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 metasubmetacentric 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. brevifasciata* 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

<table>
<thead>
<tr>
<th>Species</th>
<th>No. of individuals</th>
<th>No. of cells investigated</th>
<th>No. of chromosomes</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Kichulchoia brevifasciata</em></td>
<td>5</td>
<td>98</td>
<td>3</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Species</th>
<th>2N</th>
<th>Karyotypic formula*</th>
<th>FN**</th>
<th>Localities</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Kichulchoia brevifasciata</em></td>
<td>48</td>
<td>16msm-32stt</td>
<td>64</td>
<td>Goheung-gun</td>
<td>Present study</td>
</tr>
<tr>
<td><em>Niwaella delicata</em></td>
<td>50</td>
<td>6m-14ms, st-30a</td>
<td>68</td>
<td>Kado river</td>
<td>Kimizuka <em>et al.</em> (1982)</td>
</tr>
<tr>
<td><em>N. multifasciata</em></td>
<td>50</td>
<td>38msm-12stt</td>
<td>88</td>
<td>Milyang river, Nam river</td>
<td>Nam <em>et al.</em> (1991)</td>
</tr>
<tr>
<td><em>I. longicorpa</em></td>
<td>50</td>
<td>20msm-30stt</td>
<td>70</td>
<td>Maryeong-myeon, Namwon-si</td>
<td>Kim and Lee (1986)</td>
</tr>
<tr>
<td><em>I. pamila</em></td>
<td>50</td>
<td>22msm-28stt</td>
<td>72</td>
<td>Buan-gun</td>
<td>Lee (1988)</td>
</tr>
<tr>
<td><em>I. choi</em></td>
<td>50</td>
<td>14msm-36stt</td>
<td>68</td>
<td>Chongwon, Miho stream</td>
<td>Lee (1986)</td>
</tr>
<tr>
<td><em>I. hugowolfeldi</em></td>
<td>50</td>
<td>24msm-26stt</td>
<td>74</td>
<td>Yeongsang river</td>
<td>Kim <em>et al.</em> (2003)</td>
</tr>
<tr>
<td><em>I. yongdokensis</em></td>
<td>100</td>
<td>44msm-56stt</td>
<td>144</td>
<td>Yeongdokship stream</td>
<td>Kim <em>et al.</em> (1999)</td>
</tr>
<tr>
<td><em>Cobitis takatsensis</em></td>
<td>48</td>
<td>12m-18ms, st-18a</td>
<td>78</td>
<td>Takatsu river</td>
<td>Kimizuka <em>et al.</em> (1982)</td>
</tr>
<tr>
<td><em>C. lutheri</em></td>
<td>50</td>
<td>16msm-30stt</td>
<td>66</td>
<td>Youngjin-myeon, Wanju-gun</td>
<td>Kim and Lee (1986)</td>
</tr>
<tr>
<td><em>C. pacifica</em></td>
<td>50</td>
<td>24msm-26stt</td>
<td>74</td>
<td>Myongju-gun</td>
<td>Lee (1988)</td>
</tr>
<tr>
<td><em>C. tetrilineata</em></td>
<td>50</td>
<td>16msm-34stt</td>
<td>66</td>
<td>Maryeong-myeon, Jinan-gun</td>
<td>Kim and Lee (1986)</td>
</tr>
</tbody>
</table>

* 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 phylogenetic 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 phylogenetic 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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