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Morphological and Genetic Variations among Local Individuals of Genko Sole, *Cynoglossus interruptus* (Pisces, Pleuronectiformes, Cynoglossidae)

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Abstract. Morphological and genetic variations among four local samples (from the Seto Inland Sea, Tokyo Bay, Tosa Bay and Kagoshima Bay) of genko sole, *Cynoglossus interruptus*, were examined. Morphologically, significant differences were detected among the samples in many characters. Also, differences were recognized among the samples in proportions of individuals which lack the cephalodorsal line, and the coloration of the ocular and blind sides. Results of the Principal component analysis, which compares the morphological characters analytically, showed that the examined individuals are generally separated into two groups, *viz*. a group comprised those from the Seto and Tosa samples and another one comprised those from the Tokyo and Kagoshima samples. Isozymes detected by electrophoresis were used as the genetic markers, and unique allelic compositions by sample were observed at the *ACP**, *CK**, *FBALD-2**, *IDDH**, *MEP-1** and *SOD-1** loci, indicating independency of the samples. The genetic distances (*D* values) between the samples, calculated from isozymic allele frequencies, were 0.0073–0.0081 (average 0.0077), may being figures greater than the inter-populational level. The results given by the present study showed that the examined four *Cynoglossus interruptus* samples considerably diverged from one another, inferring presence of some regional populations in *C. interruptus*, which have morphologically and genetically diverged from one another.

Key words: *Cynoglossus interruptus*, morphology, genetics, isozyme, local variation.

Former report (Yokogawa *et al*., 2008*b*) examined genetic differences between two forms (dilinear and trilinear) of a tongue sole, "*Cynoglossus interruptus*" (Pisces: Pleuronectiformes: Cynoglossidae), revealed considerable differences between them, and strongly inferred that they represent distinct species from each other. Consequently, the trilinear form was described as a new species, *Cynoglossus ochiaii* (Yokogawa *et al*., 2008*a*).

Although "*Cynoglossus interruptus*" has been supposed to be widely distributed in shallow waters around Japan, Yokogawa *et al*. (2008*a*) revealed that true *C. interruptus* inhabits coastal shallower waters (*ca* 10–50 m) and *C. ochiaii* inhabits deeper waters (*ca* 50–150 m). For *C. interruptus*, its local variations might include differences at subspecific and specific levels like *C. ochiaii*. Because such variations have not been revealed yet, examinations would

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necessitate. Therefore, some local samples of *C. interruptus* from Japan were obtained and morphological and genetic variations among them were examined.

Materials and Methods

Data from specimens used for the present study are shown in Table 1. Specimens were collected from four localities around Japan, *viz*. Tokyo Bay, Seto Inland Sea, Tosa Bay and Kagoshima Bay (Fig. 1). All the specimens were collected with bottom trawl. Most of the specimens have been deposited in Laboratory of Marine Biology, Faculty of Science, Kochi University (BSKU) and the Kagoshima University Museum (KAUM) (Table 1). Methodologies of the morphological measurements including terminology of lateral line systems and definition of scale counts totally followed Yokogawa *et al*. (2008*a*), and that of the genetic examinations by using isozymes totally followed Yokogawa *et al*. (2008*b*). For the specimens from Kagoshima, the isozyme analysis could not be performed because all of them had been directly fixed (Table 1).

The present study introduced the principal component analysis by the usual method (Arima and Ishimura, 1997) to compare the morphology of the specimens analytically. For the analysis, examined all items of length-measured and meristic characters were used as variates; however, number of pelvic fin rays was just omitted from the variates, because its variance resulted in zero owing to no variation in all samples. When a value of a character in a specimen were unavailable owing to damage, an average value of the character for all the specimens substituted for the value.

Table 1. Collection and handling data for specimens examined.

