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Morphological Characters and Geographical Distributions of Korean Ricefish (Pisces, Adrianichthyidae), *Oryzias*

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The Korean ricefish, *Oryzias*, consisted of 2 species, *O. latipes* and *O. sinensis*. Through investigation on their distributions, it was confirmed that *O. sinensis* was restricted to western areas, and *O. latipes* was distributed throughout Korea. Interestingly, Daecheon region near the West Sea was sympatric, existing *O. latipes* and *O. sinensis*. In addition to their distributions, *O. latipes* was well distinguished from *O. sinensis* as the following morphological characteristics: mean 31 vertebrae (vs. 30), mean 15 gill rakers (vs. 12), lots of dots in both sides of body (vs. absent), and yellowish ventral and anal fins (vs. blackish).

Key words : Ricefish, *Oryzias*, gill raker, vertebra, Korea

Introduction

Adrianichthyidae consists of 4 genera, *Xenopoecilus*, *Oryzias*, *Adrianichthys*, and *Horaichthys*, and contains 25 species in the world (Parenti and Soeroto, 2004). The genus *Oryzias* is found in Asia including Japan, China, Indonesia and Korea and inhabited mostly freshwater but rarely brackish water. The genus *Oryzias*, an egg-laying fish, has been known as an useful experimental material for genetics, physiology, embryology, and development due to its small size, strong tolerance to unstable environmental change, ease of the breeding and induction of spawning, and relatively short generation time (Yamamoto, 1975). In particular, Japanese medaka, *Oryzias latipes*, has been used as an experimental animal.

In Korean Peninsula, only one species *Oryzias latipes* had been known in the genus *Oryzias*. Since then, two species were reported as follows: China-West Korean population (*O. sinensis*) and East Korean population (*O. latipes*) through re-examination on their distribution and morphology in Korea (Kim and Lee, 1992; Kim and Kim, 1993). However, although there are distinct differences between *O. latipes* and *O. sinensis*, two species have been often in conflict (Matsuda *et al*., 1997; Takehana *et al*., 2004). Recently, results of mtDNA and allozymic analysis made clear the geographical and reproductive isolation between two Korean ricefishes, *O. latipes* and *O. sinensis* (Sakaizumi and Jeon, 1987; Matsuda *et al*., 1997; Takehana *et al*., 2004). These results well accorded with the past karyological and morphological studies (Uwa 1986; Kim and Moon, 1987; Uwa and Jeon, 1987).

As it is necessary for giving a useful taxonomic key between *O. latipes* and *O. sinensis*, we are going to describe morphological characters and review distributions on Korean ricefishes.

Materials and Methods

The specimens were taken from the specimens deposited at Faculty of Biological Sciences, Chonbuk National University, Jeonju, Korea (CNUC), and collected in the freshwater and brackish water of several Korean streams from September 2004 to July 2005 (Table 1) by a 1 mm × 1 mm-sized hand net. The specimens were fixed with

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Table 1. The researched sites of *Oryzias latipes* and *O. sinensis* in Korea

