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Genetic Relationships of the Genus *Tridentiger* (Pisces, Gobiidae) Based on Allozyme Polymorphism

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ABSTRACT—The genetic relationships and taxonomic status of 7 taxa belonging to the genus *Tridentiger* (Pisces, Gobiidae) were investigated by means of analysis of allozymic variation at 14 loci. The results suggest that the two taxa *"T. obscurus"* and *"T. brevispinis"* which are sympatric and morphologically similar are reproductively isolated and are highly divergent from each other (the genetic distance values are 0.501-0.707). It is also suggested that *"T. brevispinis"* and *"T. kuroiwae"* are genetically different enough from each other to deserve subspecies at least. The other 4 taxa, *"T. barbatus"*, *"T. nudicervicus"*, *"T. trigonocephalus"* and *"T. bifasciatus"*, are genetically divergent each and are considered to be 4 biological species. A dendrogram showing the phylogenetic relationships of the 7 taxa was constructed from the genetic distances.

INTRODUCTION

The gobiid fishes (Pisces, Gobiidae), which are distributed throughout the tropical and temperate waters of the world, have adapted to various environments and have acquired various life histories. Among them, the genus *Tridentiger* Gill is one of the dominant genera inhabiting the brackish-water environment around Japan. Only three species, *T. obscurus* (Temminck et Schlegel), *T. trigonocephalus* (Gill) and *T. nudicervicus* Tomiyama, were generally recognized as valid before 1972 (Matsubara, 1955; Fowler, 1962) when Katsuyama *et al.* (1972) reexamined *T. obscurus* and found that it consisted of two subspecies *T. obscurus obscurus* and *T. obscurus brevispinis* Katsuyama, Arai et Nakamura. They considered *T. kuroiwae* Jordan et Tanaka, which had generally been regarded as a junior synonym of *T. obscurus* (Tomiyama, 1936; Matsubara, 1955), to belong to *T. o. obscurus.* Akihito *et al.* (1984, 1988) in their comprehensive reviews raised the three taxa of *T. obscurus* to three species *T. obscurus, T. brevispinis* and *T. kuroiwae* on the basis of their distributions and color patterns. In addition to these species, *T. barbatus* (Günther) which used to represent the genus *Triaenopogon* Bleeker was included in *Tridentiger* in accordance with their definition of the genus by the morphological character of the outer trilobed teeth in both jaws. *T. trigonocephalus* was further classified into two separate species *T. trigonocephalus* and *T. bifasciatus* Steindachner by Akihito and Sakamoto (1989). Akihito *et al.* (1993a), in conclusion, recognized 7 species, *T. obscurus, T. brevispinis, T. kuroiwae, T. trigonocephalus, T. bifasciatus, T. nudicervicus* and *T. barbatus.* On the other hand, Kawanabe and Mizuno (1989) treated *T. kuroiwae* and *T. brevispinis* as subspecies *"T. kuroiwae kuroiwae"* and *"T. k. brevispinis"* within a single species because of their allopatric distribution (*"T. kuroiwae"* is restricted to the Ryukyu Islands, *"T. obscurus"* and *"T. brevispinis"* around the Japanese Archipelago except Ryukyus) and similar ecological characters.

Some ecological and ethological studies have been done on *"T. obscurus"* and *"T. brevispinis".* The social behavior of these two taxa are similar in an aquarium (Kishi, 1979) though they show clear habitat segregation whenever they occur in the same river system (Itai and Kanagawa, 1989; Tamada, 1993). These facts make us eager to know whether these two taxa are genetically different or not.

Allozyme analyses have been used for the classification of gobiid fishes *Chaenogobius* (Aizawa *et al.,* 1994), *Rhinogobius* (Masuda *et al.*, 1989; Katoh and Nishida, 1994) and *Pomatoschistus* (Wallis and Beardmore, 1984), and have resolved the confused taxonomic status and clarified phylogenetic relationships among morphologically similar species. Allozyme polymorphism detected by electrophoresis also supplies a useful measure of genetic differentiation among populations or species in terms of the genetic distance (Nei, 1987).