Sample name	Date of collection	Locality	*n*	Preservation until electrophoresis	Catalogue No.	Remarks[1]
Tokyo	Aug. 29, 1997	Off Yokohama City, Tokyo Bay	5	Frozen at −20°C	BSKU 89574–89578	M,
	Sep. 11, 1997	Off Yokohama City, Tokyo Bay	12	Frozen at −20°C	BSKU 89579–89590	M, G
Seto	Oct. 3, 1997	Off Marugame City, Seto Inland Sea	42	Frozen at −80°C	Uncatalogued	M, G
	Nov. 27, 1997	Off Marugame City, Seto Inland Sea	6	Directly fixed	BSKU 89744 (single lot)	M
Tosa	Mar. 20, 1997	Off Irino, Tosa Bay	1	Directly fixed	Uncatalogued	M
	June 22, 1997	Off Kamikawaguchi, Tosa Bay	2	Frozen at −20°C	BSKU 89877, 89883	M, G
Kagoshima	Sep. 18, 1997	Off Kochi City, Tosa Bay	7	Frozen at −20°C	BSKU 89878–89882, 89884, 89885	M, G
	Oct. 23, 1997	Off Kochi City, Tosa Bay	25	Frozen at −20°C	BSKU 89886–89910	M, G
	Mar. 15, 2006	Off Tarumizu City, Kagoshima Bay	1	Directly fixed	KAUM–I. 466	M
	Sep. 11, 2006	Off Tarumizu City, Kagoshima Bay	1	Directly fixed	KAUM–I. 532	M
	Sep. 11, 2006	Off Kagoshima City, Kagoshima Bay	6	Directly fixed	KAUM–I. 620–625	M
	July 7, 2007	Off Kagoshima City, Kagoshima Bay	2	Directly fixed	KAUM–I. 5168, 6033	M

[1]M: Specimens used for morphological analysis, G: Specimens used for genetic analysis.

Results

Morphological characters The four samples differed from one another as follows: Tokyo sample—greater values for body width (BWT), midlateral line (MLL) scale number and abdominal vertebra (AV) number, and smaller values for distance from tip of fleshy snout to angle of mouth (DSM) and CDL scale number; Seto sample—greater values for body depth (BD), snout length (SNL), DSM and CDL scale number, and smaller values for head length (HL) and AV number; Tosa sample—greater values for dorsal fin length (DFL), anal fin length (AFL), pelvic fin length (PFL) and HL, and smaller

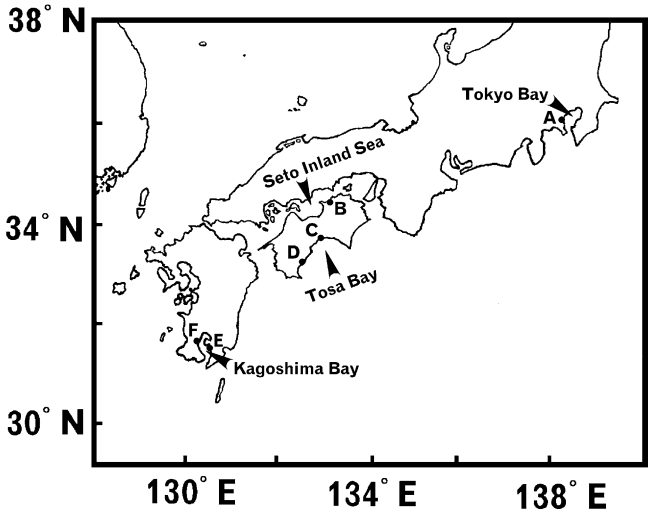

Fig. 1. Collection localities of the specimens examined. Dark circles indicate fishing bases; A: Yokohama City; B: Marugame City; C: Kochi City; D: Tosa-saga and Irino (localities are close to each other); E: Tarumizu City; F: Kagoshima City.

values for BD, BWT and MLL scale number; Kagoshima sample—greater values for BD, DFL, AFL, CDL and MLL scale numbers, and smaller value for SNL (Fig. 2; Table 2).

In particular, the cephalodorsal line (CDL) scale number may be significant. The character being highly variable in all, the Tokyo sample included many individuals which lacked the cephalodorsal line (CDL scale number is zero). Such individuals comprised 40.0% of the Tokyo sample, 13.9% (Seto), 14.3% (Tosa) and 0% (Kagoshima).

The body size of the four samples also apparent to be different (Table 2). Although individuals in each sample were presumably variously aged, the Tosa sample comprised particularly smaller individuals; while the Tokyo and Kagoshima samples comprised larger individuals (Table 2). This may reflect their biological feature as populations.