O. latipes	*O. sinensis*
1 Daebudo, Ansan, Gyeonggido	35 Jangheung reservoir, Ganghwa, Gyeonggido
2 Anmyeondo, Chungcheongbukdo	36 Inchon
3 Degi, Taean, Chungcheongnamdo	37 Daeduck, Ansung, Gyeonggido
4 Waljoen, Boryung, Chungcheongnamdo	38 Jangam, Tanbu, Boeun, Chungcheongbukdo
5 Daecheon, Boryung, Chungcheongnamdo	39 Daecheon, Boryung, Chungcheongnamdo
6 Chungso, Boryung, Chungcheongnamdo	40 Janghang, Seocheon, Chungcheongnamdo
7 Hwachon, Soesan, Chungcheongnamdo	41 Jeungsuk, Hoehyun, Gunsan, Jeonbuk
8 Daehang, Buan, Jeollabukdo	42 Aeun, Oku, Gunsan, Jeollabukdo
9 Sukpo, Buan, Jeollabukdo	43 Munyodo, Gunsan, Jeollabukdo
10 Seonyudo, Gunsans Jeollabukdo	44 Jangdeung, Buan, Jeollabukdo
11 Sukchang, Hampyung, Jeollanamdo	45 Geumchon reservoir, Kimje, Jeollabukdo
12 Unbong, Namwon, Jeollabukdo	46 Seopo, Juksan, Gimje, Jeollabukdo
13 Inwal, Namwon, Jeollabukdo	47 Nampo, Sungduk, Gimje, Jeollabukdo
14 Gidong, Busan, Jangheung, Jeollanamdo	48 Osung reservoir, Jeonju, Jeollabukdo
15 Anjwado, Sinan, Jeollanamdo	49 Gui, Wanju, Jeollabukdo
16 Jindo, Jeollanamdo	50 Maryung, Jinan, Jeollabukdo
17 Wando, Wando, Jeollanamdo	51 Bongnam, Soyang, Jeollabukdo
18 Kumdangdo, Wando, Jeollanamdo	52 Ogeum, Jungeup, Jeollabukdo
19 Geogeumdo, Goheumg, Jeollanamdo	53 Sinheung, Gobu, Jungeup, Jeollabukdo
20 Waenarodo, Goheumg, Jeollanamdo	54 Taechang, Taein, Jeollabukdo
21 Dolsando, Jeollanamdo	55 Negyo, Hampyung, Jeollanamdo
22 Bogildo, Wando, Jeollanamdo	56 Singwang, Dasi, Naju, Jeollanamdo
23 Haechang, Youngam, Jeollanamdo	57 Youngsanho, Mokpo, Jeollanamdo
24 Gyechon, Geumnam, Hadong, Jeollanamdo	58 Gurye, Jeollanamdo
25 Hanlim, Kimhae, Gyeongsangnamdo	59 Akyang, Hadong, Jeollanamdo
26 Sangdong, Kimhae, Gyeongsangnamdo	
27 Imrang, Yangsan, Gyeongsangnamdo	
28 Jangan, Gyeongsangnamdo	
29 Haseo, Gyeongju, Gyeongsangbukdo	
30 Yongchon, Pohang, Gyeongsangbukdo	
31 Wonhwang, Youngdok, Gyeongsangbukdo	
32 Nakpung, Okgye, Gangreun, Gangwondo	
33 Jusu, Okgye, Gangreun, Gangwondo	
34 Dongduk, Sachon, Gangreung, Gangwondo	

10% neutral formalin solution for morphological studies and some live specimens were carried into laboratory for karyotype study.

For karyotype analysis, injection of 1% colchicine to the body cavity of the live specimens, KCl hypotonization, acetic alcohol fixation and Giemsa staining were performed according to Ojima *et al.* (1972). The nomenclature of the chromosomes followed the methods of Levan *et al.* (1964).

The measurements and counts were carried out under the dissecting microscope using 1/10 mm digital calipers by the methods of Hubbs and Lagler (1964).

Results

Counts and measurements

There were no significant differences between two species except for the number of vertebra and gill raker (Table 2). In the vertebra, *O. latipes* was more one than that of *O. sinensis*; *O. sinensis* ranged from 28 to 30 in number, mean 29, and *O. latipes* from 29 to 32, mean 30. In the gill raker, *O. latipes* was more three than that of *O. sinensis*; *O. latipes* ranged from 14 to 16, mean 15, and *O. sinensis* from 12 to 14, mean 12. Interestingly, sexual dimorphism in the orbit dimension to head length appeared in the male of *O. sinensis* (Table 3), having a larger orbit diameter as the following values: 1.7% in Hampyung, 5.2% in Gunsan and 4.1% in Gimje. But *O. latipes* did not show sexual dimorphim as in the following values: 1.5% in Hampyung, 1.2% in Daecheon, 2.1% in Youngdok and 1.5% in Hadong.