In the present study, we applied such technique to the above-mentioned 7 taxa of the genus *Tridentiger,* in an attempt to clarify their taxonomy, especially that of the most confused *"T. obscurus"*, *"T. brevispinis"* and *"T. kuroiwae"*.

MATERIALS AND METHODS

A total of 200 specimens representing 21 populations of 7 taxa of *Tridentiger* which were tentatively identified as species after Akihito *et al.* (1993a) (*T. obscurus, T. brevispinis, T. kuroiwae, T. trigonocephalus, T. bifasciatus, T. nudicervicus* and *T. barbatus*)

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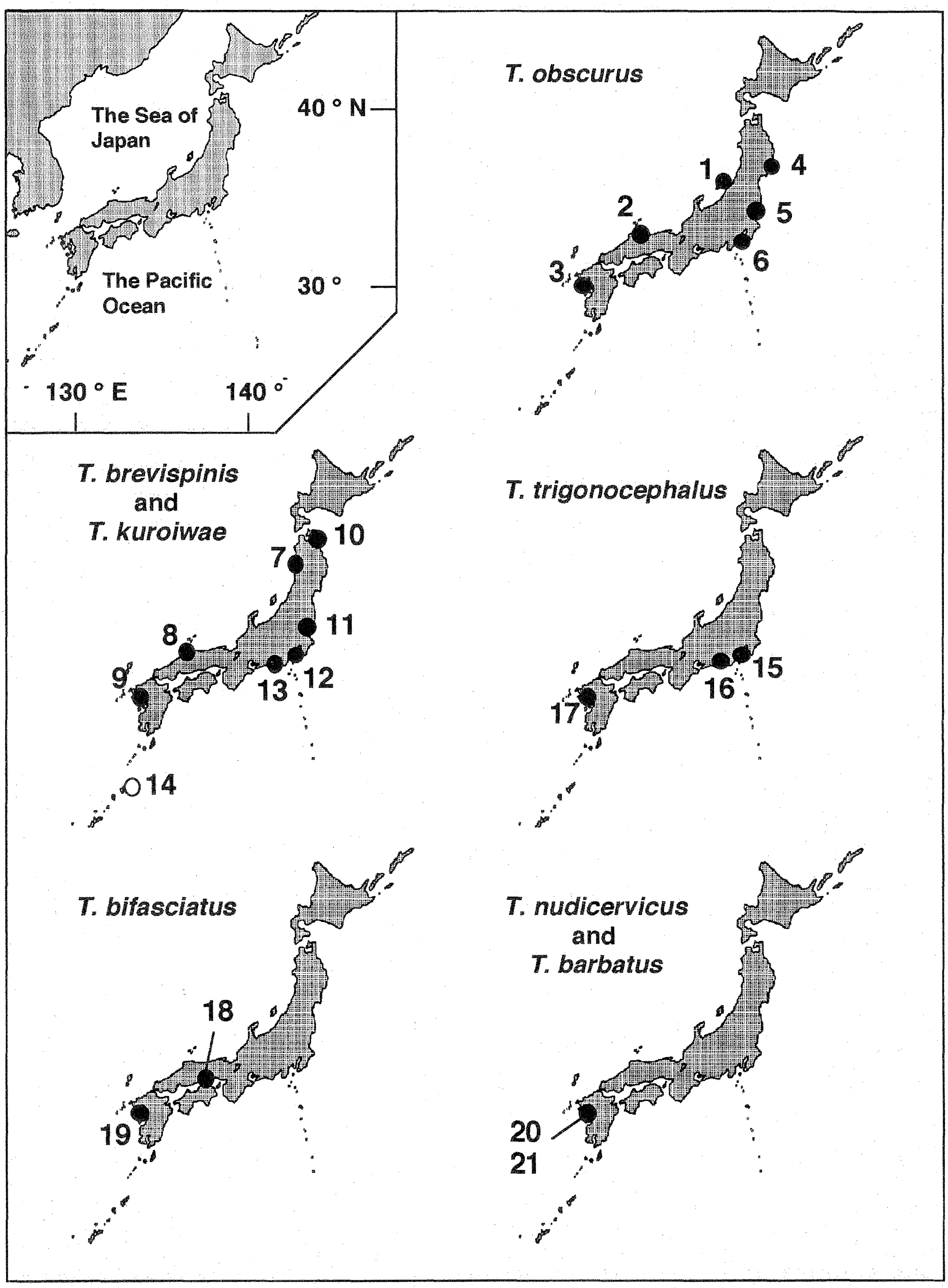

