# Morphological and Genetic Differences between Japanese and Chinese Sea Bass of the Genus *Lateolabrax*

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Abstract Morphological and genetic differences between Japanese and Chinese sea bass of the genus Lateolabrax were examined. Pronounced differences were recognized between the two forms in some morphological characters, in particular the number of pored lateral line scales and gill rakers, which differed sufficiently for unequivocal differentiation of the forms, when used in combination. Isozyme analyses of genetic characters indicated a complete replacement of alleles at the PROT-1\* locus, and extreme differences in allelic frequencies at the GPI-1\* and LDH\* loci. The genetic distance (D value) between the Japanese and Chinese forms, calculated from isozymic allele frequencies, was 0.174, a figure significant at the inter-specific level. The considerable morphological and genetic differences suggested that the sea bass from China is a distinct species from L. japonicus.

During recent aquaculture operations in Japan, inexpensive and easily obtained seeds of various fish species from foreign countries have been vigorously introduced. In particular, sea bass *Lateolabrax japonicus* (Cuvier) have been abundantly imported from China, Korea and Taiwan, mainly to western Japan (Matsuoka, 1993). Importation from China began in 1990, when about 1 million young sea bass were imported through a joint venture in Hong Kong, the quantity thereafter increasing with each succeeding importation and reaching 4 million in 1992.

Most imported sea bass are characterized by many clear black dots on the lateral body region (Fig. 1). It is common knowledge in the sea bass nurseries that such dots do not disappear with growth, as in sea bass from Japanese waters. In particular, the sea bass from China, which constitute the bulk of imported sea bass, display this feature prominently.

Sea bass with many clear black dots have been recently caught by anglers and fishermen in fishing grounds in western Japan. These fish are considered to be Chinese sea bass, since such are known to have escaped from some nurseries.

Consequently, the effects of the existence of freeliving Chinese sea bass in Japanese waters is of concern, necessitating examination of their biological features. For this reason, the present study was carried out to clarify morphological and genetic differences between sea bass from Japan and China.

## Materials and Methods

Morphological characters.—Data from specimens used for morphological analyses are shown in Table 1. The sea bass from Japan (hereafter called Japanese form) were collected from a branch of the Yoshino River, Tokushima City, and from southern Harima Sea, Seto Inland Sea. The sea bass from China (hereafter called Chinese form) were sampled from aquaculture seeds, which were transported from China via Hong Kong to Kagawa Prefecture. Although the exact collection locality is uncertain, information from buyers suggested that the imported fish were caught from coastal waters of the Bohai Sea or Yellow Sea. The specimens examined in this study have been deposited in the Tokushima Prefectural Museum (TKPM) and the Kagawa Prefectural Fisheries Experimental Station (KPFES).

The methods of measurements and counts followed Hubbs and Lagler (1970). Vertebrae were counted using radiographs. The total of recognizable black dots on the left side of the body were

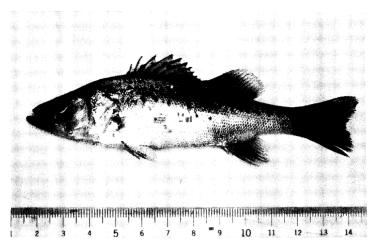


Fig. 1. General aspect of Lateolabrax sp. (KPFES 90003, 124.8 mm TL).

counted, including those on the mid-dorsal aspect of the caudal peduncle. Because squamation on the pectoral base differed between the Japanese and Chinese forms, a new character, pectoral scaly area length (PSAL), was defined (Fig. 2).

Genetic characters.—Data from specimens used for genetic analyses are shown in Table 1. Japanese forms were collected from the southern Harima Sea by trawl. Chinese forms comprised mostly those

used for the morphological analyses. The specimens were preserved in a refrigerator at either  $-80^{\circ}$ C or  $-15^{\circ}$ C.

Isozymes detected by horizontal starch-gel electrophoresis were used as genetic markers. The experimental methods were based mainly on Taniguchi and Okada (1980), using citric acid-aminopropylmorpholine buffer (pH 6.0) in each case.

