Karyotypes of Three Species of *Gobiobotia* (Pisces: Cyprinidae) in Korea

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The karyotypes of three species of *Gobiobotia* in Korea were investigated: *G. macrocephala, G. brevibarba,* and *G. nakdongensis.* In these species, the mitotic chromosomes from 25 groups with two chromosomes each indicated that it is a diploid. The karyotypes of *Gobiobotia macrocephala* are 2n = 50 (9M+7SM+9ST) with NF = 100, *G. brevibarba* 2n = 50 (10M+7SM+4ST+4T) with NF = 92, and *G. nakdongensis* 2n = 50 (5M+9SM+9ST+2T) with NF = 96. Chromosome sizes ranged from 3.3 to 7.5 µm, 2.7 to 6.3 µm and 3.5 to 7.3 µm in length, respectively. This is the first report on the chromosomes of *G. macrocephala* and *G. nakdongensis*.

Key words : Chromosome, Cyprinidae, *Gobiobotia macrocephala, G. brevibarba, G. nakdongensis*, Korea

Introduction

The Cyprinid fishes represent the world's largest primary freshwater fish family (more than 2,000 species; Nelson, 1994), and also an important element in the Korean ichthyofauna. In Korea, 65 species of Cyprinidae are recognized (Kim, 1997). In the Korean Peninsula, the genus *Gobiobotia* Kreyenberg, 1911 has been reported by three species: *G. macrocephala* Mori 1935, *G. brevibarba* Mori, 1935 and *G. nakdongensis* Mori, 1935 (Mori, 1935; Uchida, 1939). They are endemic and endangered species of Korea. There have been a few previous studies on the feeding habit and reproductive ecology in these species (Choi and Baek, 1972; Choi *et al.*, 2001, 2004).

Cytogenetic studies of fishes have been important in aspects of phylogenetics and cytogenetic relationships among the species (Ozouf-Costaz and Foresti, 1992; Collares-Pereira *et al.*, 1998; Gozukara and Cavas, 2004). The chromosome numbers of animal species are, in general, uniform and constant, each species having a characteristic chromosome number. The chromosome numbers of about 50 species belonging to the Korean Cyprinidae family have been reported previously (Table 1). And the chromosome numbers of these species range from 2n = 44 to 2n =76 (Lee *et al.*, 1982; Lee *et al.*, 1983; Lee, 1984; Lee *et al.*, 1984b; Ueno and Ojima, 1984; Kim *et al.*, 2004). Ueno and Ojima (1984) reported the chromosome numbers of *G. brevibarba.* However, cytogenetic studies of *G. macrocephala* and *G. nakdongensis* have not been examined.

This paper presents the chromosome numbers and the karyotypes of *G. macrocephala, G. brevibarba* and *G. nakdongensis* in Korea.

Materials and Methods

The specimens used in this study were collected in Korea from July 2004 to January 2005, and examined shortly after collection. Fifteen specimens of *G. brevibarba* were collected in the Geoun-ri, Yeongwol-eup, Yeongwol-gun, Gang-

