

## Cytogenetic Study of *Pleuronectes obscurus*, *Konosirus punctatus* and *Pseudoblennius percoides*

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Our objective was to clarify the cytogenetic characteristics, including karyotypes, cellular DNA content, and nuclear size of erythrocytes, of black plaice *Pleuronectes obscurus*, dotted gizzard shad *Konosirus punctatus*, and perch sculpin *Pseudoblennius percoides*, collected from the coastal areas of Jo Island, Busan, Korea. Karyotypes of *P. obscurus* and *K. punctatus* both had a diploid number of 48 and a fundamental number (FN) of 48, with a chromosome formula of 48T. The karyotype of *P. percoides* had a diploid number of 46 and FN of 56, with a chromosome formula of 10SM + 36T. No sex-associated heteromorphic pairs were detected for any species. The variation in DNA values (*P. obscurus*=1.15 pg/nucleus, *K. punctatus*=1.56 pg/nucleus, *P. percoides*=1.11 pg/nucleus) was positively related to variation in chromosome FN.

Key words: *Pleuronectes obscurus*, *Konosirus punctatus*, *Pseudoblennius percoides*, DNA content, Karyotype

### Introduction

Fish cytotaxonomy refers to the study of phenetic and/or phylogenetic relationships among species based on comparisons of chromosome number and morphology, genome size, or the amount of DNA per nucleus (Gold, 1979; Kim and Park, 1990; Park, 1992; Park et al., 1999). In particular, there is a highly significant positive correlation between chromosome number and genome size in diploid teleost fish (Gold and Amemiya, 1987; Hinegardner and Rosen, 1972; Perderson, 1971).

Cytogenetic studies of fish from coastal areas around Jo Island are limited to *Parapercis sexfasciata* (Temminck et Schlegel), *Sebastiscus marmoratus* (Cuvier), and *Pleuronectes yokohamae* (Günther) (Park and Lee, 2005).

The black plaice *Pleuronectes obscurus* (Herzenstein) (Pleuronectiformes: Pleuronectidae) is widely distributed throughout the South, East, and West Seas and around Japan in the East China Sea (Choi et al., 2002). This fish generally inhabits coastal areas, but has been introduced into estuaries. Its average total length is about 40 cm and it spawns in spring; its

eggs are of the demersal-adhesive type (Choi et al., 2002).

The gizzard shad *Konosirus punctatus* (Temminck et Schlegel) (Clupeiformes: Clupeidae) is widely distributed in the East China Sea, Middle Sea around Japan, and the South Sea of Korea. Its average total length is about 25 cm and spawning mainly occurs in river estuaries from March to June; its eggs are of the separation-floating type (Choi et al., 2002).

The perch sculpin *Pseudoblennius percoides* (Günther) (Scorpaeniformes: Cottidae) is distributed across the South Sea, including around Jeju Island. This species inhabits rocky coastal regions and its average total length is about 20 cm (Choi et al., 2002).

We sought to clarify the cytogenetic aspects of *P. obscurus*, *K. punctatus*, and *P. percoides* collected off the coast of Jo Island, Busan, Korea. Details of karyological features and flow cytometry are described.

### Materials and Methods

#### Sampling and species identification

*Pleuronectes obscurus*, *K. punctatus*, and *P. percoides* were collected with traps and nets near-shore around Korea Maritime University (KMU) on

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Jo Island, Busan, Korea, from September 2005 to January 2006. The fish were transported to the Fishery Genetics and Breeding Laboratory, KMU, where they remained alive until they were analyzed.

The classification of the three species were based on Choi et al. (2002). After anesthesia with 200 ppm lidocaine-HCl/1000 ppm NaHCO<sub>3</sub> at 22°C, body length of each specimen was measured to the nearest 0.1 cm digital by a Vernier calipers (CD-20CP, Japan).

### Chromosome analysis

Ten specimens of each species (five females and five males) were subjected to chromosome number and karyotype analyses. The sex was determined by gonadal inspection. Fish were intraperitoneally injected with 0.02% colchicine (1 mL per 100 g body weight), left in a well-aerated aquarium for 3 h, and then killed with an overdose of 200 ppm lidocaine-HCl/1000 ppm NaHCO<sub>3</sub>. The kidneys were removed and minced in hypotonic 0.075 M KCl solution until a good cell suspension was obtained, and then allowed to hypotonize for 20 min at 37°C (Almeida-Toledo et al., 1995). The kidney cells were fixed with methanol/ acetic acid (3:1, v/v) and shaken gently. The suspension was replaced three times on ice for 15 min and then centrifuged.