Color pattern and sexual dimorphism

When they are alive, their colors were gener-

Table 2. Comparison of measurements and meristic counts between *Oryzias sinensis* and *O. latipes*

Locality	*O. sinensis*			*O. latipes*		
	Hampyung	Gunsan	Gimje	Hampyung	Daecheon	Hadong
Indivisual numbers	20	20	20	14	20	18
Standard length (mm)	22.1±1.3 (20.3~25.1)	25.9±2.6 (21.4~30.5)	22.2±1.4 (20.1~26.3)	22.0±2.8 (18.8~30.4)	26.1±1.8 (23.2~29.4)	26.8±1.4 (24.1~28.4)
% to Standard length						
Body depth	20.6±1.4 (18~23.3)	23.5±1.9 (21.4~27)	22.4±1.2 (20.3~24.3)	20.2±1.1 (18.8~23.2)	25.7±1.9 (23.1~28.5)	25.1±1.7 (21.5~26.9)
Predorsal length	75.6±1.2 (73~77.3)	77.6±2.8 (64.6~80.7)	77.8±2 (75.4~84.7)	76.4±0.8 (74.9~77.5)	76.4±1.3 (74.3~78.7)	75.6±1.3 (73.8~77.9)
Dorsal origin to caudal base	25.6±0.8 (24~27.1)	23.7±1.4 (21.4~26.6)	24.3±1.4 (20.4~26.3)	25±0.6 (24.4~26.2)	25.6±1.2 (24~28)	25.7±0.7 (24.5~26.8)
Preanal length	57.3±1.6 (54.1~59.8)	60.5±1.9 (56.8~63.9)	59.2±1.7 (56.7~62)	58.9±1.6 (57.2~62.5)	60.2±1.0 (58.5~61.4)	60.1±1.1 (58.6~61.8)
Preventral length	45.83±1.4 (43.6~48.3)	48±2.5 (44.1~52.9)	46.3±1.6 (43.2~49.4)	47.6±1.5 (45.1~50.2)	48±1.6 (45.6~50.2)	48.8±1.3 (46.4~50.6)
Prepectoral length	28±1.1 (25.9~30)	28±1.1 (26~30.4)	28.4±0.9 (27.2~30.7)	29.5±1.4 (27.5~31.9)	27.5±1.1 (25.9~29.0)	28.0±0.5 (27.6~29.1)
Caudal peduncle length	17.5±1.3 (15.4~19.4)	16.7±2 (13.5~25.24)	16.8±1.4 (13.6~19.1)	16.8±1.2 (14.6~18.7)	15.8±1.1 (14.4~17.9)	16.1±0.7 (15.4~17.8)
Caudal peduncle depth	9.4±0.6 (8.3~10.5)	9.6±1.5 (8.4~16.8)	9.8±0.6 (8.8~11.1)	9.1±0.4 (8.5~9.9)	10.6±0.7 (9.8~12.0)	10.6±0.3 (9.9~11.3)
Length of dorsal fin base	7.6±0.9 (5.7~9.3)	6.9±0..8 (5.5~9)	7.3±0.9 (5.9~8.9)	6.7±0.7 (5.6~8.1)	8.0±0.9 (7.0~9.9)	8.3±0.8 (7.0~9.5)
Length of anal fin base	26.6±1.8 (23.8~30.2)	25.5±2.5 (20.7~29.9)	27.1±1.3 (25.4~29.4)	26.6±1.3 (24.6~29)	27.5±1.1 (26.0~29.0)	26.5±0.8 (24.8~27.8)
Head length	26.4±1.1 (23.4~28.6)	24.8±1.1 (22.3~26.9)	26.2±0.9 (24.4~28.3)	26.0±0.8 (24.3~27.7)	26.1±1.1 (24.6~28.3)	25.6±0.6 (24.9~26.3)
% to Head length						
Orbit diameter	37.4±2.1 (34.4~42.7)	36.2±3.2 (29.4~41.4)	38.4±2.2 (35.2~42.0)	36.9±1.8 (32.8~39.2)	36±1.4 (34.0~38.4)	37.1±1.6 (35.3~40.0)
Interorbital width	47±3.1 (42.7~56.9)	43.3±4.4 (25~49.2)	46±2 (41.9~49)	47.4±1.4 (44.1~49.5)	47.3±1.0 (46.0~49.3)	49.1±2.2 (45.6~51.7)
Counts characters						
Dorsal fin rays	6	6	6	6	6	6
Anal fin rays	18 (16~18)	17 (16~18)	18	18 (17~19)	18 (17~18)	17 (17~18)
Pectoral fin rays	9	9	9	9	9	9
Gill rakers	12 (12~13)	13 (12~14)	13 (12~14)	15 (14~16)	15 (15~16)	15 (14~16)
Vertebrae	30 (29~30)	30 (29~30)	29 (28~30)	31 (29~32)	31 (29~32)	31 (30~32)