Enzymes and a protein detected by electrophoresis

Table	1.	Collection	and	treatment	data	of	specimens
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	Morphological examinations				
T 154	Ja	pan	China		
Locality -	Tokushima	Harima Sea	Chinese coast		
Date of collection	30 Oct. 1988	11 Dec. 1990	17 June 1990		
Method of sampling	Angling	Trawl net	- Unknown		
Number of individuals	20	45	62		
Size range (TL, mm)	149.8-280.0	247.0-493.0	90.4-143.0		
Average size (TL, mm)	198.7	440.6	116.2		
Preservation	70% ethanol	10% formalin	10% formalin		
Catalog No.	TKPM-P352	KPFES 90007	KPFES 90001-6, TKPM-P1655		

Electrophoretic analyses Japan China Locality Harima Sea Harima Sea Chinese coast Date of collection 11 Dec. 1990 17 Dec. 1991 17 June 1990 Method of sampling Trawl net Trawl net Transported from Hong Kong Number of individuals 45 27 58 Size range (TL, mm) 247.0-493.0 Unmeasured 90.4-143.0 Average size (TL, mm) 440.6 Unmeasured 116.3 Preservation Frozen in −15°C Frozen in −80°C Frozen in −80°C Catalog No. **KPFES 90007** TKPM-P1655 Not preserved

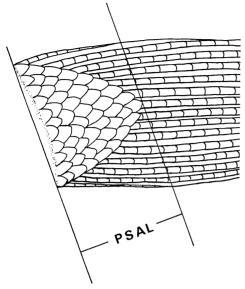


Fig. 2. Definition of pectoral scaly area length (PSAL).

were as follows; aspartate aminotransferase, E.C. 2.6.1.1 (sAAT), alcohol dehydrogenase, E.C. 1.1.1.1 (ADH), glyceraldehyde-3-phosphate dehydroge-

nase, E.C. 1.2.1.12 (GAPDH), glucose-6-phosphate isomerase, E.C. 5.3.1.9 (GPI), L-iditol dehydrogenase, E.C. 1.1.1.14 (sIDDH), isocitrate dehydrogenase (NADP<sup>+</sup>), E.C. 1.1.1.42 (IDHP), lactate dehydrogenase, E.C. 1.1.1.27 (LDH), malate dehydrogenase, E.C. 1.1.1.37 (sMDH), malic enzyme (NADP<sup>+</sup>), E.C. 1.1.1.40 (sMEP), mannose-6-phosphate isomerase, E.C. 5.3.1.8 (MPI), phosphogluconate dehydrogenase, E.C. 1.1.1.44 (PGDH), phosphoglucomutase, E.C. 2.7.5.1 (PGM), superoxide dismutase, E.C. 1.15.1.1 (sSOD), general protein (PROT).

Gene nomenclature followed Shaklee et al. (1990), the alleles being symbolized as relative mobility percentages compared with the most dominant alleles identified.

#### Results

Morphological characters.—Proportional measurements are shown in Table 2. Some proportional differences between the two forms were recognized, notably the orbital diameter (OD), which was greater in the Chinese form (Table 2, Fig. 3). In addition,

