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Karyotypes of Five Species in Odontobutidae and Cottidae of Korea

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The chromosome numbers of five species in two families of Korea are investigated: *Odontobutis platycephala*, *O. interrupta*, and *O. obscura* in Odontobutidae, and *Cottus koreanus* and *C. hangiongensis* in Cottidae. In *Odontobutis* species, the three species showed the diploid chromosome number, 2n=44 (NF=44) telocentric chromosomes. In *Cottus* species, the mitotic chromosomes from 24 groups with two chromosomes each indicated that it is a diploid. The karyotype of *C. koreanus* and *C. hangiongens*is is 2n=48 and NF=52. These species is the first report on the chromosomes and the karyotype analysis except *O. platycephala*. Our findings provide cytotaxonomic evidence for the species distinctness of these five species whose descriptions were based primarily on external morphology.

Key words : karyotype, Cottidae, Odontobutidae, *Cottus koreanus*, *C. hangiongensis*, *Odontobutis platycephala*, *O. interrupta*, *O. obscura*, Korea

Introduction

Odontobutidae is a small family of fishes in the order Perciformes. They are native to fresh water rivers flowing into the South China Sea and the northwestern Pacific Ocean. There are twenty species in six genera. In Korea, 4 species in two genera of Odontobutidae are recognized (Kim, 1997; Choi, 1998). Molecular phylogenetic and morphological character analyses have been recently been studied using DNA sequencing in Odontobutidae (Thacker, 2003; Thacker and Hardman, 2005). In the Korean peninsula, the genus *Odontobutis* (Bleeker, 1874) has been reported by three species: *Odontobutis platycephala* Iwata and Jeon, 1985, *O. interrupta* (Iwata and Jeon, 1985), and *O. obscura* (Temminck and Schlegel, 1845; Kim, 1997). *O. platycephala* and *O. interrupta* are known endemic species from Korea. The genus *Odontobutis* has a wide geographic distribution in Korean peninsula. The genus also reveals closely similar species from taxonomic point of view. *Odontobutis interrupta* has been previously known to serve as the second intermediate host of *Echinostoma hortense* (Ahn *et al*., 1985; Ahn and Ryang, 1986). The family Cottidae inhabits mostly marine habitats in the temperate and cold waters of the Northern Hemisphere. This group included with 70 genera and 300 species in the worldwide (Nelson, 1994). Thirty six species belonging to 21 genera in the family Cottidae from Korea are reviewed (Kim and Youn, 1992). The genus *Cottus* belongs to the family Cottidae in Korea contains only three described species. *Cottus czerskii* is distributed in Duman River of Korea. *C. koreanus* and *C. hangiongensis* are endangered species in Korea. There have been a few previous studies of the parasites (Muzzall *et al*., 1997), genetic variation (Gyllenstewn and Ryman, 1988), and molecular evolution (Hunt *et al*., 1997; Yokoyama and Goto,

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2005).

In recent years, through a considerable number of works, a large amount of information has been accumulated on the chromosomes of the fishes. Cytogenetic studies of fishes have been important in aspects of phylogenetics and cytogenetic relationships among the species (Collares-Pereira *et al*., 1998; Gozukara and Cavas, 2004). Lee *et al*. (1983) and Lee (1986) reported the chromosome numbers and karyotypes of *O. platycephala*, genus *Odontobutis*. However, karyological studies of these two species of genus *Odontobutis* and two species of genus *Cottus* have not been examined. In this work, five species of two families of Korea were examined to determine and analyze the karyotypes for future comparative study of fish karyology.

Materials and Methods

The specimens used in this study were collected in Korea from July to October 2005, and examined shortly after collection. Six specimens of *Odontobutis platycephala* were collected in the Tonggok-ri, Namsan-myeon, Chuncheon-si, Gangwon-do, five specimens of *O. interrupta* in Wolmyeong-ri Yanggu-eup, Yanggu-gun, Gangwon-do, seven specimens of *O. obscura* in Gucheon-ri, Dongbu-myeon, Geoje-si, Gyeongsangnam-do, 3 specimens of *Cottus koreanus* in Jingdong-ri, Girin-myeon, Inje-gun, Gangwon-do, and five specimens of *C. hangiongensis* in Hosan-ri, Wondeok-eup, Samcheok-si, Gangwon-do. The chromosomes preparations were made on gills of the fish by the usual flame drying method (Ojima *et al*., 1972). The prepared slides were observed under an Olympus BX-51 microscope. Nomenclature of chromosome morphological types follows Levan *et al*. (1964). To estimate the NF value, the chromosomes of the group meta- and submetacentric were scored as bi-armed and the chromosomes of the group acrocentric as uni-armed. Voucher specimens of the five species used in this investigation have been placed in the Department of Parasitology, Kwandong University College of Medicine, Korea.