The samples also differed in body coloration. The differences in three samples, which could be photographed in fresh condition, were noted as follows: Ocular side—Seto sample light brown with dark dapples (Fig. 3-A); Tokyo sample generally somewhat darkish with darker dapples (Fig. 3-C); Tosa sample light brown almost without dark dapples in most of the individuals (Fig. 3-G), with a few individuals having similar coloration to Seto and Tokyo samples (Fig. 3-E). Blind side—Seto sample typically bright reddish-orange on basal parts of dorsal and anal fins (Fig. 3-B); Tokyo and Tosa samples with less prominent orange coloration (Figs 3-D, F), in particular, blind side of Tosa sample almost uniformly pale (Fig. 3-F).

On the other hand, ocular side of the Kagoshima sample in preserved condition is more darkish than the other three samples, without dark dapples (Fig. 3-H). The coloration of the Kagoshima sample is similar to that of *Cynoglossus ochiaii* (Yokogawa *et al*., 2008*a*).

Figure 4 illustrates results of principal component analysis indicated with plots of individual principal component scores. Regarding these, the examined individuals are generally separated into two groups by the principal component-1, that is, a group comprised individuals from the Seto and Tosa samples, and another one comprised those from the Tokyo and Kagoshima samples (Fig. 4). These matters infer morphological difference between the two groups and similarity between the each two samples of each group.

Table 2. Morphometric measurements and counts for four samples of *Cynoglossus interruptus*.

Sample	Tokyo		Seto		Tosa		Kagoshima	
	Average	Range	Average	Range	Average	Range	Average	Range
Body size (SL in mm)	125.8	99.4 – 141.7	106.4	68.3 – 153.4	94.3	63.1 – 118.6	122.0	95.0 – 138.0
Proportion								
Total length[1]	106.11	103.72 – 108.17	106.86	104.88 – 109.47	106.98	104.62 – 109.39	107.18	106.02 – 108.42
Body depth[1]	27.59	24.71 – 30.23	27.95	24.30 – 31.56	26.60	23.74 – 29.01	28.16	26.59 – 30.33
Body width[1]	6.29	4.77 – 9.76	6.02	3.37 – 7.76	5.64	4.83 – 6.42	6.16	5.05 – 7.00
Dorsal fin length[1]	7.02	5.75 – 8.45	7.45	5.98 – 9.57	8.10	6.72 – 10.73	8.31	7.44 – 9.57
Anal fin length[1]	7.12	5.89 – 8.41	7.57	5.85 – 9.59	8.36	6.93 – 11.35	8.45	7.44 – 9.57
Pelvic fin length[1]	4.90	3.59 – 7.51	5.60	3.35 – 7.44	6.36	4.86 – 7.70	5.82	4.86 – 6.72
Head length[1]	19.55	17.32 – 21.52	19.30	16.09 – 22.06	20.46	18.95 – 22.35	20.02	18.57 – 20.82
Snout length[2]	30.91	24.69 – 35.56	32.58	26.15 – 40.00	30.63	23.19 – 36.84	30.16	27.87 – 32.10
Upper eye diameter[2]	14.91	11.65 – 18.18	15.63	11.56 – 20.32	15.93	12.86 – 20.49	14.49	13.54 – 16.07
Lower eye diameter[2]	15.12	12.40 – 17.75	15.73	11.90 – 22.54	16.36	13.01 – 20.37	14.93	13.47 – 16.80
Distance from tip of fleshy snout to angle of mouth[2, 3]	39.60	30.00 – 48.06	46.01	34.24 – 59.53	43.38	34.97 – 50.00	44.04	41.58 – 48.56
Distance from tip of lower jaw to angle of mouth[2, 3]	20.85	15.56 – 25.11	22.01	17.11 – 31.15	22.26	18.58 – 26.84	20.81	18.85 – 21.83
Meristic counts								
Dorsal fin rays	108.53	103 – 114	108.92	103 – 114	108.91	105 – 112	109.20	108 – 111
Caudal fin rays	9.93	9 – 10	9.85	8 – 11	9.86	9 – 11	9.90	9 – 10
Anal fin rays	85.59	80 – 91	85.00	77 – 91	84.36	80 – 88	86.10	84 – 88
Pelvic fin rays	4.00	4 – 4	4.00	4 – 4	4.00	4 – 4	4.00	4 – 4
Pored scales on								
cephalodorsal line (CDL)[3]	2.70	0 – 7	4.17	0 – 9	3.07	0 – 5	6.33	5 – 7
mandibulo-opercular line (MOL)[3]	12.44	11 – 15	13.29	10 – 18	13.13	11 – 15		
supraorbital commissure (SOC)[3]	10.53	9 – 12	10.83	9 – 12	11.14	10 – 12	8.33	7 – 10
preopercular line (POL)[3]	5.90	5 – 7	5.29	4 – 7	5.14	4 – 8	5.00	5 – 5
dorsolateral line (DLL)[3]	29.00	18 – 37	31.08	24 – 43	29.38	23 – 35	26.57	19 – 33
midlateral line (MLL)[3]	74.27	68 – 81	71.26	60 – 77	70.12	66 – 77	72.89	69 – 78
Scales above DLL	2.14	2 – 3	2.14	2 – 3	2.04	2 – 3	2.00	2 – 2
Scales between DLL and MLL	11.29	10 – 12	11.36	10 – 13	11.17	10 – 13	12.00	12 – 12
Vertebrae								
abdominal	9.18	8 – 10	8.51	7 – 10	8.59	8 – 9	9.00	9 – 9
caudal	43.41	40 – 45	43.81	39 – 46	43.88	42 – 45	44.00	43 – 45
total	52.59	49 – 54	52.30	48 – 55	52.47	51 – 54	53.00	52 – 54