mean±SD, (min~max)

ally yellowish brown in the body region, silvery white in the ventral side, and light yellow in all the fins. However, there were clear differences in the color patterns between *O. sinensis* and *O. latipes*. *O. sinensis* had dark spots on the yellowish body. Whereas *O. latipes* had just yellow fins and lots of spots on the lateral body sides.

During the breeding season, the sexual dimorphism appeared. *O. sinensis* had thick, black

Table 3. Comparison of interspecific and sexual difference of orbit diameter (%) to head length

Orbit diameter	*O. sinensis*			*O. latipes*		
Locality	Hampyung	Gunsan	Gimje	Hampyung	Daecheon	Hadong
♂	38.7±2.4 (34.4~42.7)	38.9±1.8 (35.8~41.4)	41.1±0.6 (40.2~42.0)	37.9±1.6 (35.5~39.2)	36.5±1.4 (34.2~38.4)	38.7±1.1 (37.2~39.9)
♀	36.7±1.4 (35.0~39.8)	33.6±1.8 (29.4~36.8)	37.0±1.1 (35.2~37.7)	36.4±1.8 (32.8~38.7)	35.3±1.0 (34.0~36.1)	37.2±1.4 (34.5~39.3)
No. of specimen	9, 11	15, 15	10, 10	9, 5	10, 10	10, 8
T-test	**	**	**	NS	NS	*

No. of specimen is each male and female orderly.
T-test: '*': significant (t0.05< ts< t0.01), '**': highly significant (ts> t0.01), 'NS': non-significant.