Results

1. *Odontobutis platycephala*

Diploid chromosome number of this species was 44 (NF=44) and consisted of 22 pairs telocentric chromosomes (Fig. 1). Table 1 shows the mean lengths and relative lengths of each chromosome as examined in three cells. Observed chromosomes ranged from 3.03 to 5.46 μm. The mean total chromosome length based on the measurements of three cells was 99.84±2.97 μm. Fig. 1 B is the karyotype constructed from the chromosomes shown in Fig. 1 A, which was one of the most elongated complements. The chromosomes were arranged by size. This species is the second report on the chromosomes. Lee *et al*. (1983) and Lee (1986) reported the same chromosome numbers of 2n=44.

2. *O. obscura*

O. obscura had 44 diploid chromosomes consisting of 22 pairs of telocentric chromosomes (Fig. 2). Table 2 shows the mean lengths and relative lengths of each chromosome as examined in three cells. Observed chromosomes ranged from 3.21 to 6.17 μm. The mean total chromosome length based on the measurements of three cells was 100.96±4.00 μm. Fig. 2 B is the karyotype constructed from the chromosomes shown in Fig. 2 A, which was one of the most elongated complements.

3. *O. interrupta*

Chromosomes in six cells were observed and the chromosome number is 44 (NF=44). The karyotype of this species consists of 22 pairs of telocentric chromosomes (Fig. 3). Table 3 shows the mean lengths and relative lengths of each chromosome as examined in two cells. Observed chromosomes ranged from 3.02 to 6.45 μm. Fig. 3 B is the karyotype constructed from the chromosomes shown in Fig. 3 A, which was one of the most elongated complements. The karyotypes of this species arranged by size. There were no differences in diploid chromosome number or in chromosome morphology between males and females.

4. *Cottus koreanus*

Chromosomes in eight cells were observed and the chromosome number is 48 (NF=52). The karyotype of this species consists of two pairs of subtelocentric chromosomes and 22 pairs of telocentric chromosomes (Fig. 4). Table 4 shows the mean lengths and relative lengths of each

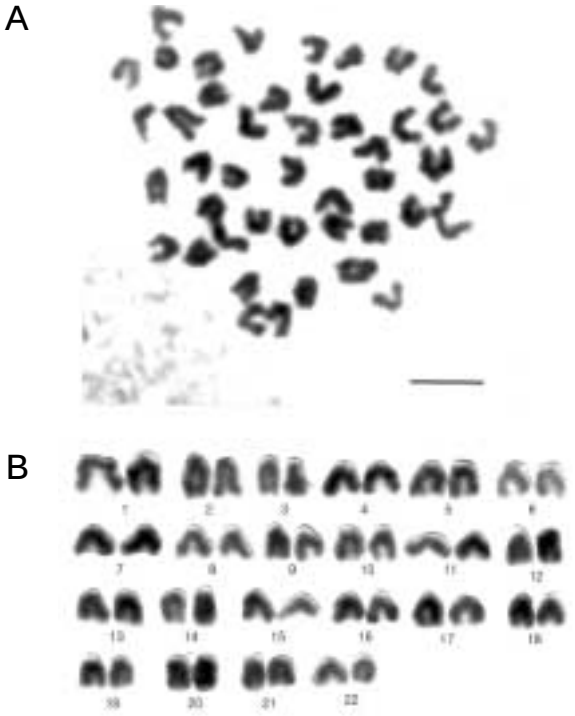

Fig. 1. A, Metaphase chromosome of *Odontobutis platycephala*; B, Karyotype constructed from A. Scale bar indicates 10 μm.