[1]Percentage of standard length (SL).
[2]Percentage of head length (HL).
[3]Definition followed Yokogawa *et al*. (2008*a*).

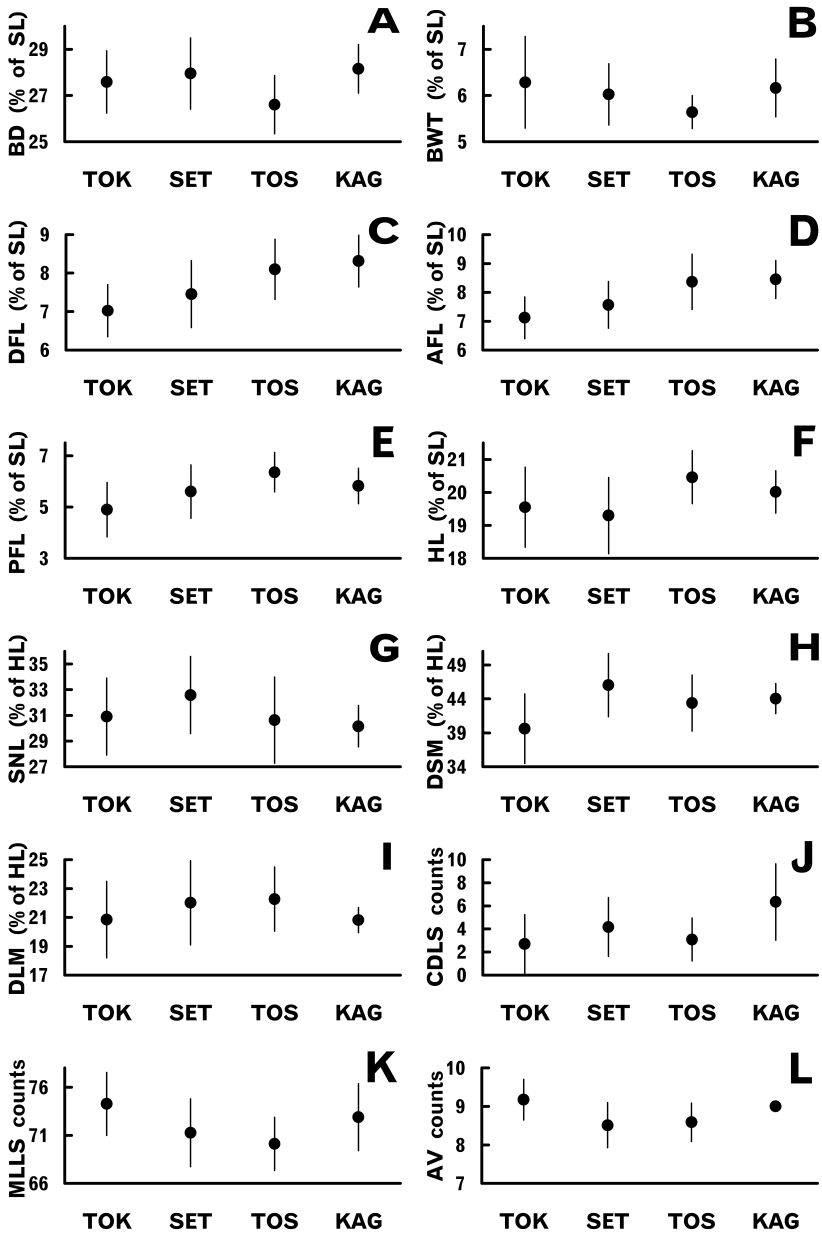

Fig. 2. Graphic comparisons of significant morphological characters in four samples of *Cynoglossus interruptus*. Dark circles indicate average values of each sample and longitudinal bars indicate standard deviations (unbiased value). Sample names are abbreviated as TOK (Tokyo), SET (Seto), TOS (Tosa) and KAG (Kagoshima). A: Body depth (BD); B: Body width (BWT); C: Dorsal fin length (DFL); D: Anal fin length (AFL); E: Pelvic fin length (PFL); F: head length (HL); G: Snout length (SNL); H: Distance from tip of fleshy snout to angle of mouth (DSM); I: Distance from tip of lower jaw to angle of mouth (DLM); J: Pored scales on cephalodorsal line (CDLS); K: Pored scales on midlateral line (MLLS); L: Abdominal vertebrae (AV).

Genetic characters Because the isozyme analysis was unavailable for the Kagoshima sample, genetic examinations were performed for three samples of Tokyo, Seto and Tosa. Allelic frequencies of the 33 loci detected by electrophoresis, together with values to indicate genetic features in the three samples are shown in Table 3. The fitness of the allelic frequencies in the polymorphic loci, according to the Hardy-Weinberg equilibrium, was examined by chi-square test. Because no χ^2 values were significant at the 5% level, all of the samples were considered to have originated from simple Mendelian populations.

Although the values to indicate genetic features were similar to one another in the three samples, some differences were found in the allelic frequencies among the three samples at some loci such as *ACP**, *CK**, *FBALD-2**, *IDDH**, *MEP-1** and *SOD-1** (Table 3). Table 4 briefly summarizes the results of chi-square heterogeneities for the allelic frequencies between the samples. Significant differences between the samples were recognized at some of the loci such as those noted above, indicating independency of the three samples.

The genetic distances (*D* values) between the three samples with *Cynoglossus ochiaii* as an out group (data from Yokogawa *et al*., 2008*b*), calculated from isozymic allele frequencies after Nei (1972), have been shown in Table 4. Subsequently, a dendrogram based on the genetic distances was created by the UPGMA method (Fig. 5).