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Classification	Chromosome No. (2n)	References
Acheilognathinae		
Rhodeus ocellatus	48	Lee <i>et al.</i> , 1982; Lee, 1983
R. uyekii	46, 48	Lee et al., 1982; Lee, 1982; Lee et al., 1983
R. notatus	46	Lee, 1982; Lee <i>et al.</i> , 1982; Lee, 1983
Acheilognathus lanceolatus	48	Lee et al., 1982; Lee, 1983; Kim, 1991
A. signifier	48	Lee <i>et al.</i> , 1983; Kim, 1991
A. koreensis	48	Lee, 1982; Lee <i>et al.</i> , 1982; Kim, 1991; Yang, 2004
A. somjinensis	48	Kim, 1991; Yang, 2004
A. yamatsutae	44, 48	Lee et al., 1982; Lee, 1983; Lee et al., 1983; Kim, 1991
A. rhombeus	44	Lee et al., 1982; Lee, 1983; Kim, 1991
A. macropterus	44	Lee et al., 1982; Lee, 1983
A. gracilis	44	Lee et al., 1982; Lee, 1983
Gobioninae		
Pseudorasbora parva	50	Lee <i>et al.</i> , 1983
Pungtungia herzi	50	Lee <i>et al.</i> , 1983; Lee, 1984
Pseudopungtungia nigra	50	Lee <i>et al.</i> , 1983; Kim and Shim, 1991
P. tenuicorpa	50	Kim and Shim, 1991
Coreoleuciscus splendidus	50	Lee et al., 1983; Lee, 1984
Sarcocheilichthys variegatus wakiyae	50	Lee et al., 1984a
S. nigripinnis morii	50	Lee, 1984
Ganthopogon strigatus	50	Lee, 1984
Squalidus gracilis majimae	50	Lee, 1984
S. japonicus coreanus	50	Lee <i>et al.</i> , 1984a
S. chankaensis tsuchigae	50	Kim <i>et al.</i> , 2004
S. multimaculatus	50	Kim <i>et al.</i> , 2004
Hemibarbus labeo	50	Kang and Park, 1973; Lee <i>et al.</i> , 1984a
H. longirostris	50	Lee <i>et al.</i> , 1983
H. mylodon	50	Ueno and Ojima, 1984
Pseudogobio esocinus	50	Lee <i>et al.</i> , 1984a; Lee, 1984
Gobiobotia macrocephala	50	Present study
<i>G. brevibarba</i>	50	Ueno and Ojima, 1984; Present study
G. nakdongensis	50	Present study
Microphysogobio yaluensis	50	Lee <i>et al.</i> , 1983
M. jeoni	50	Lee <i>et al.</i> , 1983
M. longidorsalis	50	Lee et al., 1983; Im et al., 2004
-		
Leuciscinae	50	Les et al. 1004b. Les et al. 1000
Tribolodon hakonensis	50 50	Lee <i>et al.</i> , 1984b; Lee <i>et al.</i> , 1986
T. brandti	50 50	Lee <i>et al.</i> , 1984b; Lee <i>et al.</i> , 1986
Phoxinus phoxinus	50	Lee <i>et al.</i> , 1984b; Lee <i>et al.</i> , 1986
Rhynchocypris oxycephalus	48, 50	Kang and Park, 1973; Lee <i>et al.</i> , 1983; Lee <i>et al.</i> , 1984b; Lee <i>et al.</i> , 1986; Lee <i>et al.</i> , 1987
R. steindachneri	50	Kang and Park, 1973; Lee <i>et al.</i> , 1984a; Lee <i>et al.</i> , 1984b Lee <i>et al.</i> , 1986; Lee <i>et al.</i> , 1987
R. kumgangensis	50	Lee et al., 1983; Lee et al., 1984b; Lee et al., 1986
R. semotilus	50	Lee <i>et al.</i> , 1984b
Danioninae		
Aphyocypris chinensis	48	Lee et al., 1984a; Lee et al., 1986
Zacco temmincki	48	Lee <i>et al.</i> , 1983; Lee <i>et al.</i> , 1986
Z. platypus	48	Lee <i>et al.</i> , 1983; Lee <i>et al.</i> , 1986
<i>Opsariichthys uncirostris amurensis</i>	76	Lee <i>et al.</i> , 1983; Lee <i>et al.</i> , 1986

Table 1. Chromosome number of Korean Cyprinid fishes by references

weon-do, Korea, twelve specimens of *G. macro-cephala* in Boktan-ri, Sotae-myeon, Chungjusi, Chungcheongbuk-do, Korea and five specimens of *G. nakdongensis* in Seoktap-ri, Andong -si, Gyeongsangbuk-do, Korea. Chromosome preparations were made from gill tissues by the

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Species	No. of chromosome	Karyotype*	NF value	Locality
Gobiobotia macrocephala	2n = 50	9M+7SM+9ST	100	Han river
Gobiobotia brevibarba	2n = 50	$10M \! + \! 7SM \! + \! 4ST \! + \! 4T$	92	Han river
Gobiabotia nakdongensis	2n = 50	$5M\!+\!9SM\!+\!9ST\!+\!2T$	96	Nakdong river

Table 2. Karyotypes of the three species in the genus Gobiobotia (Cyprinidae) of Korea

*M: metacentric chromosome; SM: submetacentric chromosome; ST: subtelocentric chromosome; T: telocentric chromosome

Table 3. Relative lengths and total lengths (μm) of chromosomes of *Gobiobotia macrocephala**