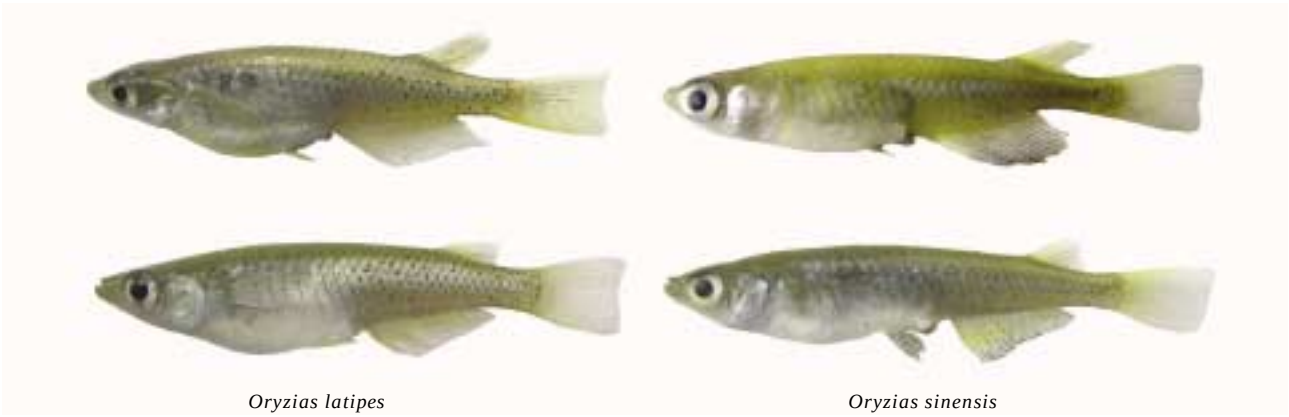

Fig. 1. The interspecific difference of dots and colors in *O. latipes* and *O. sinensis*. The upper one is male, lower one is female.

ventral and anal fin, but *O. latipes* had thick and yellow fins. In particular, the anal fin in the male of *O. latipes* was relatively light black. Also, the spots dispersed along with the lateral body region became larger and thicker, and one or two spots larger than those of lateral body region were situated behind the pectoral fin (Fig. 1).

Distribution

Two Korean *Oryzias* showed a distinctive difference in their geographical distributions. *O. latipes* was found not only in the streams flowing into the East and South Sea but also in streams flowing into the West Sea, whereas *O. sinensis* was only in the streams flowing into the West Sea and the inland up to the Mountain area. As in the Fig. 2 and Table 1, *O. latipes* was found in such isolated streams as Daebudo, Anmyeondo, Taean, Daecheon, Boryung, Seosan, Buan, Seonyudo and such main streams as the Yongsan River (Hampyung) and Sumjin River (Hadong), which are close to the estuary. Whereas *O. sinensis* was not found in the Geum River, Dongin River and Mangyung River but in the Sumjin River, Youngsan River and their inland regions. Interestingly, Daecheon region close to the West Sea had sympatric habitat, which were coexisted two species, *O. latipes* and *O. sinensis*.

In addition to the geographical isolation and the morphological differences between the two species, there was a distinctive difference in karyotypic analysis. *O. latipes* had 2n=48, consisted of FN 70 and none large-metacentric chromosome and *O. sinensis* had 2n=46, FN 68 and one large-metacentric chromosome. Moreover, even though the Dacheon population shows sympatric distribution, two species showed a clear difference in karyotype, *O. latipes* (2n=48) and *O. sinensis* (2n=46) (Fig. 3), as in the two species of isolated regions.

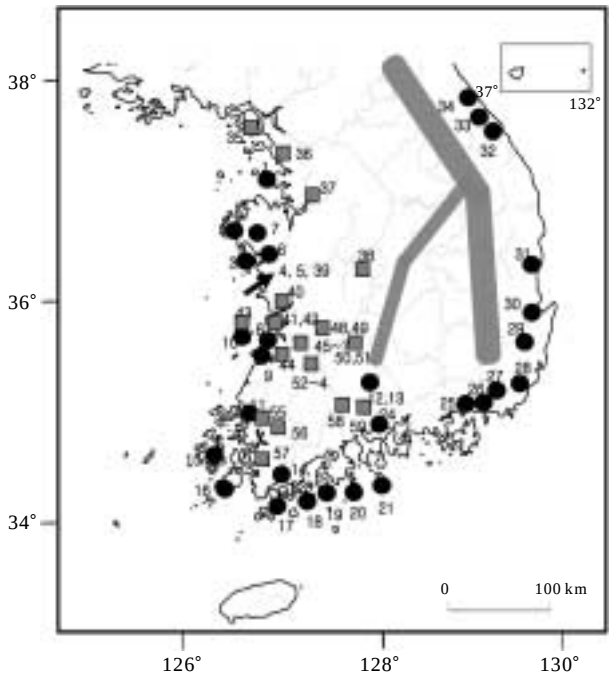

Fig. 2. The distribution of the two Korean ricefishes. Circles, *O. latipes*; rectangles, *O. sinensis*; arrow, sympatric site. The detailed localities see Table 1.