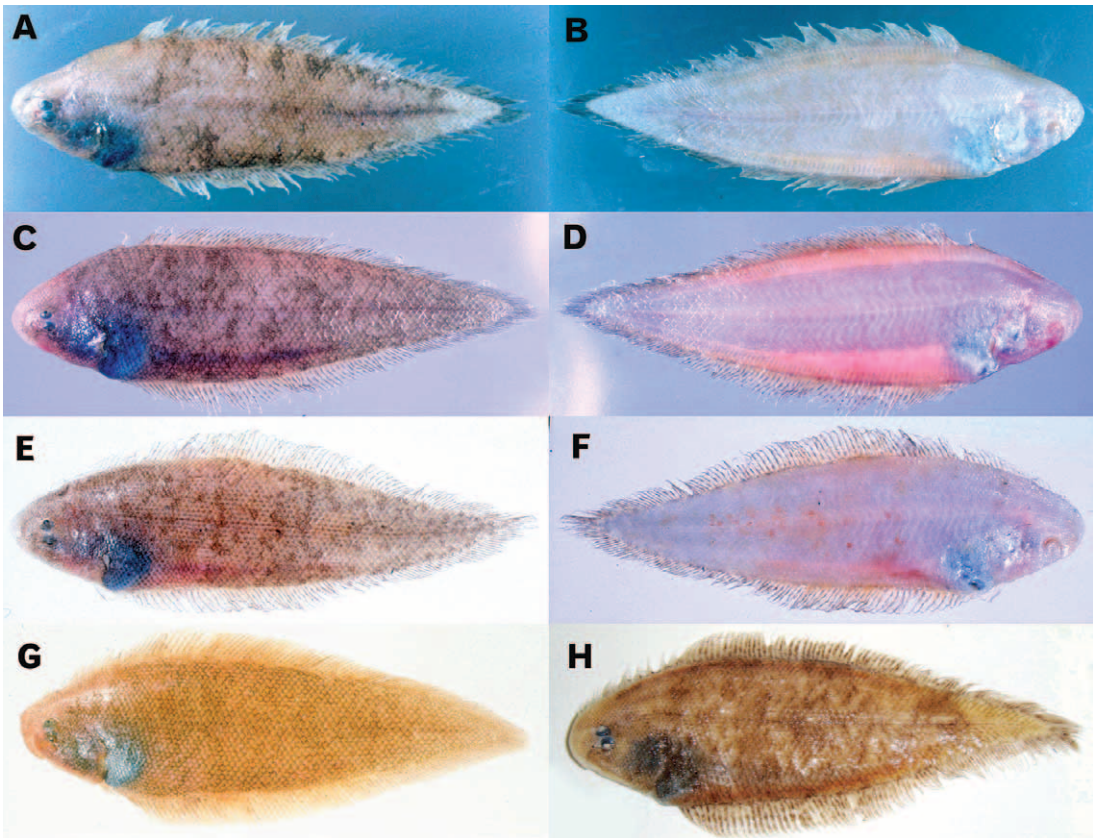

Fig. 3. General aspects of *Cynoglossus interruptus* from four localities. A, B: Tokyo Bay (A: Ocular side; B: Blind side); C, D: Seto Inland Sea (C: Ocular side; D: Blind side); E, F, G: Tosa Bay (E, G: Ocular side; F: Blind side); H: Kagoshima Bay (Ocular side). A–G are in fresh condition and H is in preserved condition.

Table 3. Allelic frequencies with values to indicate genetic features of three samples of *Cynoglossus interruptus*.