Chromosome	RL±SE(%)	TL±SE	Туре
1	5.62 ± 1.04	7.5 ± 0.37	ST
2	5.57 ± 0.80	7.4 ± 0.21	Μ
3	4.97 ± 0.76	6.6 ± 0.16	ST
4	87 ± 0.72	6.5 ± 0.15	ST
5	4.77 ± 0.67	6.3 ± 0.12	ST
6	4.72 ± 0.67	6.3 ± 0.12	Μ
7	4.62 ± 0.43	6.1 ± 0.19	SM
8	$4.52 \!\pm\! 0.39$	6.0 ± 0.17	Μ
9	$4.42 \!\pm\! 0.39$	5.9 ± 0.17	SM
10	4.31 ± 0.34	5.7 ± 0.14	Μ
11	$4.22 \!\pm\! 0.84$	5.6 ± 0.19	ST
12	$4.12 \!\pm\! 0.79$	5.5 ± 0.17	Μ
13	4.01 ± 0.73	5.3 ± 0.16	Μ
14	3.82 ± 0.66	5.1 ± 0.10	Μ
15	3.67 ± 0.39	4.9 ± 0.12	SM
16	3.61 ± 0.35	4.8 ± 0.09	SM
17	3.52 ± 0.35	4.7 ± 0.09	ST
18	3.46 ± 0.33	4.6 ± 0.08	ST
19	3.41 ± 0.57	4.5 ± 0.15	SM
20	3.31 ± 0.33	4.4 ± 0.14	SM
21	3.22 ± 0.25	4.3 ± 0.16	ST
22	3.11 ± 0.29	4.1 ± 0.14	SM
23	$2.91 \!\pm\! 0.24$	$3.9 \!\pm\! 0.15$	Μ
24	2.71 ± 0.74	3.6 ± 0.16	Μ
25	2.51 ± 0.69	3.3 ± 0.15	ST

^{*}Based on measurement of six karyotyped cells. RL \pm SE, relative length of the chromosome (percentage of the total length of the autosomes in diploid); TL, total length of the autosomes in diploid; SE, standard error.

air-dry method with minor modification (Collares -Pereira, 1992; Park, 1994). Specimens were treated with 0.1 mL of 0.05% colchicine solution to intra-abdominal injected to fishes and set aside for 10 hours in an aquarium at room temperature. The treated gill tissues were dissected and minced with needles in a hypotonic 0.01% NaCl solution. Separated cells were collected by centrifugation at 1,000 rpm, for 10 mins. These cells were fixed in freshly mixed modified Carnoy's fixative (three parts of methanol and one part of glacial acetic acid). The supernatant was replaced by fresh fixative. The centrifugation (1,000 rpm, 10 mins) was repeated two more times. A drop of the cell suspension was then pipetted by a microhematocrit capillary tube and dropped onto a clean slideglass pre-cooled at 4°C. The cells left on the slide were air-dried and then stained for 10 minutes with 4% Giemsa (Gurr's R66) solution made up in 0.1 M phosphate buffer, pH 7.0. The prepared slides were observed under an Olympus (VANOX, Japan) microscope with a $100 \times$ (n.a. 1.25) oil immersion objective and a $10 \times$ ocular.

Morphological features of the chromosomes used to compare karyotypes were the total lengths and the relative lengths of the chromosomes, as well as the positions of their centromeres (primary constrictions). Nomenclature of chromosome morphological types follows Levan *et al.* (1964). To estimate the NF value, the chromosomes of the group meta- and submetacentrics were scored as bi-armed and the chromosomes of the group acrocentrics as uni-armed. For sex chromosomes, males and females of three species were separately observed.

Voucher specimens of the three species used in this investigation have been placed in the Department of Parasitology, Kwandong University College of Medicine, Korea.

Results

1. Gobiobotia macrocephala

In 25 cells, 50 chromosomes were observed (NF = 100). The karyotype of this species consists of nine pairs of metacentric chromosomes, seven pairs of submetacentric chromosomes and nine pairs of subtelocentric chromosomes (Table 2, Fig. 1). Table 3 shows the mean lengths and relative lengths of each chromosome as examined in six cells. Observed chromosomes ranged from 3.3 to 7.5 μ m. The mean total chromosome length based on the measurements of six cells was 132.9 \pm 3.85 μ m. Fig. 1B is the karyotype constructed from the chromosomes shown in Fig. 1A, which

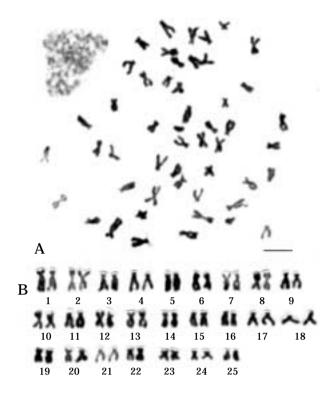


Fig. 1. A, Metaphase chromosome of *Gobiobotia macrocephala*; B, Karyotype constructed from A. Scale bar indicates 5 μm.

was one of the good prepared complements. The chromosomes were arranged by size. This is the first report on the chromosomes of *G. macrocephala*.