Table 1. Relative lengths and total lengths (μm) of chromosomes of *Odontobutis platycephala**

Chromosome No.	RL±SE	TL±SE	Type
1	6.12±1.01	5.46±0.27	T
2	6.05±0.82	5.44±0.31	T
3	5.97±0.76	5.42±0.14	T
4	5.87±0.74	5.34±0.20	T
5	5.77±0.61	5.23±0.12	T
6	5.52±0.60	5.04±0.13	T
7	5.41±0.41	4.87±0.09	T
8	5.39±0.36	4.82±0.17	T
9	5.28±0.37	4.81±0.06	T
10	5.17±0.33	4.68±0.12	T
11	5.10±0.82	4.57±0.13	T
12	5.09±0.77	4.56±0.11	T
13	4.91±0.73	4.39±0.15	T
14	4.82±0.63	4.38±0.10	T
15	4.67±0.39	4.24±0.11	T
16	4.61±0.35	4.23±0.09	T
17	4.52±0.34	4.14±0.07	T
18	4.46±0.33	4.03±0.08	T
19	4.31±0.50	3.89±0.12	T
20	4.25±0.31	3.78±0.14	T
21	3.89±0.22	3.59 ±0.16	T
22	3.31±0.29	3.03±0.10	T

*Based on measurement of three karyotyped cells. RL±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; T, telocentric chromosomes.

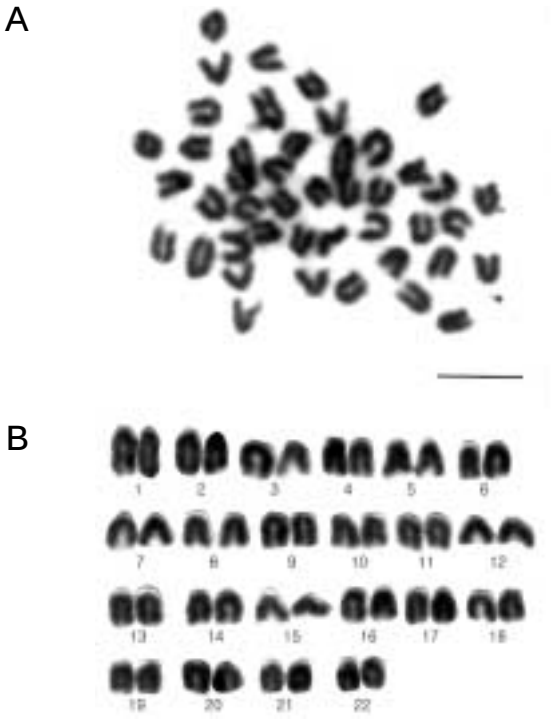

Fig. 2. A, Metaphase chromosome of *Odontobutis obscura*; B, Karyotype constructed from A. Scale bar indicates 10 μm.

Table 2. Relative lengths and total lengths (μm) of chromosomes of *Odontobutis obscura**

Chromosome No.	RL±SE	TL±SE	Type
1	6.00±0.67	6.17±0.35	T
2	5.72±0.38	5.68±0.24	T
3	5.45±0.28	5.42±0.37	T
4	5.43±0.46	5.46±0.29	T
5	5.35±0.40	5.31±0.12	T
6	5.30±0.34	5.24±0.17	T
7	5.13±0.52	5.10±0.20	T
8	5.05±0.43	5.04±0.16	T
9	4.99±0.23	4.89±0.17	T
10	4.86±0.31	4.76±0.14	T
11	4.75±0.32	4.71±0.21	T
12	4.59±0.16	4.52±0.16	T
13	4.37±0.21	4.34±0.13	T
14	4.31±0.22	4.27±0.11	T
15	4.26±0.13	4.19±0.09	T
16	4.15±0.26	4.14±0.17	T
17	3.98±0.25	3.88±0.13	T
18	3.74±0.12	3.66±0.15	T
19	3.59±0.13	3.59±0.18	T
20	3.43±0.21	3.42±0.17	T
21	3.31±0.32	3.34±0.14	T
22	3.20±0.11	3.21±0.15	T

*Based on measurement of three karyotyped cells

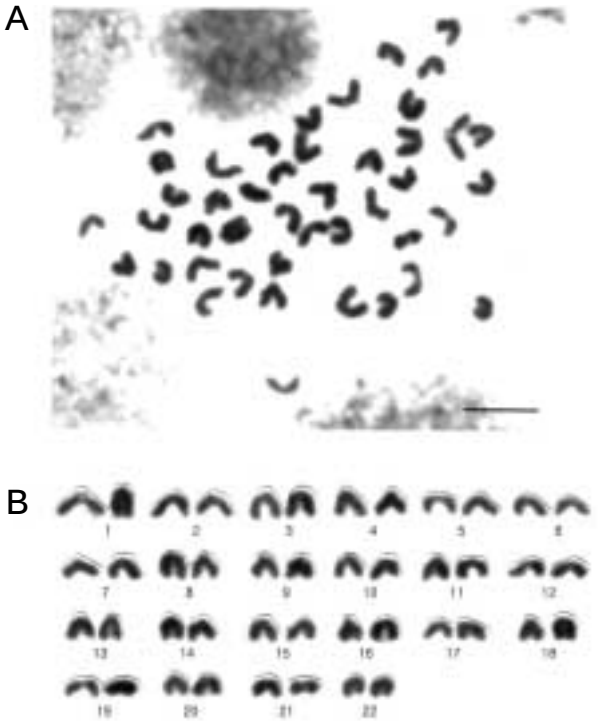

Fig. 3. A, Metaphase chromosome of *Odontobutis interrupta*; B, Karyotype constructed from A. Scale bar indicates 10 μm.