Table 2. Proportional measurements of Japanese and Chinese forms of Lateolabrax

	Japanese		Chinese	
	Average	Range	Average	Range
Total length <sup>1</sup>	121.69	117.25-128.00	119.17	114.61-125.4
Fork length <sup>1</sup>	115.11	111.14-121.09	114.00	111.11-118.33
Pre-anus length <sup>1</sup>	66.04	62.35- 69.31	66.76	63.70- 69.94
Body depth <sup>1</sup>	24.24	20.35- 29.12	26.28	24.34- 28.76
Body width <sup>1</sup>	13.38	10.42- 16.43	13.46	12.11- 18.78
Caudal peduncle depth <sup>1</sup>	9.28	7.77- 11.04	10.16	9.35- 10.89
Caudal peduncle length <sup>1</sup>	21.61	18.70- 24.64	22.09	20.21- 25.70
Pre-dorsal length <sup>1</sup>	35.24	33.33- 38.31	35.00	31.99- 39.29
First dorsal fin length <sup>1</sup>	14.10	11.99- 17.04	12.62	10.24- 14.73
Second dorsal fin length <sup>1</sup>	11.82	9.19- 13.89	12.57	10.58- 14.9
Anal fin length <sup>1</sup>	12.49	10.05- 14.90	14.33	11.84- 16.67
Pectoral fin length <sup>1</sup>	17.05	14.84- 19.51	16.08	13.67- 17.8
Pelvic fin length <sup>1</sup>	17.57	15.05- 19.58	18.33	16.30- 21.03
Head length <sup>1</sup>	31.98	29.16- 35.46	32.56	30.67- 33.65
Snout length <sup>2</sup>	26.25	21.95- 29.64	24.06	19.43- 26.81
Orbital diameter <sup>2</sup>	17.70	14.34- 22.71	24.83	21.31- 30.49
Interorbital width <sup>2</sup>	21.20	17.38- 24.48	21.64	18.18- 24.14
Sub-orbital width <sup>2</sup>	11.26	6.64- 14.45	10.72	6.96- 15.9
Upper jaw length <sup>2</sup>	42.36	38.36- 46.51	44.21	40.63- 50.00
Lower jaw length <sup>2</sup>	46.43	42.44- 49.51	46.82	43.71- 51.70
Pectoral scaly area length <sup>3</sup>	26.73	18.69- 36.51	19.43	14.79- 23.78

<sup>&</sup>lt;sup>1</sup> Percentage of standard length; <sup>2</sup> percentage of head length; <sup>3</sup> percentage of pectoral fin length.

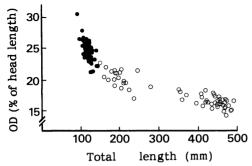


Fig. 3. Relationship between total length and percentage of orbital diameter (OD) of head length. *Open circles*—Japanese form; *dark circles*—Chinese form.

the PSAL in the Chinese form was distinctly shorter than in the Japanese form (Table 2, Fig. 4). Histograms of PSAL frequencies in the two forms are shown in Figure 5. Both resulted in normal distributions and indicated clear differences in shape and mode.

Average values and ranges of the meristic counts, except number of lateral dots, are shown in Table 3, the average values of the meristics differing between the two forms. The differences in pored lateral line scales (LLS) and total gill rakers (GR) were obvious, the average values of these characters differing by nearly 10 and 6, respectively, between the two forms. Although LLS and GR ranges overlapped (Figs. 6, 7), the combination of GR and LLS counts separated the two forms completely (Fig. 8).

A histogram of the number of lateral dots on the

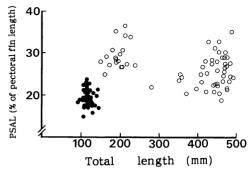


Fig. 4. Relationship between total length and percentage of pectoral scaly area length (PSAL) of pectoral fin length. *Open circles*—Japanese form; *dark circles*—Chinese form.

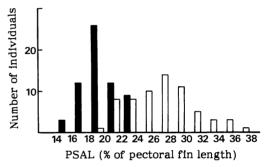


Fig. 5. Frequency distribution of pectoral scaly area rate (PSAL). Open bars—Japanese form; dark bars—Chinese form.

Chinese form indicated a normal distribution, ranging from 0 to nearly 100, with a mode of 40-50 (Fig. 9). This character was a typical feature of the

Table 3. Meristic counts of Japanese and Chinese forms of Lateolabrax

	Japanese		Chinese	
	Average	Range	Average	Range
Dorsal fin spines	12.85	12-14	12.95	12-14
Dorsal fin rays	12.78	12-14	13.06	12-14
Anal fin spines	3.00	3-3	2.98	2- 3
Anal fin rays	7.73	7- 9	7.53	6- 8
Pectoral fin rays	16.85	15-18	16.31	15-18
Pelvic fin spines	1.00	1- 1	1.00	1- 1
Pelvic fin rays	5.00	5- 5	5.00	5- 5
Pored scales on lateral line	83.08	76-92	72.85	66-82
Scales above lateral line	14.85	13-17	15.82	13-17
Scales below lateral line	19.37	17-22	19.18	16-22
Gill rakers (upper limb)	9.66	8-11	6.38	5- 8
Gill rakers (lower limb)	17.52	16-20	14.70	13-17
Gill rakers (total)	27.18	24-30	21.07	19-24
Vertebrae	35.89	35-37	34.95	34-36

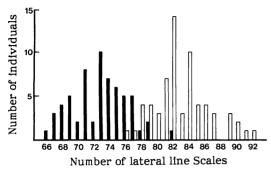


Fig. 6. Frequency distribution of lateral lines scales (LLS). *Open bars*—Japanese form; *dark bars*—Chinese form.