Chromosome slides were made by the conventional air-drying technique. Detailed procedures for the preparation are provided by Im et al. (2001) and Park et al. (2003). The final suspension was dropped on clean dry slides and placed on a 60°C slide warmer. Chromosome preparations were stained with 10% Giemsa (Gurr's R66) for conventional analysis and at least 20 countable metaphase spreads were obtained per fish.

Well-spread chromosomes at metaphase were selected and photographed. Chromosome morphology was determined on the basis of arm ratios, as proposed by Levan et al. (1964). Chromosomes were grouped into three categories, i.e., metacentric, submetacentric and telocentric, and arranged in decreasing order of size.

### Flow cytometry

Flow cytometric analysis was performed to

estimate the average cellular DNA content of 10 individuals from each species, as described by Park and Lee (2005). After anesthetizing the fish with 200 ppm lidocaine-HCl/1000 ppm NaHCO<sub>3</sub>, a 0.5-1.0 mL sample of whole blood was collected from the caudal vein of each of 10 individuals. Blood cells were fixed in 10 mL of cold 70% ethanol and filtered through a 30 µm filter. The cell solution was stored at 4°C.

One million cells were collected and stained using a high-resolution DNA staining kit (Partec GmbH, Münster, Germany) under dark conditions at room temperature for 15 min. Stained samples were analyzed on a Partec PA-II flow cytometer (Partec GmbH) to determine the relative DNA content. The red blood cells (2.8 pg DNA/nucleus) of Chinese muddy loach *Misgurnus mizolepis* (Günther) were used as a standard reference (Park et al., 1999).

To improve the standard DNA content of *M. mizolepis*, we attempted to culture human white blood cells (WBCs). We obtained 10 mL of whole blood from a human male. The isolation of WBCs was achieved from 10 mL of whole blood diluted with 5 mL of serum-free culture medium in a clear culture tube using the stirring method. After centrifugation, the buffy coat on the surface of the erythrocytes was floated in plasma by gentle stirring with a pipette along the inside wall of the tube, and the lymphocyte-rich plasma was then collected in a culture tube (Abe et al., 2001). Plasma was harvested after incubation for 3 to 4 days at 37°C. Cells from human leukocytes were fixed with 70% ethanol. The fixed cells were treated using a CyStain DNA two-step kit (Partec GmbH).

Whole blood was sampled from *M. mizolepis* and then fixed with 70% ethanol. The fixed cells were treated using a CyStain DNA two-step kit (Partec GmbH). The DNA content of *M. mizolepis* was confirmed as for WBCs using standards. The DNA content of control human WBCs was 7.0 pg/nucleus, whereas that of *M. mizolepis* was 2.8 pg/nucleus.

## Results

The mean total body length of *P. obscurus*, *K. punctatus*, and *P. percoides* was 25.7±1.29, 22.5±

Table 1. Karyotypes and frequency distribution (%) of diploid chromosome numbers of *Pleuronectes obscurus*, *Konosirus punctatus* and *Pseudoblennius percoides*

Species	Frequency distribution (%)							Fundamental number	Total cell count
	42	44	46	48	50	52	54		
<i>Pleuronectes obscurus</i>	1	3	5	83	6	3	2	48	400
<i>Konosirus punctatus</i>	0	1	3	86	6	2	0	48	360
<i>Pseudoblennius percoides</i>	1	4	88	3	3	2	1	56	380