Locus	Allele	Tokyo	Seto	Tosa
*AAT-3**	**–20*	0.147	0.150	0.155
	**–100*	0.471	0.350	0.397
	**–120*	0.176	0.288	0.276
	**–150*	0.176	0.150	0.086
	**–200*	0.029	0.063	0.086
*ACP**	**145*	0.029	0.013	0.031
	**120*	0.029	0.163	0.125
	**100*	0.676	0.538	0.656
	**80*	0.206	0.238	0.094
	**70*	0.059	0.038	0.094
	**50*	0	0.013	0
*AH-1**	**110*	0	0.053	0.063
	**100*	0.967	0.908	0.906
	**90*	0.033	0.039	0.031
*AH-2**	**100*	0.429	0.586	0.414
	**75*	0.536	0.414	0.569
	**65*	0.036	0	0.017
*CAT-1**	**100*	1.000	1.000	1.000
*CAT-2**	**–100*	1.000	1.000	1.000
*CK**	**–70*	0.029	0.085	0.045
	**–100*	0.735	0.732	0.758
	**–155*	0.176	0.171	0.076
	**–165*	0.059	0.012	0.121
*EST-3**	**105*	0	0	0.015
	**100*	0.853	0.829	0.833
	**90*	0.118	0.110	0.045
	**80*	0.029	0.061	0.106
*FBALD-1**	**–100*	1.000	1.000	1.000
*FBALD-2**	**–100*	0.824	0.702	0.591
	**–140*	0.176	0.298	0.409
*FH-2**	**250*	0.059	0	0
	**100*	0.941	1.000	1.000
*G3PDH-1**	**100*	1.000	1.000	1.000
*G3PDH-2**	**100*	1.000	0.988	0.970
	**40*	0	0.012	0.030
*G3PDH-3**	**125*	0	0.012	0
	**–100*	0.912	0.929	0.955
	**–160*	0	0	0.030
	**–220*	0.088	0.060	0.015
*GPI-1**	**100*	0.500	0.583	0.500
	**90*	0	0	0.015
	**80*	0.382	0.310	0.394
	**75*	0.118	0.060	0.076
	**65*	0	0.048	0
	**50*	0	0	0.015
*GPI-2**	**–60*	0	0.036	0.015
	**–100*	1.000	0.940	0.924
	**–160*	0	0.024	0.061
*IDDH**	**215*	0.094	0.056	0.019
	**160*	0.156	0.208	0.058
	**100*	0.750	0.736	0.923
*IDHP-1**	**140*	0	0.012	0
	**135*	0.206	0.214	0.152
	**100*	0.706	0.714	0.788
	**90*	0.088	0.060	0.045
	**65*	0	0	0.015
*IDHP-2**	**100*	1.000	1.000	1.000
*LDH-1**	**–100*	1.000	1.000	1.000
*LDH-2**	**–100*	1.000	1.000	1.000
*MDH-1**	**120*	0.029	0.012	0.045
	**100*	0.971	0.963	0.909
	**70*	0	0	0.015
	**60*	0	0.024	0.030
*MDH-2**	**130*	0.029	0.024	0
	**100*	0.971	0.878	0.939
	**80*	0	0.098	0.061