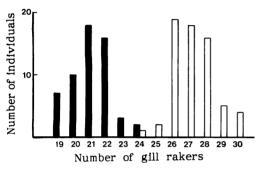


Fig. 7. Frequency distribution of total gill rakers (GR). Open bars—Japanese form; dark bars—Chinese form.

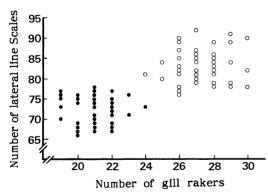


Fig. 8. Relationship between gill rakers (GR) and lateral line scales (LLS). *Open circles*—Japanese form; *dark circles*—Chinese form.

Chinese form, few individuals lacking dots.

The squamation on the dorsal surface of the head differed in the two forms (Fig. 10). In the Japanese form, two scale rows extended from the interorbital region, reaching anteriorly beyond the nostrils (Fig.

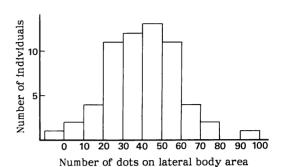


Fig. 9. Frequency distribution of dot numbers on lateral body area in Chinese form.

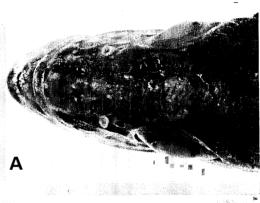




Fig. 10. Dorsal aspects of head. A) Japanese form (TKPM-P352, 149.8 mm TL); B) Chinese form (KPFES 90004, 112.5 mm TL).

10A). In the Chinese form, however, the scale rows were restricted to the interorbital region, not extending beyond the nostrils (Fig. 10B). This difference in squamation provides a means of identifying the two forms in the fingerling and juvenile stages.

Key characters for identification of *Lateolabrax* japonicus and *L. latus* (Katayama, 1957, 1960a, 1960b) were also examined in the two forms. Dorsal fin rays numbered 12–14 in both (Table 3), with scale counts

below the lateral line in each almost exactly corresponding with those of *L. japonicus* given by Katayama (1957, 1960a, 1960b). No individuals of either form had scales on the ventral surface of the lower jaw.

**Table 4.** Allelic frequencies in Japanese and Chinese forms of *Lateolabrax* 

•	A 11 1	Frequency		
Locus	Allele	Japanese	Chinese	
sAAT-1* (L)	*100	0.792	0.981	
	*85	0.208	0.019	
sAAT-2* (L)	*-100	1.000	1.000	
ADH* (L)	*-50	0.011	0.000	
	*-100	0.659	1.000	
	*-150	0.330	0.000	
GAPDH-1* (L)	*100	1.000	1.000	
<i>GAPDH-2</i> * (M)	*100	0.986	1.000	
	*-200	0.014	0.000	
<i>GPI-1</i> * (M)	*130	0.000	0.060	
	*110	0.100	0.922	
	*100	0.900	0.018	
<i>GPI-2*</i> (M)	*-100	0.937	0.940	
	*-250	0.063	0.060	
sIDDH* (L)	*165	0.037	0.200	
	*100	0.963	0.800	
IDHP* (L)	*100	0.912	0.588	
	*70	0.088	0.412	
<i>LDH</i> * (M)	*100	0.097	0.966	
	*-100	0.903	0.034	
<i>sMDH-1</i> * (L)	*100	0.986	1.000	
	* <i>70</i>	0.014	0.000	
<i>sMDH-2</i> * (L)	*-100	1.000	1.000	
sMEP*(M)	*150	0.079	0.050	
	*100	0.886	0.900	
	*50	0.035	0.050	
<i>MPI-1</i> * (L)	*125	0.306	0.363	
	*100	0.694	0.638	
<i>MPI-2</i> * (L)	*100	1.000	0.987	
	*75	0.000	0.013	
PGDH* (L)	*120	0.000	0.011	
	*100	1.000	0.989	
<i>PGM</i> * (M)	*115	0.021	0.060	
	*100	0.465	0.500	
	*75	0.507	0.431	
	*55	0.007	0.009	
<i>PROT-1</i> * (M)	*170	0.000	1.000	
	*100	1.000	0.000	
<i>PROT-2</i> * (M)	*100	1.000	1.000	
sSOD* (L)	*100	1.000	0.964	
	*20	0.000	0.036	