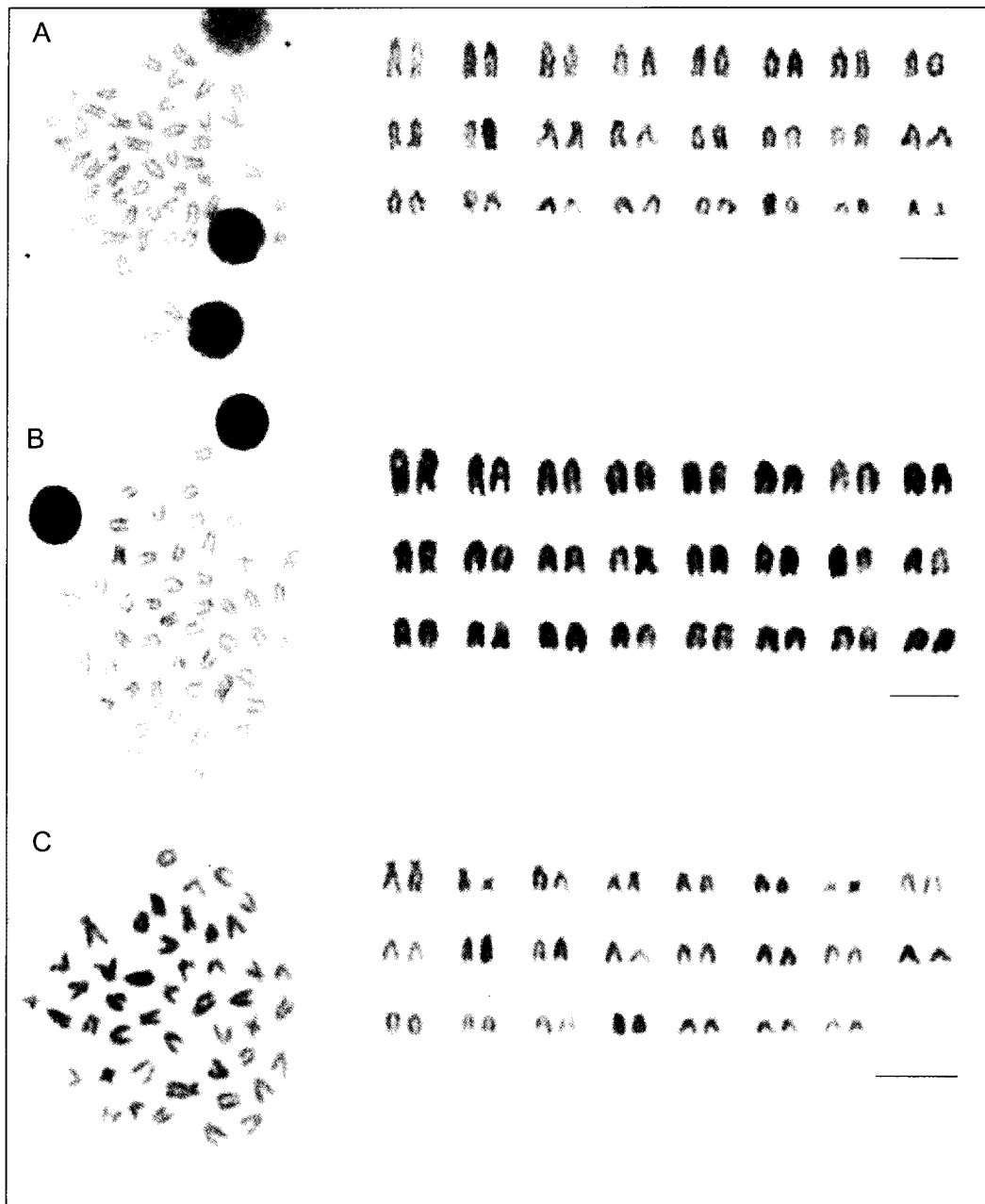


Fig. 1. Methaphase and idiograms of (a) *Pleuronectes obscurus*, (b) *Konosirus punctatus*, (c) *Pseudoblennius percoides*. Scale bars are 10  $\mu$ m.

1.69, and  $16.2 \pm 1.23$  cm, respectively.

The modal chromosome number of *P. obscurus* was  $2N=48$  with a chromosome distribution frequency mode of 83% (Table 1), consisting of 24 pairs of telocentrics. The fundamental number (FN) of *P. obscurus* was 48 (Table 1, Fig. 1A). The modal chromosome number of *K. punctatus* was  $2N=48$  with a chromosome distribution frequency mode of 86% (Table 1), consisting of 24 pairs of telocentrics. The FN of *K. punctatus* was 48 (Table 1, Fig. 1B).

The modal chromosome number of *P. percoides* was  $2N=46$  with a chromosome distribution frequency mode of 88% (Table 1), consisting of five pairs of submetacentrics and 18 pairs of telocentrics. The FN of *P. percoides* was 56 (Table 1, Fig. 1C). There was no evidence of polymorphism, including aneuploidy or sex-related heteromorphic chromosomes, in any species examined (Fig. 1).

The mean DNA content of each species was examined using mud loach as a standard reference

Table 2. Nuclear DNA content of *Pleuronectes obscurus*, *Konosirus punctatus* and *Pseudoblennius percoides*

Species	DNA content (pg/nucleus)*
<i>Pleuronectes obscurus</i>	1.15 ± 0.103
<i>Konosirus punctatus</i>	1.56 ± 0.132
<i>Pseudoblennius percoides</i>	1.11 ± 0.160
Standard <i>Misgurnus mizolepis</i> **	2.80

\*Means ± SD (n=10).

\*\**Misgurnus mizolepis* (from Park et al., 1999).