Discussion

In Korean Peninsula, although two ricefishes, *O. latipes* and *O. sinensis*, has been known, a taxonomic confusion remained still (Kim and Moon, 1987; Kim and Lee, 1992; Kim and Kim, 1993; Matsuda *et al.*, 1997; Takehana *et al.*, 2004). However, interspecific characteristics became more clear by re-examining such the following features as measurement and meristic count, color pattern, distribution, and karyotype.

Firstly, in the color pattern, two species showed a clear difference: *O. latipes* had lots of spots on the lateral body sides, whereas *O. sinensis* had no spots. In the ventral and anal fin during the breeding season, *O. latipes* was thick and yellow, and tinged, but *O. sinensis* was thick and black. These differences described in this paper were little known in the previous reports (Kim and Moon, 1987; Kim and Lee, 1992; Kim and Kim, 1993).

Secondly, in number of the vertebrae and gill raker, two species showed interspecific differ-

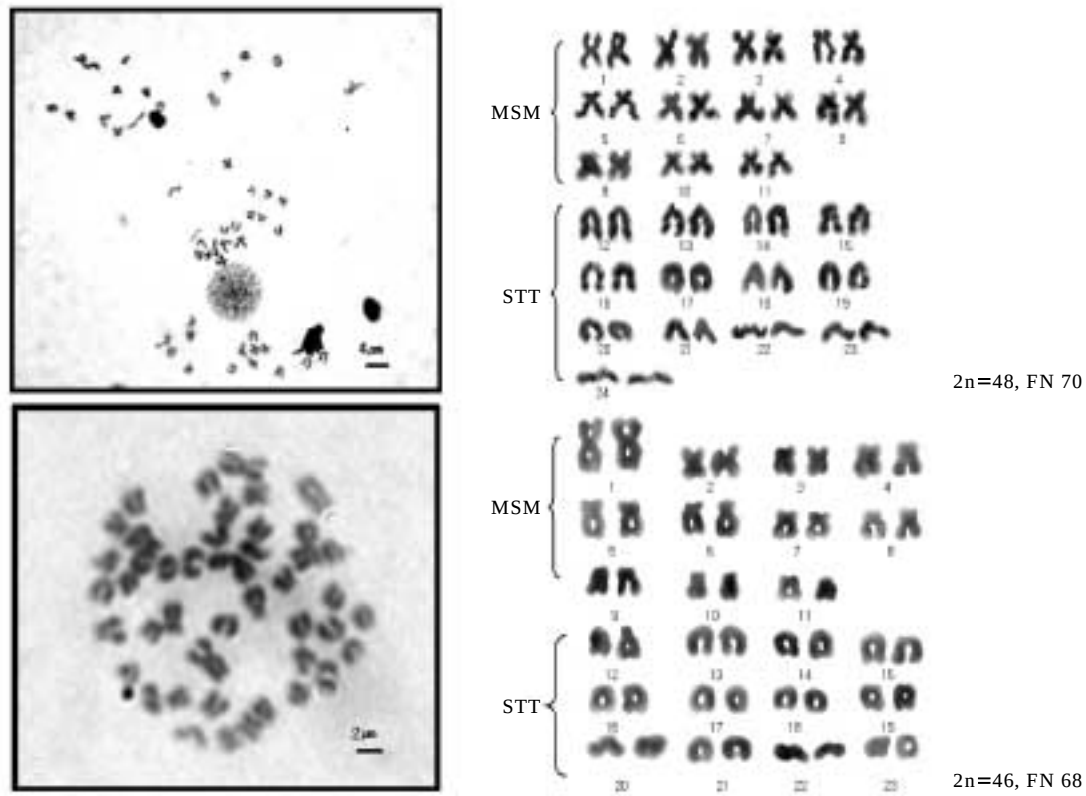

Fig. 3. A figure of metaphase chromosome in the slide and its idiogram of *Oryzias latipes* (A) and *O. sinensis* (B) from southern population. MSM: meta-submeta centric, STT: subtelo telo cenctric.

ences: in the vertebrae, *O. latipes* was 29～32 (mean 30) and *O. sinensis* 28～30 (mean 29), and in the gill raker, *O. latipes* 14～16 (mean 15) and *O. sinensis* 12～14 (mean 12). Although the number of the vertebrae and gill rakers between the two species are more or less overlapped, it is considered as useful characters to compare with interspecific populations as in other fishes (Kim and Park, 2002; Kim *et al*., 2005).