2. Gobiobotia brevibarba

Chromosomes in 28 cells were observed and the chromosome number is 50 (NF = 92). The karyotype of this species consists of 10 pairs of metacentric chromosomes, seven pairs of submetacentric chromosomes, four pairs of subtelocentric chromosomes and four pairs of telocentric chromosomes (Table 2, Fig. 2). Table 4 shows the mean lengths and relative lengths of each chromosome as examined in five cells. Observed chromosomes ranged from 2.7 to 6.3 µm. The mean total chromosome length based on the measurements of six cells was $112.5 \pm 4.28 \,\mu\text{m}$. Fig. 2B is the karyotype constructed from the chromosomes shown in Fig. 2A, which was one of the good prepared complements. The karyotypes of this species arranged by size.

3. Gobiobotia nakdongensis

Chromosomes in 21 cells were observed and the

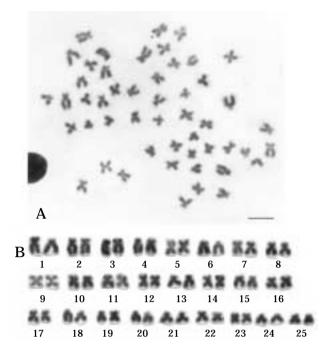


Fig. 2. A, Metaphase chromosome of *Gobiobotia brevibarba*; B, Karyotype constructed from A. Scale bar indicates 5 μm.

Table 4. Relative lengths and total lengths (μm) of chromosomes of *Gobiobotia brevibarba**

mosomes of <i>Gobiobotia brevibarba</i> *			
Chromosome	RL±SE(%)	TL±SE	Туре
1	5.48 ± 0.77	6.3 ± 0.35	ST
2	5.30 ± 0.68	6.1 ± 0.24	SM
3	5.25 ± 0.38	6.1 ± 0.27	SM
4	5.13 ± 0.46	5.9 ± 0.19	SM
5	5.02 ± 0.47	5.8 ± 0.18	Μ
6	$4.91\!\pm\!0.44$	5.7 ± 0.17	ST
7	4.73 ± 0.52	5.5 ± 0.20	Μ
8	4.50 ± 0.53	5.2 ± 0.18	SM
9	4.33 ± 0.51	5.0 ± 0.17	Μ
10	4.21 ± 0.41	4.9 ± 0.14	М
11	4.15 ± 0.38	4.8 ± 0.16	Μ
12	4.09 ± 0.26	4.7 ± 0.14	Μ
13	3.87 ± 0.32	4.5 ± 0.13	SM
14	3.75 ± 0.36	4.3 ± 0.11	Μ
15	3.69 ± 0.33	4.3 ± 0.09	ST
16	3.57 ± 0.31	4.1 ± 0.17	Μ
17	3.46 ± 0.34	4.0 ± 0.17	Μ
18	3.40 ± 0.32	3.9 ± 0.15	Т
19	3.29 ± 0.33	3.4 ± 0.18	SM
20	3.23 ± 0.31	3.3 ± 0.17	Т
21	3.18 ± 0.46	3.2 ± 0.14	Т
22	3.05 ± 0.32	3.1 ± 0.15	SM
23	2.88 ± 0.45	2.9 ± 0.13	М
24	2.83 ± 0.26	2.8 ± 0.16	Т
25	2.71 ± 0.35	2.7 ± 0.14	ST

*Based on measurements of five karyotyped cells.