Fig. 4. A, Metaphase chromosome of *Cottus koreanus*; B, Karyotype constructed from A. Scale bar indicates 10 μm.

Table 3. Relative lengths and total lengths (μm) of chromosomes of *Odontobutis interrupta**

Chromosome No.	RL±SE	TL±SE	Type
1	5.90±0.50	6.45±0.37	T
2	5.59±0.34	6.11±0.28	T
3	5.32±0.27	5.82±0.18	T
4	5.29±0.39	5.79±0.26	T
5	4.89±0.18	5.35±0.17	T
6	4.76±0.27	5.21±0.14	T
7	4.56±0.19	4.99±0.20	T
8	4.51±0.18	4.93±0.23	T
9	4.36±0.37	4.77±0.11	T
10	4.24±0.30	4.64±0.21	T
11	4.13±0.16	4.52±0.20	T
12	4.08±0.24	4.46±0.12	T
13	3.89±0.13	4.25±0.14	T
14	3.78±0.21	4.13±0.16	T
15	3.64±0.19	3.98±0.13	T
16	3.57±0.21	3.90±0.17	T
17	3.48±0.33	3.80±0.12	T
18	3.36±0.21	3.67±0.10	T
19	3.25±0.22	3.55±0.13	T
20	3.14±0.19	3.43±0.21	T
21	2.86±0.21	3.13±0.24	T
22	2.76±0.18	3.02±0.22	T

*Based on measurement of two karyotyped cells.

Table 4. Relative lengths and total lengths (μm) of chromosomes of *Cottus koreanus**

Chromosome No.	RL±SE	TL±SE	Type
1	5.63±0.20	6.51±0.17	T
2	5.51±0.24	6.36±0.26	T
3	5.35±0.17	6.19±0.13	T
4	5.27±0.19	6.09±0.15	T
5	5.08±0.08	5.87±0.16	T
6	4.95±0.12	5.72±0.15	T
7	4.87±0.09	5.63±0.17	T
8	4.69±0.18	5.42±0.14	T
9	4.59±0.17	5.31±0.18	T
10	4.53±0.13	5.24±0.21	ST
11	4.25±0.06	4.92±0.24	T
12	4.12±0.14	4.76±0.18	T
13	4.06±0.08	4.70±0.12	T
14	3.93±0.11	4.55±0.21	T
15	3.79±0.12	4.38±0.17	T
16	3.71±0.23	4.29±0.23	T
17	3.61±0.13	4.18±0.17	T
18	3.48±0.11	4.02±0.18	T
19	3.33±0.12	3.85±0.24	T
20	3.23±0.10	3.74±0.17	T
21	3.10±0.17	3.59±0.15	ST
22	2.99±0.12	3.46±0.19	T
23	2.94±0.08	3.40±0.22	T
24	2.87±0.13	3.32±0.24	T

*Based on measurement of four karyotyped cells. ST, subtelocentric chromosomes.

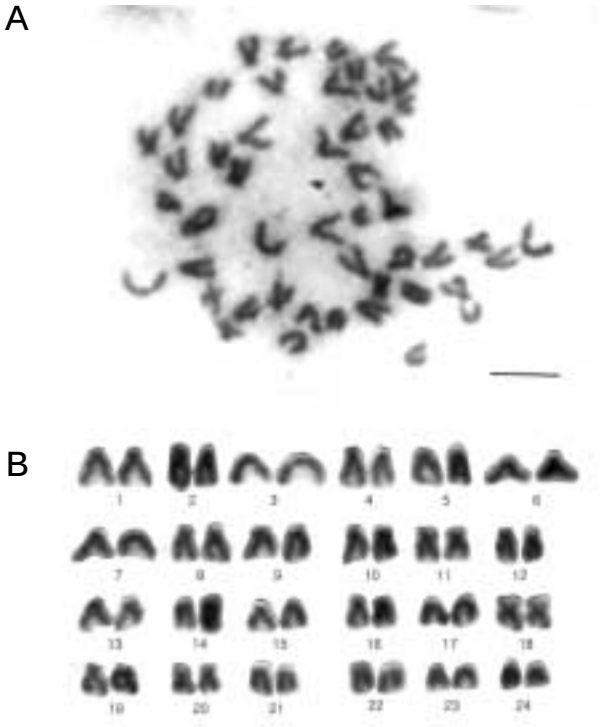

Fig. 5. A, Metaphase chromosome of *Cottus hangiongensis*; B, Karyotype constructed from A. Scale bar indicates 10 μm.