Letters in parenthese indicate tissue sample used. L: liver; M: white skeletal muscle.

Genetic characters.—Allelic frequencies of 20 loci detected by electrophoresis with  $\chi^2$  heterogeneities between both forms are shown in Table 4, with some electrophoregrams of significant isozymes illustrated in Figure 11.

Initially, the fitness of the allelic frequencies in polymorphic loci, according to Hardy-Weinberg equilibrium, was examined by chi-square test. Because no  $\chi^2$  values were significant at the 5% level, it was considered that the two sea bass forms originated from simple Mendelian populations.

Regarding to the heterogeneities between the groups, chi-square tests indicated significant differences in many of the loci examined (Table 4). Some of these loci and alleles are described as follows.

Alcohol dehydrogenase (ADH): The  $ADH^*$  locus of the Japanese form had a major allele of \*-100, and two minor ones of \*-50 and \*-150, whereas the locus in the Chinese form was monomorphic, being entirely occupied by the \*-100 allele (Fig. 11; Table 4).

Glucose-6-phosphate isomerase (GPI): At the GPI-1\* locus of the Japanese form, a major allele (\*100) with a frequency of 0.900, and a minor one (\*110) were found, whereas the Chinese form had the \*110 allele as the major allele, with a frequency of 0.922, and minor ones of \*100 and \*130 (Fig. 11; Table 4).

Lactate dehydrogenase (LDH): At the  $LDH^*$  locus of the Japanese form, the major allele of \*-100 had a frequency of 0.903, with a minor allele of \*100. In contrast, in the Chinese form, the major and minor alleles were essentially reversed, with the major allele being \*100 (frequency 0.966) and the minor one \*-100 (Fig. 11; Table 4).

General protein (PROT): The PROT-1\* locus of the Japanese form was occupied by an allele of \*100. That of the Chinese form was occupied by an allele of \*170, the alleles having completely replaced each other (Fig. 11; Table 4).

Of the other isozymes, significant differences in allelic frequencies between the two forms were found in sAAT-1\*, IDDH\*, IDHP\* and sSOD\* (Table 4).

The genetic distance (D value) between the two forms, calculated from isozymic allele frequencies after Nei (1972) was 0.174, far above the D values of some Japanese sea bass populations examined by Tsuda (1989), and significant at the inter-specific level (Nei, 1990).

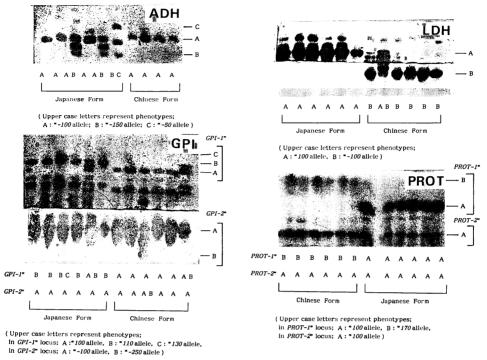


Fig. 11. Selected electrophoregrams of some loci in Japanese and Chinese forms.

## Discussion

The morphological and genetic differences indicated that the Chinese form is a distinct species from Lateolabrax japonicus. This is also supported by the absence of genetic exchange between the groups, there being complete allele replacement at the PROT-1\* locus. The Chinese form also differed from L. latus in the number of dorsal fin rays and scales below the lateral line, and scalation on the ventral side of the lower jaw.