*MDH-3**	**–70*	0.059	0.012	0
	**–100*	0.941	0.988	1.000
*MEP-1**	**120*	0	0.012	0
	**110*	0.088	0.179	0.061
	**100*	0.676	0.738	0.682
	**80*	0.088	0.060	0.242
	**65*	0.147	0.012	0.015
*MPI**	**100*	1.000	1.000	1.000
*ODH**	**130*	0.125	0.090	0.103
	**100*	0.875	0.882	0.897
	**55*	0	0.010	0
*PGDH**	**160*	0	0.012	0
	**150*	0.176	0.357	0.303
	**100*	0.794	0.607	0.667
	**80*	0.029	0.024	0.030
*PROT-1**	**100*	1.000	1.000	1.000
*PROT-2**	**100*	1.000	1.000	1.000
*PROT-3**	**100*	0.941	0.929	0.864
	**85*	0.059	0.071	0.136
*SOD-1**	**115*	0.118	0.303	0.045
	**100*	0.853	0.645	0.788
	**80*	0.029	0.053	0.167
*SOD-2**	**100*	1.000	1.000	1.000
Alleles/Locus		2.121	2.424	2.394
Percentage of polymorphic loci	P*	0.515	0.545	0.545
	P	0.091	0.091	0.061
	P+P*	0.606	0.636	0.606
Average Heterozygosity	Ho	0.199	0.207	0.190
	He	0.186	0.208	0.197
	Ho/He	1.070	0.996	0.965

P* : Polymorphism less than 0.95.
P : Polymorphism greater than 0.95.
Ho : Observed heterozygosity.
He : Expected heterozygosity.

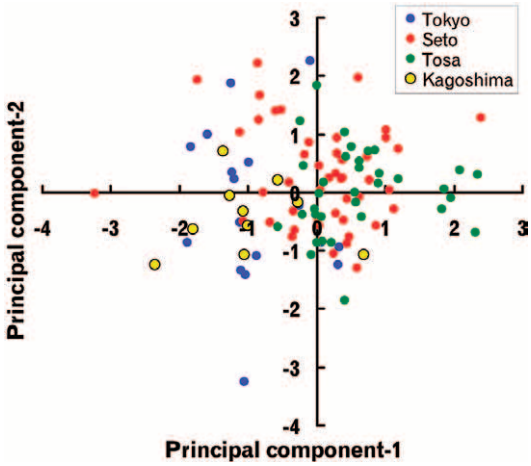

Fig. 4. A distribution graph plotted with principal component scores 1 and 2 for morphological data of four samples of *Cynoglossus interruptus*.

The *D* values among the three *Cynoglossus interruptus* samples resulted in 0.0073–0.0081 (Table 4). They might be regarded to be greater than the interpopulational level (Nei, 1990), indicating some considerable genetic divergence. And the degree of divergence between the samples were regarded to be similar to one another (Table 4), the three samples formed a single cluster in the dendrogram (Fig. 5). While, the *D* values between *C. ochiaii* and the three *C. interruptus* samples resulted in 0.4207–0.4353 (Table 4), it was confirmed that *C. ochiaii* genetically so diverges from any local samples of *C. interruptus*.