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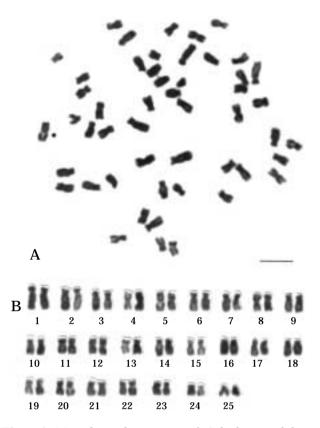


Fig. 3. A, Metaphase chromosome of *Gobiobotia nakdon-gensis*; B, Karyotype constructed from A. Scale bar indicates $10 \,\mu$ m.

chromosome number is 50 (NF = 96). The karyotype of this species consists of five pairs of metacentric chromosomes, nine pairs of submetacentric chromosomes, nine pairs of subtelocentric chromosomes and two pairs of telocentric chromosomes (Table 2, Fig. 3). Table 5 shows the mean lengths and relative lengths of each chromosome as examined in six cells. Observed chromosomes ranged from 3.5 to 7.3 µm. Fig. 3B is the karyotype constructed from the chromosomes shown in Fig. 3A, which was one of the good prepared complements. The karyotypes of this species arranged by size. This species is the first report on the chromosomes. The mitotic metaphase chromosomes were observed in both sexes but sex chromosomes were not observed. Sex chromosomes were not determined in the studied species.

Discussion

An increase in the number of published works

Table 5. Relative lengths and total lengths (μm) of chro)-
mosomes of <i>Gobiobotia nakdongensis</i> *	

Chromosome	RL±SE(%)	TL±SE	Туре
1	5.0 ± 0.55	7.31 ± 0.57	ST
2	4.9 ± 0.64	7.23 ± 0.48	ST
3	4.9 ± 0.57	7.21 ± 0.38	ST
4	4.9 ± 0.49	7.17 ± 0.36	Μ
5	4.8 ± 0.78	7.02 ± 0.27	Μ
6	4.7 ± 0.37	6.93 ± 0.54	ST
7	4.6 ± 0.29	6.77 ± 0.50	ST
8	4.6 ± 0.18	6.66 ± 0.33	Μ
9	4.5 ± 0.47	6.53 ± 0.21	ST
10	4.4 ± 0.34	6.47 ± 0.31	ST
11	4.3 ± 0.26	6.27 ± 0.30	Μ
12	4.2 ± 0.24	6.15 ± 0.21	Μ
13	$3.9\!\pm\!0.33$	5.68 ± 0.22	SM
14	3.8 ± 0.21	5.61 ± 0.16	ST
15	3.8 ± 0.09	5.52 ± 0.23	SM
16	3.7 ± 0.57	5.45 ± 0.21	Т
17	3.7 ± 0.37	5.36 ± 0.12	SM
18	3.6 ± 0.45	5.20 ± 0.12	ST
19	3.5 ± 0.28	$5.15 \!\pm\! 0.37$	SM
20	3.5 ± 0.19	5.06 ± 0.51	SM
21	3.4 ± 0.24	$4.93 \!\pm\! 0.47$	SM
22	3.3 ± 0.18	$4.82 \!\pm\! 0.32$	SM
23	3.2 ± 0.23	$4.68 \!\pm\! 0.42$	SM
24	$2.6\!\pm\!0.21$	3.78 ± 0.36	SM
25	2.4 ± 0.14	3.51 ± 0.25	Т

*Based on measurements of six karyotyped cells.

and laboratory techniques relevant to chromosome investigation in recent years has furthered our knowledge of chromosome number and morphology in many fish species. However, a taxonomic revision has been needed to enhance a thorough interpretation of the phylogenetic interrelationships between various groups. Levan *et al.* (1964) proposed the chromosomal classification. Considering the potential application of the cytological approach to cyprinid systematic (Buth *et al.*, 1991), this study provides also data for a correct specific definition, due to the relatively high conservative character of karyotypes in the family (Rab and Collares–Pereira, 1995).