(Table 2). The mean DNA content of *P. obscurus* was 1.15±0.103 pg/nucleus. The mean DNA contents of *K. punctatus* and *P. percoides* were 1.56±0.132 and 1.11±0.160 pg/nucleus, respectively.

### Discussion

Chromosome number and variability in chromosome number distinguish certain major taxonomic groups of fish (Gold, 1979; Park and Lee, 1996; Park et al., 1995; 1999; 2000). Chromosome number and karyotype have unique numerical forms in each species and can provide useful data with which to identify species (Gold, 1979). To our knowledge, this is the first report of the karyotypes of *P. obscurus*, *K. punctatus*, and *P. percoides*.

The modal chromosome number of *P. obscurus* was 2N=48 (83%), consisting of 24 pairs of telocentrics (FN=48), that of *K. punctatus* was 2N=48 (86%), consisting of 24 pairs of telocentrics (FN=48), and that of *P. percoides* was 2N=46 (88%), consisting of five pairs of submetacentrics and 18 pairs of telocentrics (FN=56). There was no evidence of polymorphism, including aneuploidy or sex-related heteromorphic chromosomes, in any species examined.

Both *Pseudoblennius percoides* and *Pseudoblennius cottoides* (Richardson) belong to the order Scorpaeni-formes in the family Cottidae and are similar in terms of life history and habitat use. The karyotype of *P. percoides* was 2N=46, 10SM+36T, FN=56, and that of *P. cottoides* is 2N=46, 4M+8SM+34ST (subtelocentric), FN=58 (Arai and Fujiki, 1978). The FN of chromosomes is very important when comparing species within a genus and indicates the progenitor type from which the acrocentric chromosome number increases (Kim et al., 2004; Ohno, 1974). The FN increases with further differentiation of species (Arai, 1983). In the comparison of *P. percoides* and *P. cottoides*, the chromosome configuration of *P. cottoides* is more variable than that of

*P. percoides*, and the FN value of *P. cottoides* is higher than that of *P. percoides*. Thus, *P. cottoides* differentiated from *P. percoides*. However, our knowledge of cytogenetic traits remains insufficient, especially with regard to comparative karyotypes (Park et al., 1999). Accordingly, further detailed molecular studies are needed.

The DNA nuclear content, which is species specific, may be correlated with the morphological basis for species definitions, i.e., it may be related to the karyotype and regarded as its total dimension or as correlated to a total dimension such as the area or total length of chromosomes (Chiarelli and Capanna, 1973). The direct measurement of genome size by flow cytometry analyzes the unique genetic material of each specimen using a large amount of nuclear material within a short time, which is advantageous (Lovett et al., 1980; Thorgaard et al., 1982; Park, 2004; Park et al., 1999; Wolters et al., 1982). Flow cytometry analyses resulted in 1.15 pg/nucleus in *P. obscurus*, 1.56 pg/nucleus in *K. punctatus*, and 1.11 pg/nucleus in *P. percoides*. No intraspecific differences in DNA content were found between the sexes.

The use of flow cytometry to analyze DNA content is considered the most effective method in terms of not perturbing the study animal and successfully identifying individuals (Kim et al., 2004). Moreover, it is a convenient way to analyze species cytogenetically (Kim et al., 2004). Evidence indicates that the DNA nuclear content is statistically constant in all cells exhibiting identical ploidy in various tissues from the same species, whereas it is significantly divergent among different species (Chiarelli and Capanna, 1973; Park and Lee, 2005). In addition, the DNA content analysis can be used in phylogenetic studies (Vendrey and Vendrey, 1948). Based on the amount of nuclear DNA and the karyotype of a given individual, and this correlation ensures the number and shape of the chromosome characteristics of the species (Lovett et al., 1980; Park et al., 1999; 2000; 2003).

Large numbers of *P. obscurus* are captured in Korea in the winter and *Konosirus punctatus* is frequently captured in the fall in coastal areas around Jo Island (Park, 2005), and their commercial values are high. No studies have attempted cytogenetic research on *K. punctatus*. Therefore, our cytogenetic study of *K. punctatus* may provide useful basic data for the aquaculture industry. A previous cytogenetic study of fish in Jo Island area targeted the saddled weever *Parapercis sexfasciata*, the marbled rockfish

*Sebastiscus marmoratus*, and the marbled sole *Pleuronectes yokohamae* (Park and Lee, 2005). This previous report and our current investigation can be used as a basis for continued cytogenetic studies of coastal fish around Jo Island for species classification or maintenance, as well as to provide basic data for the commercial production of new species.

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