

Karyotype Analysis of an Endemic Korean Torrent Catfish *Liobagrus hyeongsanensis* (Siluriformes: Amblycipitidae)

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ABSTRACT For the karyotype analysis of *Liobagrus hyeongsanensis*, an endemic Korean torrent catfish, ten females and five males were used from Yangbuk-myeon, Gyeongju-si, Gyeongsangbuk-do, Korea. The diploid number of chromosomes was 42, and its karyotype consisted of 30 metacentrics and 12 submetacentrics; 84 FN (fundamental number), with having no polyploidy and sexual dimorphism. The chromosome number was the same as other closely-related species, *L. mediadiposalis* and *L. somjinensis*, but their karyotypes showed a clear difference by species. Such result may be related to chromosomal rearrangements by Robertsonian rearrangement with geographical isolation.

Key words: *Liobagrus hyeongsanensis*, chromosome, karyotype, Korea

INTRODUCTION

The genus *Liobagrus* belonging to the family Amblycipitidae includes currently 13 species in the world (Nelson *et al.*, 2016), of which five endemic species are reported in the Korean Peninsula: *L. andersoni* (Regan, 1908), *L. obesus* (Son *et al.*, 1987), *L. mediadiposalis* (Mori, 1936), *L. somjinensis* (Park and Kim, 2010) and *L. hyeongsanensis* (Kim *et al.*, 2015). Except for two species, *L. andersoni* and *L. obesus* that have a distinct geological isolation with limited water systems, *L. mediadiposalis* has a wide distribution throughout streams, which led to morphological problems. Recently, with re-examination of all populations for *L. mediadiposalis*, two new species, *L. somjinensis* and *L. hyeongsanensis*, were reported (Park and Kim, 2010; Kim *et al.*, 2015). The former has been known only from western and southern rivers or coasts such as Geogum Island, Somjingang, Yeongsangang, Dongingang, and Tamjingang Rivers, whereas the latter from Hyeongsangang and Taehwagang

Rivers, and Daejongcheon Stream (Kim, 2013).

The karyotype analysis in teleost plays an important role in taxonomic and genetic studies (Gold *et al.*, 1990), showing its own unique genetic trait that has a specific pattern of chromosomal rearrangements within different evolutionary lines (Chiarelli and Capanna, 1973). The order Siluriformes of *Liobagrus*, has a variety of chromosome number, $2n = 28 \sim 132$, but most species are within $2n = 56 \pm 2$ (LeGrande, 1981). Also, the XX/XY heteromorphy (Chen *et al.*, 2008) and cytogenetic analysis (Wang *et al.*, 2011) have been studied in *L. marginatus* and *L. marginatoides*. Meanwhile, for Korean *Liobagrus*, the diploid chromosome number of *L. andersoni* and *L. obesus* are 28, 20, respectively (Son and Lee, 1989). *L. mediadiposalis* and *L. somjinensis* have chromosome of the same number, $2n = 42$ but the both showed a different karyotype (Kim, 2013; Cho and Park, 2016). Kim (2013) described *L. hyeongsanensis*, formerly *Liobagrus* sp. 1, may have sex chromosomes due to an unmatched pair in its males. Therefore, this study will be focused on the chromosome number and karyotype of *L. hyeongsanensis* as part of acquisition of basic data for phylogenetic and evolutionary information of the genus Korean *Liobagrus*.

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Table 1. The chromosome number of *Liobagrus hyeongsanensis* from Yangbuk-myeon, Gyeongju-si, Gyeongsangbuk-do, Korea

No. of samples	Sex	No. of metaphase counted	Frequency of chromosome number				
			40	41	42	43	44
10	Female	43	6	8	23	5	1
5	Male	48	5	4	32	5	2
Total		91	11	12	55	10	3