Discussion

The results given by the present study revealed that the morphological and genetic characteristics of the four local *Cynoglossus interruptus* samples differed considerably from one another, implying biological divergence of the four samples. This suggests presence of some independent populations of *C. interruptus* around Japan. Because *C. interruptus* is a benthonic species and may have less mobility, a populational isolation may potentially occur, subsequently it can cause such divergence.

The analytical morphological examination of the principal component analysis (PCA) suggested that the four samples are separated into two groups, *viz*. a group comprised individuals from the Seto and Tosa samples and another one comprised those from the Tokyo and Kagoshima samples (Fig. 4). Although morphological similarity between the Seto and Tosa samples may be related to geographic closeness (Fig. 1), that between the Tokyo and Kagoshima samples may be difficult to explain. For the latter case, it may be likely that some environmental factors have affected formation of morphology of this species. Otherwise, the Tokyo and Kagoshima samples are in fact similar to each other biologically. In order to reveal this matter, genetic examinations for the Kagoshima sample necessitate. However, such morphological similarity has been given analytically; actually there are many differences in morphological characters by sample, even between the "similar" samples by PCA (Figs 2, 3; Table 2).

Concerning the populational genetic structure of heterosomate fishes, some works have been per-

Table 4. Nei's genetic distance (*D* value) between three samples of *Cynoglossus interruptus* (Tokyo, Seto and Tosa) with *C. ochiaii*, with numbers of loci in which significant differences in allelic frequencies between samples were recognized by chi-square test (above oblique line).

	Tokyo	Seto	Tosa	*C. ochiaii*
Tokyo		2	4	20
Seto	0.0073		4	22
Tosa	0.0077	0.0081		21
C. ochiaii	0.4353	0.4273	0.4207	

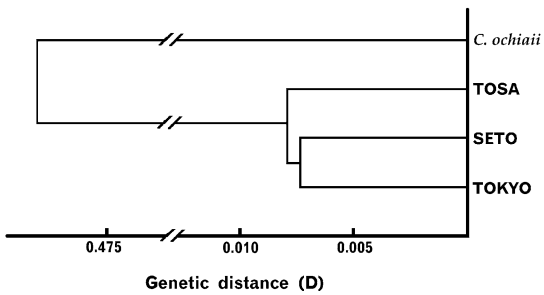

Fig. 5. A dendrogram based on genetic distances (*D* values) between three samples of *Cynoglossus interruptus* with *C. ochiaii* from Tosa Bay as an out group.

formed, such as for olive flounder, *Paralichthys olivaceus* (Fujio *et al*., 1989) and littlemouth flounder, *Pleuronectes herzensteini* (Kijima & Park, 1989), by using the isozymes as the genetic markers. In the case of *Pa. olivaceus*, four local samples around Japan (including the samples from the Japan Sea and Pacific) were examined and 23 loci were detected. The genetic distances between the samples resulted in 0.0002–0.0013 (average 0.0006), showing very little genetic divergence among the local samples (Fujio *et al*., 1989). For *Pl. herzensteini*, samples from the Japan Sea, Pacific and Yellow Sea were examined and 14 loci were detected. The genetic distances between the samples resulted in 0.0003–0.0006 (average 0.0004), showing the similar tendency to *Pa. olivaceus* (Kijima & Park, 1989). In the case of *Cynoglossus interruptus*, the genetic distances between the samples resulted in 0.0073–0.0081 (average 0.0077) (Table 4), being much higher than the divergence degrees of those species. This would suggest presence of a mechanism of the populational isolation in *C. interruptus*.

In general, cynoglossid fishes originated in the tropical waters of the Indo-Pacific (Ochiai, 1957*c*, 1963; Menon, 1977), *C. interruptus* (might includes *C. ochiaii*, which have been mis-identified as *C. interruptus*) being classified as a subtropical "form" (Ochiai, 1957*a*, 1966). Ochiai (1957*b*, 1966) considered this "form" to have reached Japanese waters from the south via the continental shelf. *C. interruptus*, which adopted a shallow waters environment expanded its distribution throughout Japanese coastal waters, some morphological and genetic divergence apparent in local populations maybe having been established by regional isolation. This can be the preceding process in the specific divergence mechanism. In order to reveal such an interesting matter, more local samples of *C. interruptus* including those from neighbor nations to Japan should be examined hereafter.

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