Fig. 1. Locations of collection sites for the 7 taxa of *Tridentiger.* 1= Lake Kamo, 2= Lake Nakaumi, 3= Kano-o River, 4= Moune Bay, 5= Lake Hinuma, 6= Koajiro Bay, 7= Lake Hachirou-gata, 8= Lake Shinji, 9= Sakai River, 10= Takase River, 11= Lake Hinuma, 12= Maeda River, 13= Hatauchi River, 14= Yanma River, 15= Aburatsubo Bay, 16= Shimizu Port, 17= Isahaya Bay, 18= Ushimado, 19, 20 and 21= Isahaya Bay. Nos. 5 and 11 are the same place. Nos. 17, 19, 20 and 21 are also the same place. The collection site for *T. kuroiwae* (No. 14) is shown by an open circle to be distinguished from the sites for *T. brevispinis* (solid ones). The species names are tentatively applied to each taxon after Akihito *et al.* (1993a).

Table 1. Enzymes and proteins examined with their locus designations and tissue sources used in the electrophoretic analyses

Enzyme and protein	Locus	Tissue
Aspartate aminotransferase	*AAT-1**	muscle
	*AAT-2**	muscle
Esterase	*EST**	muscle
Fumarate hydratase	*FH**	muscle
Glucose-6-phosphate isomerase	*GPI**	eye
Lactate dehydrogenase	*LDH-1**	eye
	*LDH-2 **	eye
	*LDH-3 **	eye
Malic enzyme (NADP$^+$)	*MEP**	muscle
Octanol dehydrogenase	*ODH**	liver
Sorbitol dehydrogenase	*SDH**	liver
Superoxide dismutase	*SOD**	liver
Xanthine dehydrogenase	*XDH**	liver
Sarcoplasmic protein	*PROT**	muscle

were collected in 1993 and 1994 at the locations shown in Figure 1. *T. obscurus, T. brevispinis, T. trigonocephalus* and *T. bifasciatus* which were distributed widely around the Japanese Archipelago were collected each from several geographically distant populations. On the other hand, single populations were chosen for *T. kuroiwae* which was distributed only in the Ryukyu Islands and *T. nudicervicus* and *T. barbatus* which were each distributed only in the Ariake Sound and the Seto Inland Sea (Akihito *et al.,* 1993a). Living specimens were transported to the laboratory and stored at -20°C or -80°C until used. The liver, eyes and skeletal muscle of each individual were separated, homogenized with deionized distilled water and centrifuged at 15,000 g for 10 min at 4°C. The supernatant was then removed and analyzed by polyacrylamide-slab gel electrophoresis (Davis, 1964). The electrophoresis was performed using 6 or 12 % polyacrylamide gel and applying 10 or 15 mA current for about 2hr. Proteins and enzymes were stained by the methods of Shaw and Prasad (1970) and Ayala *et al.* (1972).

Presumed loci and tissue sources of the enzymes used in this study are shown in Table 1. Locus and gene nomenclature followed Shaklee *et al.* (1989). Loci and alleles were numbered and alphabetized, respectively, in the order of decreasing anodal mobility of the products of each allele.

To estimate the intrapopulational genetic variability, the percentage of polymorphic loci and expected average heterozygosity were calculated in each population. Deviations from Hardy-Weinberg equilibrium were tested with Chi-square. Genetic identities and distances were computed for all pairs of samples after the formula of Nei (1987). From these genetic distance data, a dendrogram was derived using UPGMA (Sneath and Sokal, 1973).