MATERIALS AND METHODS

15 specimens (10 females, 5 males) of *Liobagrus hyeongsanensis* were collected in Daejongcheon of Yangbuk-myeon, Gyeongju-si, Gyeongsangbuk-do, Republic of Korea (35°49'58" N, 129°24'41" E), where the holotype specimen is distributed, from May to June 2016, ranging 51 to 90 mm in standard length. The specimens are kept alive and transported to the laboratory at Department of Biological Sciences, Chonbuk National University. For chromosomal and karyotype analysis, 1% concanavalin A solution was injected into the abdominal cavity at a dose of 0.1 mL per 1 g body weight for individuals anesthetized with MS222 (Woogenebng, Korea) after identifying the male and female. After 24 hours, a colchicine solution of 0.1 mL of 0.05% was injected to intraabdominal cavity. After 2~3 hours, each specimen's kidney was dissected out and minced with slide glass, and then the minced cells were transferred to a hypotonic solution (0.075 M KCl) of an incubator at 28°C for 30 minutes. The minced cells were gathered by centrifugation at 1,100 rpm, for 10 mins and fixed in a fresh chilled Carnoy's solution (Methanol : Acetic acid in 3 : 1 ratio). The cells obtained from the above procedure were settled on the slide according to the flame drying method (Ojima *et al.*, 1972). The slides were stained with 4% Giemsa solution for 8 minutes, and washed by distilled water, and dried at room temperature. The treated slides were observed on metaphase spreads with microscope (Carl Zeiss, AX10, Germany). Karyotype analysis was based on general methods suggested by Levan *et al.* (1964) and Arai and Kobayasi (1973).

RESULTS AND DISCUSSION

The chromosome number for total 15 specimens experimented (10 females and 5 males) is 40 to 44 from 43~48 counted metaphase spreads, of which number 42 is the highest frequency (Table 1). The chromosome number comes to $2n=42$. Cells different from $2n=42$

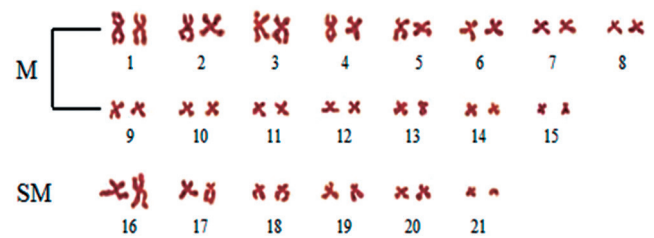
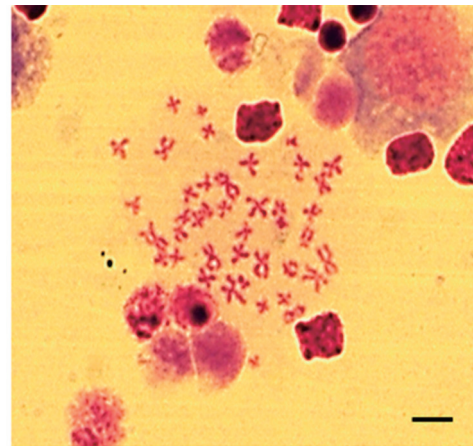


Fig. 1. Metaphase plate (above) and karyotype (below) of *Liobagrus hyeongsanensis* from Yangbuk-myeon, Gyeongju-si, Gyeongsangbuk-do, Korea. M, metacentric; SM, submetacentric. Bar = 5 μ m.

may be probably caused by losses during preparation or addition from nearby cells. Such count measure method is easily seen in other fishes (Kim *et al.*, 1999). Based on the arm ratio analysis of the chromosomes, the karyotype formula consists of 15 pairs of metacentric chromosomes and 6 pairs of submetacentric chromosomes (Fig. 1) with the number of fundamental chromosome arms, $FN=84$. Total arm length and arm ratio of the chromosome are 1.37~3.91 and 1.28~2.23, respectively (Table 2). Sex chromosomes were not observed, being suggested the existence of heteromorphic chromosomes (Kim, 2013). In fish, the genetic determinants of sex are situated on autosomal or sex chromosome (Devlin and Nagahama, 2002) and sex chromosomes have been known only in approximately 10% of about 1,700 species (Arkhipchuk,

Table 2. The average length and classification of the chromosome of *Liobagrus hyeongsanensis* from Yangbuk-myeon, Gyeongju-si, Gyeong-sangbuk-do, Korea

