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Karyotype of Dwarf Loach, *Kichulchoia brevifasciata* (Pisces: Cobitidae) from Korea

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ABSTRACT The karyotype analysis of *Kichulchoia brevifasciata* was carried out, known as an endangered small endemic cobitid fish of Korea. Five females and one male were collected from Geumsan-myeon, Goheung-gun, Jeollanam-do, Korea and its gill, kidney and digestive tract tissues were treated by flame-drying technique. The karyotype was found to be 2n=48 from 33 cells; 16 meta-submetacentric and 32 subtelo-telocentric chromosomes; 64 FN (fundamental number). This karyotype showed a remarkably different karyotype formula from *Niwaella multifasciata* which is closely related to this species.

Key words : Chromosome, *Kichulchoia brevifasciata*, karyotype, fundamental number, Korea

INTRODUCTION

The Korean endemic small cobitid fish, dwarf loach, *Kichulchoia brevifasciata* is restricted within Geogeum-Island, the extreme southwestern coast of Korea. It is actually considered to be an endangered species now. This species was firstly described as *Niwaella brevifasciata* (Kim and Lee, 1995) on the basis of the absence of a lamina circularis on the pectoral fin in males. After that, it was redescribed as *Choia* by Kim *et al*. (1997), because it has only 4 branched rays in the anal fin fewer than genus *Niwaella*. However, because *Choia* was a recognized homonym, it was transferred into the genus *Kichulchoia* (Kim *et al*., 1999).

This species lives in small streams with pebble bottoms and low water currents 5～65 cm in depth. Major food items are aquatic insects and the spawning period was May to July (Kim and Kim, 2007). There were studies on the karyotype of some cobitid fishes in Korea and Japan, while karyotype of *K. breivifasciata* have not been investigated yet (Kimizuka and Mizuno, 1982; Kim and Lee, 1986; Lee *et al*., 1986; Nam *et al*., 1991; Kim *et al*., 1999, 2003). Therefore, this study examined the chromosome number, its composition and fundamental number of this species to acquire basic data for phylogenetic information.

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MATERIALS AND METHODS

Six specimens (five females and one male) of *Kichulchoia brevifasciata* were collected in Geumsan-myeon, Goheung-gun, Jeollanam-do, Korea from May to November 2006, ranging 40.4 to 67.8 mm in the total length. Using the flame-drying method, chromosome preparations were taken from the gill, kidney and stomach tissues of the specimens (Ojima *et al*., 1972).

Colchicine was injected into the abdominal cavity of each live specimen. After 2～3 hours, the gill, kidney and digestive tissues were dissected and sheared in 0.075 M KCl. Minced cells were gathered by centrifugation (1,200 rpm, 10 min), and supernatants were removed to collect cell sediments fixed in a fresh methanol-acetic acid solution (Carnoy's solution, 3 : 1). The supernatants were discarded and filled by fresh Carnoy's solution three times. The cells were settled on the slide by pipetting using the flying dry method (Ojima *et al*., 1972) and dried at room temperature. The fixed cells on the slide were stained with 4% Giemsa solution for 40 minutes.

The treated slides were observed under a microscope and karyotypes examined to investigate chromosome numbers. The karyotypes were analyzed following Levan *et al*. (1964). Classification for a fundamental number of chromosomes was observed using the relative lengths of chromosomes on the metaphase, such as meta-submetacentric and subtelo-telocentric.