chromosome as examined in four cells. Observed chromosomes ranged from 3.32 to 6.51 μm. The mean total chromosome length based on the measurements of three cells was 115.50±4.43 μm. Fig. 4 B is the karyotype constructed from the chromosomes shown in Fig. 4 A, which was one of the most elongated complements. The karyotypes of this species arranged by size. The mitotic metaphase chromosomes were observed in both sexes but sex chromosomes were not observed.

5. *C. hangiongensis*

This species showed 48 chromosomes (NF=52) in both sexes, consisting of two pairs of subtelocentric chromosomes and 22 pairs of telocentric chromosomes (Fig. 5). Table 5 shows the mean lengths and relative lengths of each chromosome as examined in three cells. Observed chromosomes ranged from 3.27 to 6.32 μm. Fig. 5 B is the karyotype constructed from the chromosomes shown in Fig. 5 A, which was one of the most elongated complements. The karyotypes of this species arranged by size.

Table 5. Relative lengths and total lengths (μm) of chromosomes of *Cottus hangiongensis**

Chromosome No.	RL±SE	TL±SE	Type
1	5.62±0.25	6.32±0.35	T
2	5.48±0.24	6.16±0.26	T
3	5.32±0.21	5.98±0.16	T
4	5.24±0.19	5.89±0.23	T
5	5.04±0.23	5.67±0.12	T
6	4.91±0.17	5.52±0.12	T
7	4.80±0.09	5.39±0.12	T
8	4.65±0.14	5.23±0.20	T
9	4.55±0.17	5.11±0.13	T
10	4.48±0.10	5.04±0.11	T
11	4.29±0.12	4.82±0.14	ST
12	4.15±0.04	4.66±0.12	T
13	4.09±0.06	4.60±0.16	T
14	3.96±0.11	4.45±0.11	T
15	3.81±0.13	4.28±0.12	T
16	3.73±0.04	4.19±0.13	T
17	3.63±0.13	4.08±0.17	T
18	3.49±0.11	3.92±0.14	ST
19	3.33±0.12	3.75±0.11	T
20	3.24±0.14	3.64±0.16	T
21	3.14±0.11	3.53±0.11	T
22	3.04±0.12	3.42±0.18	T
23	2.99±0.13	3.36±0.12	T
24	2.91±0.10	3.27±0.11	T

*Based on measurement of three karyotyped cells.

Discussion

Considering the potential application of the cytological approach to cyprinid systematic (Buth *et al*., 1991), erithrinid fish (Bertollo *et al*., 2004), Curimatidae (Brassesco *et al*., 2004), and Loricariidae (Alves *et al*., 2005; Kavalco *et al*., 2005), 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). The chromosome numbers of about 13 species belonging to the Korean Perciformes have been reported previously (Table 6). Cytogenetic studies in three species of genus *Odonobutis* have shown constant diploid number 2n=44 chromosomes. Although having the same diploid number and types, the karyotype formulae a little differ in their size. In two species of genus *Cottus*, the diploid number, fundamental number and karyotype is almost identical. However, when the chromosome of these species arranged by size, the subtelocentric chromosome was difference in a location. *Cottus koreanus* is located in the pair 11 and 18 and *C. hangion-*

Table 6. Chromosome numbers of Korean Scorpaeniformes and Perciformes fishes by references and present study