No. of chromosome pair	Average length (μm)			Arm ratio	Type*
	Total	Short arm	Long arm		
1	3.91 \pm 0.31	1.63	2.28	1.41 \pm 0.15	M
2	3.46 \pm 0.29	1.53	2.00	1.28 \pm 0.17	M
3	3.22 \pm 0.35	1.42	1.79	1.30 \pm 0.20	M
4	2.85 \pm 0.39	1.26	1.59	1.28 \pm 0.23	M
5	2.61 \pm 0.32	1.08	1.54	1.43 \pm 0.19	M
6	2.44 \pm 0.32	1.01	1.43	1.43 \pm 0.14	M
7	2.27 \pm 0.31	0.93	1.33	1.44 \pm 0.17	M
8	2.13 \pm 0.30	0.88	1.25	1.42 \pm 0.21	M
9	2.05 \pm 0.28	0.89	1.16	1.32 \pm 0.17	M
10	1.98 \pm 0.24	0.84	1.14	1.35 \pm 0.19	M
11	1.89 \pm 0.26	0.84	1.05	1.28 \pm 0.17	M
12	1.81 \pm 0.23	0.77	1.04	1.37 \pm 0.22	M
13	1.73 \pm 0.23	0.73	1.00	1.38 \pm 0.13	M
14	1.61 \pm 0.25	0.70	0.91	1.30 \pm 0.19	M
15	1.37 \pm 0.24	0.59	0.78	1.33 \pm 0.17	M
16	3.45 \pm 0.34	1.08	2.33	2.11 \pm 0.39	SM
17	2.75 \pm 0.41	0.94	1.81	1.96 \pm 0.31	SM
18	2.22 \pm 0.26	0.70	1.52	2.23 \pm 0.42	SM
19	1.95 \pm 0.30	0.65	1.30	2.02 \pm 0.24	SM
20	1.82 \pm 0.29	0.60	1.22	2.03 \pm 0.26	SM
21	1.34 \pm 0.19	0.45	0.89	1.95 \pm 0.17	SM

Based on the measurement of seven karyotyped cells.

*M: metacentric, SM: submetacentric.

Table 3. A comparison in the chromosome constitution of the genus *Liobagrus* in Korea

Species	2N	Karyotypic formular	FN	Localities	Reference
<i>L. andersoni</i>	28	18m-10sm	56	Han River	Son and Lee (1989)
<i>L. obesus</i>	20	20m	40	Kum River	Son and Lee (1989)
<i>L. mediadiposalis</i>	42	26m-16sm	84	Nakdong River	Son and Lee (1989)
<i>L. somjinensis</i>	42	28m-14sm	84	Yochen	Cho and Park (2016)
<i>L. hyeongsanensis</i>	42	30m-12sm	84	Daejongcheon	Present study

1995; Devlin and Nagahama, 2002). Meanwhile, heteromorphic chromosomes in *Liobagrus* were found in *L. marginatus* and *L. styani* (Chen *et al.*, 2008). Wang *et al.* (2011) also reported that *L. marginatoides* of Qingyijiang and Mingjiang in China has heteromorphic chromosome and chromosome variation. To confirm whether *L. hyeongsanensis* owns sex chromosome, additional methods will be applied in the future, including chromomycin A₃ and fluorescence *in situ* hybridization with Giemsa.

Until now, the chromosome number for four of five species in Korea has been reported: *L. andersoni* (2n = 28), *L. obesus* (2n = 20), *L. mediadiposalis* (2n = 42), and *L. somjinensis* (2n = 42) (Son and Lee, 1989; Kim, 2013; Cho and Park, 2016, Table 3). Through this study, the other species *L. hyeongsanensis* is revealed to have 2n = 42. According to Kim (2013), the karyotype formula of *Liobagrus* sp. 1, currently *L. hyeongsanensis*, is comprised

of 14 pairs of metacentric and 7 pairs of submetacentric that is different from our data showing 15 pairs of metacentric and 6 pairs of submetacentric. In general, the same number of chromosomes in many fishes is considered to be the same species. In the case that there are a karyotype variation, however, it has been known as different species (Miler and Walter, 1972). Such phenomena showed well in some Korean cobitid fishes that have chromosome of the same number (2n = 50). But as their karyotypes are different, they were considered as separate species (Kim and Lee, 1986; Kim and Park, 2002; Kim and Kim, 2008), saying karyotype variation may play a crucial role in classifying fishes. For example, the diploid chromosome number of *Iksookimia koreensis* and *I. longicorpa* were the same, 50, whereas their karyotypes are different: the former 22msm-28stt and the latter 20msm-30stt, respectively. This variation is said to be caused by centric fis-