Table 1. Chromosome number of *Kichulchoia brevifasciata* from Geumsan-myeon, Goheung-gun, Jeollanam-do, Korea

Species	No. of individuals	No. of cells investigated	No. of chromosomes										
			40	41	42	43	44	45	46	47	48	49	50
Kichulchoia brevifasciata	5	98	3	2	4	1	2	8	12	12	33	13	8

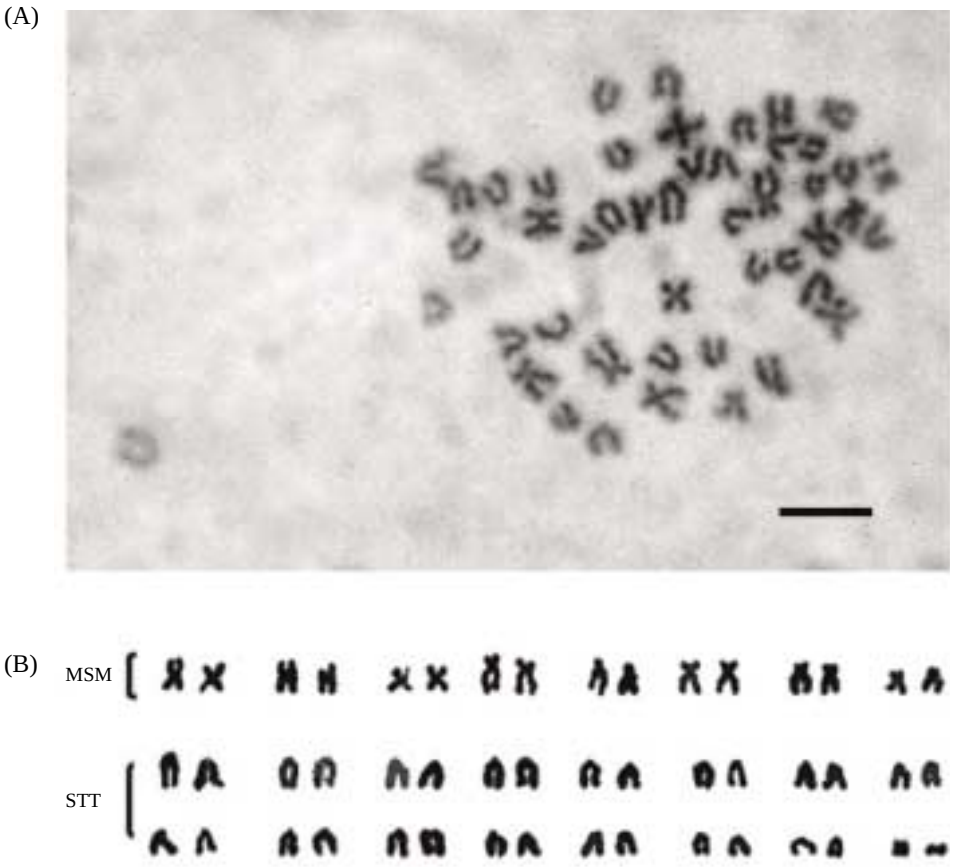

Fig. 1. A figure of metaphase chromosome in the slide (A) and its karyotype (B) of *Kichulchoia brevifasciata* from Geumsan-myeon, Goheung-gun, Jeollanam-do, Korea. MSM: meta-submetacentric, STT: subtelo-telocentric. Scale bar indicates 5 μm.

Table 2. A comparison in the chromosome constitution of cobitid fishes observed from Korea and Japan

Species	2N	Karyotypic formular*	FN**	Localities	References
Kichulchoia brevifasciata	48	16msm-32stt	64	Goheung-gun	Present study
Niwaella delicata	50	6m-14ms, st-30a	68	Kado river	Kimizuka *et al.* (1982)
N. multifasciata	50	38msm-12stt	88	Milyang river, Nam river	Nam *et al.* (1991)
Iksookimia koreensis	50	22msm-28stt	72	Gosan-myeon, Wanju-gun	Kim and Lee (1986)
I. longicorpa	50	20msm-30stt	70	Maryeong-myeon, Namwon-si	Kim and Lee (1986)
I. pumila	50	22msm-28stt	72	Buan-gun	Lee (1988)
I. choii	50	14msm-36stt	68	Chongwon, Miho stream	Lee (1986)
I. hugowolfeldi	50	24msm-26stt	74	Yeongsang river	Kim *et al.* (2003)
I. yongdokensis	100	44msm-56stt	144	Yeongdokoship stream	Kim *et al.* (1999)
Cobitis takatsuensis	48	12m-18ms, st-18a	78	Takatsu river	Kimizuka *et al.* (1982)
C. hankugensis	48	18msm-30stt	66	Hamyang-gun, Inwol-myeon, Namwon-si	Kim and Lee (1986)
C. lutheri	50	16msm-30stt	66	Youngjin-myeon, Wanju-gun	Kim and Lee (1986)
C. pacifica	50	24msm-26stt	74	Myongju-gun	Lee (1988)
C. tetralineata	50	16msm-34stt	66	Maryeong-myeon, Jinan-gun	Kim and Lee (1986)