For this reason, the Chinese form is here referred to as Lateolabrax sp. until formally described. Lateolabrax japonicus is supposedly widely distributed in coastal waters of the Japanese Archipelago, Korea, China and Taiwan (Katayama, 1965; Miyazi et al., 1976). This study showed that a distinct species, formerly confused with L. japonicus, inhabits the Chinese coast.

Although Katayama (1960a, 1960b) pointed out that sea bass from Chinese waters typically possessed many black dots along the lateral body surface, he regarded it as an intraspecific variation of *L. japonicus*. Consequently, his meristic and morphometric characters for *L. japonicus* included the two species.

Thereafter, Yamada (1986) also included both species in his description of *L. japonicus*.

According to some descriptions of so-called *L. japonicus* in China, illustrations and meristics for LLS and GR, all point to *Lateolabrax* sp. (Zhu et al., 1963; Cheng and Zheng, 1987; Zheng, 1989; Liu and Oin, 1987; Pan et al., 1991).

There is also some ecological differentiation between the two species. The general spawning season of *L. japonicus* in Japan is from December to March (Ochiai and Tanaka, 1986), whereas in the Bohai Sea, where the species is believed to be *Lateolabrax* sp., spawning is reported to occur from August to November (Liu and Qin, 1987; Deng et al., 1988; Jiang et al., 1988; Wan and Chen, 1988).

A difference in preference for fresh water is also apparent. Although *L. japonicus* is a marine species, it occasionally occurs in brackish or low salinity water in rivers (Miyazi et al., 1976). The Chinese species has a rather stronger tolerance of fresh water than *L. japonicus*, being able to survive in completely fresh water (Zhu et al., 1963; Liu and Qin, 1987). In a river in southern China, it has been reported from areas more than 300 km from the mouth (Zheng, 1989).

In Taiwan, native sea bass, which are here thought to be conspecific with *Lateolabrax* sp. owing to their appearance, are cultivated in fresh-water ponds by acclimatization. They have been reported to grow as well in fresh-water conditions as in sea water, reaching maturity and spawning (Pern et al., 1980, 1981; Tang et al., 1980; Pern and Liu, 1982, 1983; Tang, 1985; Huang et al. 1987; Huang and Tang, 1988, 1990). *Lateolabrax japonicus*, however, although euryhaline, has never been known to grow or spawn in fresh water.

Finally, a difference in growth rate of the two species has been noted. According to Ochiai and Tanaka (1986), who summarized growth data of *L. japonicus* from various localities in Japan, growth rates generally do not vary locally, lengths being around 20 cm after the first year, 30 cm after 2 years and 40 cm after 3 years. *Lateolabrax* sp. has been reported to reach around 30 cm after the first year, 40 cm after 2 years and 50 cm after 3 years (Wu et al., 1979; Liu and Qin, 1987). In artificial ponds in Taiwan, the difference is even greater, *Lateolabrax* sp. reaching around 20 cm in the first 6 months (Huang and Tang, 1990) and almost 40 cm after one year (Pern and Liu, 1982).

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## 日本産スズキと中国産"スズキ"の形態的および遺伝的 差異

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最近,養殖用種苗としてさかんに日本に移入されている中国産 "スズキ"について,日本産のものとの形態的および遺伝的差異について調べた。外部形態については,いくつかの形質において両者に大きな差異が認められ,計数形質の相違が顕著であった.特に,側線有孔鱗数と鰓耙数は明瞭に相違し,これら 2 形質の組み合わせによって両者を完全に識別することが可能であった.遺伝形質としてアイソザイムを用い,20 遺伝子座を推度したが,このうち PROT-I\* 遺伝子座では遺伝子の完全置換のGPI-I\* と LDH\* 遺伝子座では遺伝子頻度の著しい相違が認められた.アイソザイム遺伝子による両集団間の遺伝的距離(D値)は 0.174 となり,種間の水準に達していた.これらの形態的および遺伝的相違により,中国産のものはスズキ  $Lateolabrax\ japonicus\ とは別種である可能性が示唆された.$ 

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