Detailed studies of chromosome morphology and population cytology of the present fishes are very little on systematics based on the karyotypes other than chromosome numbers. In fact modern cytogenetic techniques have only recently been adopted for studies of fishes. These include application of special banding methods by Sumner (1972) and Howell and Black (1980). In the Korean cyprinid, about 50 species have been karyologically investigated; 2n range from 44 to 76: 2n = $44 \sim 48$ of Acheilognathinae; 2n = 50 of Gobioninae and Leuciscinae; $2n = 48 \sim 76$ of Danioninae (Lee et al., 1982; Lee et al., 1983; Lee, 1984; Lee et al., 1984a b; Ueno and Ojima, 1984; Kim et al., 2004). The most frequent chromosome number in the cyprinid fishes have been reported 2n = 50. For the present study, G. macrocephala, G. brevibarba and G. nakdongensis have 2n = 50 chromosomes. When the karyotypes of these three species are analyzed, G. macrocephala consists of nine pairs of metacentric chromosomes, seven pairs of submetacentric chromosomes and nine pairs of subtelocentric chromosomes, G. brevibarba has of 10 pairs of metacentric chromosomes, seven pairs of submetacentric chromosomes, four pairs of subtelocentric chromosomes and four pairs of telocentric chromosomes and G. nakdongensis has of five pairs of metacentric chromosomes, nine pairs of submetacentric chromosomes, nine pairs of subtelocentric chromosomes and two pairs of telocentric chromosomes. The chromosomes of G. brevibarba have already been revealed by Ueno and Ojima (1984). They reported the same chromosome numbers of 2n = 50. However, its karyotype is different from that of the present species. Ueno and Ojima (1984) reported that the karyotype were consists of six pairs of metacentric chromosomes, 15 pairs of submetacentric/ subtelocentric chromosomes and four pairs of telocentric chromosomes. These differences in the complements are probably due to a personal interpretation of the arm ratio. G. macrocephala and G. nakdongensis is the first report on the chromosomes. Karyotype evolution in bitterlings of cyprinid has been reported by John and Miklos (1979), Meyne et al. (1990) and Ueda et al. (2001). Most cyprinid fishes have karyotypes which are rich in M and SM (Lee et al., 1983, 1984a; Lee, 1984; Kim et al., 2004). Chromosome arm number is usually reported in addition to chromosome number. Species with vastly different chromosome numbers can have similar arm numbers, suggesting that Robertsonian fusions (creation of one metacentric by the centric fusion of two acrocentric chromosomes) occurred in evolution. Centric fission also may account for differences in chromosome number between species that have similar arm numbers (Ohno, 1974). Ohno (1974) and Arai (1983) have been reported that the archetypes have many more acrocentric chromosome and newly differentiated species have a large number of arm number. Chromosome numbers of three species

are 2n = 50 in the present study. But NF of *G. macrocephala, G. brevibarba* and *G. nakdongensis* showed 100, 92 and 96 respectively. Further study will be need C- and Ag-banding analyses for the investigation of the karyotype evolution in *Gobiobotia*.

Koehler *et al.* (1995) have been reported that European cyprinid fish species *Scardinius erythrophthalmus* have sex determining chromosomes. This species was revealed to have 2n = 50 and karyotypes of all male were identical, consisting of 48 small meta- or submetacentric chromosomes and a pair of large metacentric chromosomes (ZZ males). On the other hand, in females, 16 had a karyotype similar to the males (ZW females) and 17 had a heteromorphic pair of chromosomes, including a large metacentric Z chromosome and a small acrocentric W chromosome (ZW females). In the present study, sex determining chromosomes are not observed from males and females in three specimens.

The genus *Gobiobotia* is well known for its intra- and inter-populational morphological variability (Ramaswami, 1955; Kim and Kang, 1989). The chromosome numbers are constant to a great extent at each of the various taxonomical levels, though some of the animals are geographically very widely separated. An especially valuable first step in such an endeavor is a cytogenetic one, as demonstrated here.

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한국산 꾸구리속, *Gobiobotia* (Pisces: Cyprinidae) 3종의 핵형 송 호 복¹·박 갑 만*

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한국산 꾸구리속 (Gobiobotia) 어류인 꾸구리 (G. macrocephala), 돌상어 (G. brevibarba) 및 흰수 마자 (G. nakdongensis)의 핵형을 조사하였다. 연구 결과, 각각 25쌍의 배수체 염색체가 관찰되었다. 핵형은 꾸구리가 2n = 50 (9M+7SM+9ST), NF = 100, 염색체의 길이는 3.3~7.5 μm 크기였으며, 돌상어는 2n = 50 (10M+7SM+4ST+4T), NF = 92, 염색체의 길이는 2.7~6.3 μm의 크기였고, 흰수마자는 2n = 50 (5M+9SM+9ST+2T), NF = 96, 염색체의 길이는 3.5~7.3 μm였다. 본 연구 결과 꾸구리와 흰수마자의 핵형은 처음으로 보고되었다.