Classification	Chromosome No. (FN)	Karyotype	References
Order Scorpaeniformes			
Family Cottidae			
Cottus koreanus	2n=48 (52)	2ST+22T	Present study
C. hangiongensis	2n=48 (52)	2ST+22T	Present study
Order Perciformes			
Family Moronidae (Serranidae)			
Lateolabrax japonicus	2n=48 (48)	24A	Lee *et al*., 1984
Family Centropomidae			
Siniperca scherzeri	2n=48 (52)	2SM+22A	Park, 1981, Lee *et al*., 1984, Lee *et al*., 1997, Bang *et al*., 2001
Coreoperca kawamebari	2n=48 (54)	3SM+21T	Bang *et al*., 2001
Coreoperca herzi	2n=48	2SM+22T	Lee *et al*., 1983, Bang *et al*., 2001
Family Gobiidae			
Tridentiger abscurus	2n=44 (56)	2M+4SM+16A	Lee *et al*., 1984
T. trigonocephalus	2n=44 (56) 2n=44 (64)	2M+4SM+16A 10M (SM)+12T	Lee *et al*., 1984 Lee, 1986
Synechogobius hasta	2n=44 (46)	1M (SM)+21T	Lee, 1986
Gymnogobius heptacanthus	44 (44)	22T	Lee, 1986
Chaenogobius annularis	44 (44)	22T	Lee, 1986
Rhinogobius brunneus	2n=44 (44)	22ST	Lee *et al*., 1983
Chaenogobius urotaenia	2n=44	–	Lee *et al*., 1983
Periophthalmus modestus	2n=46 (62)	16M (SM)+30T	Lee, 1986
Family Odontobutidae			
O. platycephala	2n=44 (44)	22T	Lee *et al*., 1983, Lee, 1986, Present study
O. obscura	2n=44 (44)	22T	Present study
O. interrupta	2n=44 (44)	22T	Present study
Family Belontidae			
Macropodus ocellatus	2n=48 (54)	6~7M+6~7S+1ST	Lee *et al*., 1983

gensis is placed in the pair 10 and 21. The results obtained showed a marked chromosomal conservation with the presence of 2n=44 chromosomes in genus *Odontobutis* and 2n=48 chromosomes in genus *Cottus*. The chromosome numbers of *O. platycephala* have been reported by Lee *et al*. (1983) and Lee (1986). In comparison with this report, the chromosome numbers and karyotype had coincided. The karyotype of three species of genus *Odontobutis* was closely similar and differs only in chromosome size.

Detailed studies of chromosome morphology and population cytology of the present fishes are very little can be said on systematics based on the karyotypes other than chromosome numbers. In fact modern cytogenetic techniques have only recently been adopted for studies of fishes. In the Korean Order Perciformes, nine species have been karyologically investigated (Table 6). The diploid chromosome numbers of Korean Perciformes were found to be 2n=44 and 2n=48. Karyotypes of these nine species were found to be different.

L. japonicus of Moronidae had 24 acrocentric chromosomes, *S. scherzeri* of Centropomidae had 2 submetacentric and 22 acrocentric chromosomes, *T. abscurus* and *T. trigonocephalus* of Gobiidae had 2 metacentric, 4 submetacentricand 16 acrocentric chromosomes, and *R. brunneus* had 22 subtelocentric chromosomes, *O. obscura*, *O. platycephala* and *O. interrupta* of Odontobutidae had all 22 telocentric chromosomes. Thus, the species of Perciformes had high number telocentric chromosomes or acrocentric chromosomes. However, to the best of our knowledge, no other species of Korean Scorpaeniformes has yet been

karyotyped except for a present study. In the present study, *C. koreanus* and *C. hangiongensis* of Cottidae had 2 subtelocentric and 22 telocentric chromosomes. The karyotypes of Korean cyprinid fishes are generally constructed by more bi-armed chromosomes than mono-armed ones (Lee *et al*., 1983, 1984; Song and Park, 2005), while in almost species of Scorpaeniformes and Perciformes fish the chromosome complement consists of mono-armed chromosomes than bi-armed one.

Also, we did not found sexual dimorphism of the chromosomes in this study. Further study will be need C- and Ag-banding analyses for the investigation of the karyosystematically evolution in Korean Scorpaeniformes and Perciformes.

Acknowledgments

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한국산 동사리과 (Odontobutidae)와 둑중개과 (Cottidae) 5종의 핵형

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한국산 동사리과 (Odontobutidae)의 동사리 (*Odontobutis platycephala*), 얼록동사리 (*O. interrupta*), 남방동사리 (*O. obscura*) 그리고 둑중개과 (Cottidae)의 둑중개 (*Cottus koreanus*), 한둑중개 (*C. hangiongensis*) 등 5종의 핵형 분석을 실시하였다. 동사리, 얼록동사리, 남방동사리의 염색체 수 및 핵형은 2n=44 (22T), NF=44였으며, 둑중개와 한둑중개의 염색체 수와 핵형은 2n=48 (2ST+22T), NF=52로 밝혀졌다.