* msm: meta-submetacentric, stt: subtelo-telocentric, ac: acrocentric
** FN: fundamental number

RESULTS AND DISCUSSION

Chromosome analysis of *Kichulchoia brevifasciata* indicated 2n=48 in numbers from 33 cells (Table 1) during the metaphase (Fig. 1). Based on arm ratio analysis of the chromosomes, they consist of 16 meta-submetacentric and 32 subtelo-telocentric chromosomes. The fundamental number of this species is 64. Polyploidy phenomenon or sex dimorphism between female and male chromosomes was not observed in the present study.

Karyotype serve as significant information for understanding the phylogenic relationship and species classification between similar groups (Miller and Walter, 1972). Even if the number of chromosomes in cobitid fishes is diverse, the diploid chromosome is 2n=50, and there are more monoarm chromosomes than biarm chromosomes (Ojima and Hitotsumach, 1969). Karyotypic studies on the cobitid fishes of Korea and Japan have been reported as follows: *Iksookimia koreensis, I. longicorpa* (Kim and Lee, 1986), *I. pumila* (Lee, 1988), *I. choii* (Lee *et al*., 1986), *I. hugowolfeldi* (Kim *et al*., 2003) with altogether 50 in 2n chromosome. Among them, closely related with genus *Kichulchoia, Niwaella delicata* was 2n=50 with FN=68 (Kimizuka *et al*., 1982) in Japan and *N. multifasciata* was 2n=50 with FN=88 (Nam *et al*., 1991) in Korea (Table 2).

In this study, *Kichulchoia brevifasciata* indicated 2n =48, which is identical to *Cobitis hankugensis* (Lee *et al*., 1986) and *C. takatsuensis* in Japan (Kimizuka *et al*., 1982).

Although most cobitid fishes have 50 chromosomes, the karyotype formula and the fundamental number are different each other and are important for establishment of phylogeny position. Ohno (1974) reported that species that have more acrocentric chromosomes are regarded as archetypes. Also the more recently specified species have many more arm numbers than the archetypes (Arai, 1983).

The genus *Niwaella* is closely related to the genus *Kichulchoia* on morphology characteristics (Kim, 1997) and molecular study (Kim *et al*., 2000) on cytochrome *b* sequence. However, *Niwaella multifasciata* shows a remarkably different karyotype formula in regarding to fewer acrocentric chromosomes and a more fundamental number.

For getting of information regarding especially phylogenic relationship and analysing of karyotype, various methods such as C-banding analyses, microsatellite multi -locus and DNA fingerprinting are required in the future.

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미꾸리과 소형어류 좀수수치, *Kichulchoia brevifasciata*의 핵형

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요 약 : 멸종위기에 처한 한국 고유종인 미꾸리과(Cobitidae) 소형어류 좀수수치, *Kichulchoia brevifasciata*의 핵형을 분석하였다. 전라남도 고흥군 금산면에서 6개체를 채집하여 핵형분석 결과 염색체 수는 2n=48으로서 16 meta-submetacentric, 32 subtelo-telocentric chromosomes로 구성되어 있었으며 FN=64이었고 유연관계에 있는 수수미꾸리, *Niwaella multifasciata*와 핵형과 구성에 있어 큰 차이를 나타냈다.

찾아보기 낱말 : 좀수수치, 한국고유종, 핵형