1960)
	42	14V+28r	56	Yamada (1967)
	44	uni-+biarm		Arai and Kobayasi (1973)
	44	**38V+6r**	**82**	Present report
C. isaza	46	46r		Nogusa (1957, 1960)
Luciogobius guttatus	44	uni-+biarm		Arai and Kobayasi (1973)
Mugilogobius abei	46	46r		Nogusa (1950, 1960)
	46	uniarm		Arai and Kobayasi (1973)
Tridentigerinae				
Tridentiger obscurus	44	44r		Nogusa (1950, 1960)
T. obscurus obscurus	**44**	**26V+18r**	**70**	Present report
T. obscurus brevispinis	44	uni-+biarm		Arai and Kobayasi (1973)
T. trigonocephalus	44	uni-+biarm		Arai and Kobayasi (1973)
	44	**28V+16r**	**72**	Present report
Periophthalminae				
Periophthalmus cantonensis	46	46r		Nogusa (1975, 1960)
	46	uni-+biarm		Arai and Kobayasi (1973)
	46	**34V+12r**	**80**	Present report

Table 3. (continued).

Family, subfamily, species	2n	Forms	NF	Sources
Apocrypterinae				
Boleophthalmus pectinirostris	46	46r		Nogusa (1957, 1960)
	46	**46r**	**46**	Present report
B. boddaerti	46			Verma (1968)
	46	46V		Subrahmanyam (1969)
B. dussumieri	46			Verma (1968)
Apocryplodon madurensis	48			Verma (1968)
Pseudapocryptes borneensis	48			Verma (1968)
Gobiinae				
Glossogobius giuris	46			Kaur and Srivastava (1973)
Bathygobius fuscus	48	uniarm		Arai and Kobayasi (1973)
Ctenogobius criniger	50	34V+16r	84	Arai and Sawada (1974)
Acentrogobius pflaumi	**50**	**48V+2r**	**98**	Present report
*Gobiodon citrinus** (male)	43	1V+42r	44	Arai and Sawada (1974)
(female)	44	2V+42r	46	Arai and Sawada (1974)
*Gillichthys mirabilis**	44	12V+32r	56	Chen and Ebeling (1971)
*G. seta**	44	20V+24r	64	Chen and Ebeling (1971)
*Gobius striatus**	46			Verma (1968)
*Brachygobius nunus**	48			Post (1965)



← Fig. 6. Relationships between numbers of the diploid chromosomes and arms. Odon-o, *Odontobutis obscura*; Rhin-b, *Rhinogobius brunneus*; Rhin-g, *Rhinogobius giurinus*; Acan-f, *Acanthogobius flavimanus*; Chis-g, *Chasmichthys gulosus*; Trid-o, *Tridentiger obscurus obscurus*; Trid-t, *Tridentiger trigonocephalus*; Chae-c, *Chaenogobius castanea*; Chae-a, *Chaenogobius annularis*; Bole-p, *Boleophthalmus pectinirostris*; Peri-m, *Periophthalmus cantonensis*; Acen-p, *Acentrogobius pflaumi.*

family Eleotridae. In the family Gobiidae, the Amameleotrinae and Tridentinae have the number of 44, Periophthalminae 46, the Apocrypterinae 46～48, the Gobiinae 48～50, and the Rhinogobiinae 40～46. The Rhinogobiinae, which have the widest range in chromosome number, also have wide ranges of differentiations in morphological characters. On the other hand, the chromosome number is well stabilized at the genus level.

It has been generally assumed that in perciform teleosts the basic chromosome number is 2n=48, consisting of all uniarmed chromosomes of similar size (Post, 1965; Ohno et al., 1968). In the suborder Gobioidei, however, 2n=44 is the commonest number and possessed by most generalized species.

The arm number (NF) of 12 species examined here ranges from 44 to 98 (Fig. 6). Among 2n=44 group the two genera *Odontobutis* and *Rhinogobius* which have karyotypes consisting of all uniarmed chromosomes (NF=44) are simplest, and *Acanthogobius* with NF=54, *Chasmichthys* with NF=60, *Tridentiger* with NF=70～72, *Chaenogobius* with NF=80～82 are more complicated in their karyotypes. Among 2n=46 group, *Boleophthalmus* with NF=46 appears to be simpler than *Periophthalmus* with NF=74. Lastly, the genus *Acentrogobius* may be most complicated, having NF=98 and 2n=50 (Fig. 6).

If we follow Chen and Ebeling (1972), who judged the derived condition within a genus from number of arms, karyotype of *Tridentiger trigonocephalus* (NF=72) is considered to be more specialized than that of *T. obscurus obscurus* (NF=70), and that of *Chaenogobius annularis* (NF=82) than that of *C. castanea* (NF=80).

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日本産ハゼ亜目魚類 12 種の染色体の比較研究

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日本産ハゼ亜目魚類 12 種 (9 属, 5 亜科, 2 科) の染色体を調査し, 体細胞染色体数および NF 数を数えた. 染色体数はドンコ, ヨシノボリ, ゴクラクハゼ, マハゼ, ドロメ, ビリンゴ, ウキゴリ, チチブおよびシマハゼでは 2n=44 で, ムツゴロウ, トビハゼでは 2n=46, スジハゼでは 2n=50 である. NF 数は 44～98 の範囲にある. これらの種類と以前報告されたものを合せた 33 種と 2 亜種の染色体数を, 従来の分類体系と比較した結果, 両者の関係は属間ではよく一致しているが, 科および亜科段階では必ずしも一致していなかった. しかし, 一般的な傾向として, カワアナゴ科では 44～46, ハゼ科の中ではチチブ亜科で 44, トビハゼ亜科で 46, タビラクチ亜科で 46～48, ハゼ亜科で 48～50 であり, 比較的安定している. だが, ヨシノボリ亜科では 40～46 の広範囲にわたっている. このことはこの亜科が多くの種と属を含むため, その豊富な種的分化と関係していると考えられる. 他方, ここで調査した 12 種において, 染色体数と NF 数から, 核型はドンコ属とヨシノボリ属が最も単純で, マハゼ属, アゴハゼ属, チチブ属, ウキゴリ属, ムツゴロウ属, トビハゼ属, クツワハゼ属の順に複雑化している.

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