Thirdly, interestingly, a phenomenon of sexual dimorphism was seen in the orbit diameter. In the males, *O. sinensis* had larger orbit diameter than in its female, but *O. latipes* was not so.

Fourthly, the karyotype showed a clear interspecific difference between the two species: *O. sinensis* was 2n=46 and *O. latipes* was 2n=48. These karyological characters had been reported in the study on geographical distribution of Korean ricefishes (Uwa and 1986; Uwa and Jeon, 1987). In other fishes, the difference of the chromosome has been used as one of useful key characters in taxonomy (Kim and Park, 2002). Through mtDNA and allozyme analysis for Korean ricefish populations, some researchers surmised that these difference may caused by geographical and reproductive isolation (Sakaizumi and Jeon, 1987; Matsuda *et al*., 1997; Takehana *et al*., 2004). Nevertheless, they suggested that Korean ricefishes are not composed of two species but just one species, *O. latipes*, and regarded that *O. sinensis* is another gene type of *O. latipes*.

Fifthly, Kim and Kim (1993) reported that *O. latipes* (East Korean population) was distributed in the eastern and southern area and *O. sinensis* (China West Korean population) in western area forming a boundary of the Taebaek Mountain. However, through our investigation, their distribution data was some or less changed: *O. sinensis* was restricted to the western area, whereas *O. latipes* was distributed throughout Korea. This distributional difference was also reported by mtDNA and allozyme analysis (Sakaizumi and Jeon, 1987; Matsuda *et al*., 1997; Takehana *et al*., 2004). Kim and Kim (1993) reported that *O. sinensis* (2n=46) having a pair of large metacentric chromosome evolved from *O. latipes* (2n=48) by Robertsonian centric fusion (Kim and Kim, 1993). On the basis of the above hypothesis, they suggested that *O. latipes* had a broad range through Japan, Korea, China and Vietnam, whereas *O. sinensis* was localized to China and Western Korea. It seems that *O. sinensis* may be branched out from *O. latipes* of China and Korean *O. sinensis* may be get across to Western Korea by Paleo Hwangho River during Pleiocene (0.01～0.6 mya) (Kitamura, 2002). This hypothesis may be supported by finding the sympatric area in the Daecheon region close to the West Sea. However, more researches regarding their origins and distributions are needed.

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한국산 송사리속 어류의 형태적 특징 및 지리적 분포

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한국산 송사리속 어류 2종 *Oryzias latipes*와 *O. sinensis*에 대한 분포역을 조사한 결과 *O. sinensis*는 서해안과 그 내륙지역에 제한되어 분포한 반면에 *O. latipes*는 한반도의 대부분 지역에 분포하고 있었고, 특히 서해안 지역의 대천지역에서는 두 종이 혼서하고 있었다. 또한 송사리와 대륙송사리는 척추골수와 새파수에서 잘 구분되고 있는데 척추골 수에서는 송사리가 평균 31개, 대륙송사리가 평균 30개이었으며 새파수는 송사리가 평균 15개, 대륙송사리가 평균 12개를 갖는다. 또한 체측반문에 있어서 송사리는 체측에 반점들이 존재하였지만 대륙송사리는 없었으며, 산란시기에 송사리는 배지느러미와 뒷지느러미에 노란색을 띠는 반면에 대륙송사리는 검은색을 띠고 있었다.