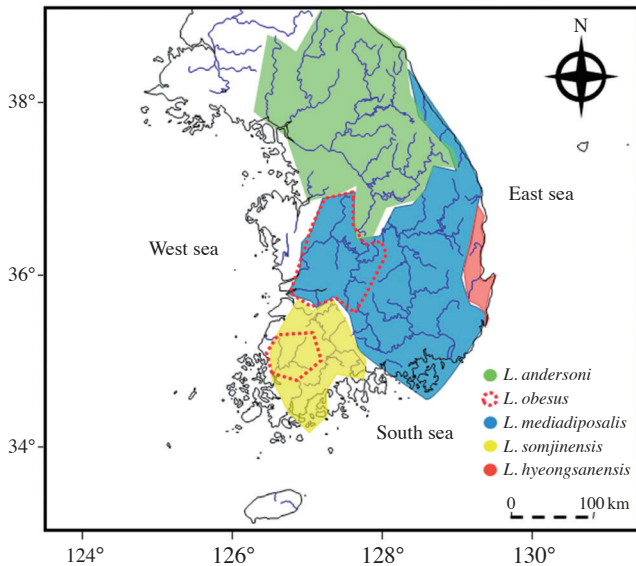


Fig. 2. A schematic distribution of the genus *Liobagrus* in Korea.

sion and fusion of Robertsonian translocation such as pericentric inversion (de Oliveira *et al.*, 2009). Such chromosomal rearrangements were shown in the X chromosome of *L. marginatus* and *L. styani* on the metacentric chromosomes and submetacentric chromosomes (Chen *et al.*, 2008). Consequently, it means that the relation between a clear or distinctive morphological difference as a separate species and its chromosome analysis does not reflect the same results consistently in species level.

The genus *Liobagrus* in Korea shows a distinct geographic isolation respectively: *L. andersoni* in Hangang and Imjingang Rivers water system; *L. obesus* in Geumgang, Yeongsangang and Mangyunggang Rivers water system; *L. mediadiposalis* in Geumgang and Nackdonggang Rivers water system; *L. somjinensis* in Seomjingang, Yeongsangang, Dongjingang and Tamjingang Rivers; and *L. hyeongsanensis* in Hyeongsangang, Taehwagang Rivers and Daejongcheon Stream (Kim *et al.*, 2015, Fig. 2). Cytochrome *b* gene analysis suggests that *L. hyeongsanensis* originated from *L. mediadiposalis*, followed by *L. somjinensis* (Kim, 2013). Such allopatric speciation may lead the three species to undergo chromosomal rearrangements that have the same chromosome number but different karyotype. Chromosomal evolution and speciation in such three closely related species still remain questionable and then various approach methods would be required, with karyotype analyses using banding and genetic diversity study.

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한국 통가리속 고유종 동방자가사리 *Liobagrus hyeongsanensis*의 핵형 분석

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요 약 : 최근 신종으로 발표된 동방자가사리 *Liobagrus hyeongsanensis*의 핵형 분석을 위해 경상북도 경주시 양북면 일대에서 암컷 10마리와 수컷 5마리를 채집하였다. 동방자가사리의 염색체 수는 $2n=42$ 이었으며, 핵형은 30개의 중부염색체와 12개의 차중부염색체로 구성되어 있었다. FN 값은 84였으며, 암수 간 성적이형과 배수체는 관찰되지 않았다. 동방자가사리는 통가리속의 자가사리, 섬진자가사리와 염색체 수는 같았으나 핵형에서 차이를 보였다. 이러한 핵형의 차이는 지리적 격리로 인한 로버트슨 전좌(Robertsonian rearrangement)와 관련 있는 것으로 생각된다.

찾아보기 낱말 : 동방자가사리, 염색체, 핵형, 한국고유종