RESULTS

Electrophoresis of allozymes

Table 2 shows the allele frequencies at 14 loci presumed to correspond to 10 enzymes and one sarcoplasmic protein for each sample. Electrophoretic patterns of some of the allozymes are shown in Figure 2. Two and three loci were scored in aspartate aminotransferase (AAT) and lactate dehydrogenase (LDH), respectively. The two loci of AAT showed independent polymorphism. *AAT-1 ** had two alleles and heterozygotes exhibited a three-banded pattern characteristic of dimeric enzymes. *AAT-2 ** had two alleles and each taxa had a fixed allele. The proteins of LDH were tetrameric, and some heterotetramers among the products of its three loci were observed. The heterozygote was observed in only one locus, *LDH-1 **, and exhibited 5 bands at the place where the homozygote exhibited one band.

In other enzymes, only one locus was scored. Esterase (EST) was monomeric and heterozygotes exhibited two bands. Glucose-6-phosphate isomerase (GPI), octanol dehydrogenase (ODH) and superoxide dismutase (SOD) were dimeric and heterozygotes exhibited three bands. Malic enzyme (MEP) and sorbitol dehydrogenase (SDH) were tetrameric and heterozygotes exhibited five bands. No heterozygote was observed in fumarate hydratase (FH) and xanthine dehydrogenase (XDH).

Although genetic control of the sarcoplasmic protein was not clear, one strongly stained band was scored as the product of one locus *PROT **. At this locus, two alleles were observed and were named **a* and **b. T. barbatus* had allele **a* and the other taxa had allele **b.*

Intrapopulational genetic variability

Table 2 also shows intrapopulational genetic variability of the 21 populations indicated by percentage of polymorphic loci (P) and expected average heterozygosity (H). Although the minimum values of P and H were 0 in *T. barbatus* (population No.21), the other populations showed polymorphisms in several loci. The maximum value of P was 28.6 % in *T. brevispinis* from the population of the Hatauchi River (population No.13) and that of H was 0.070 in *T. obscurus* from the population of Lake Nakaumi (population No.2).

Chi-square tests for deviation from panmixia revealed one case where frequencies departed significantly from Hardy-Weinberg equilibrium (P<0.01) for *MEP ** in *T. obscurus* from the population of Moune Bay (population No.4). In this sample, however, all the other loci showed no significant deviation from the equilibrium.

Interpopulational and interspecific genetic differentiation

To estimate the degree of genetic differentiation among the 7 taxa of *Tridentiger,* Nei's genetic identity and genetic distance between each pair from the 21 populations were calculated from the allele frequencies (Table 3). The genetic distances among populations of *T. obscurus* were 0.000 to 0.047 (average is 0.017), those of *T. brevispinis* were 0.000 to 0.025 (average is 0.007), those of *T. trigonocephalus* were 0.002 to 0.003 (average is 0.002), and that between the two populations of *T. bifasciatus* was 0.003. For *T. kuroiwae, T. nudicervicus* and *T. barbatus,* only one population was examined each. The genetic distances among taxa were 0.138 (*T. brevispinis* vs. *T. kuroiwae*) to 1.526 (*T. trigonocephalus* vs. *T. barbatus*). There were 1 to 11 loci at which every pair from the 7 taxa did not share alleles (Table 2).

Figure 3 shows the dendrogram of the 21 populations of *Tridentiger* constructed from the genetic distances by

Table 2. Allele frequencies for 14 loci in 21 populations of *Tridentiger*

			T. obscurus						*T. brevispinis*						
Locus	Allele	(N)	1 (17)	2 (12)	3 (12)	4 (12)	5 (3)	6 (5)	7 (10)	8 (14)	9 (19)	10 (19)	11 (5)	12 (8)	13 (13)
*AAT-1**	**a*								0.10			0.53			
	**b*		1.00	1.00	1.00	1.00	1.00	1.00	0.90	1.00	1.00	0.47	1.00	1.00	1.00
*AAT-2**	**a*								1.00	1.00	1.00	1.00	1.00	1.00	1.00
	**b*		1.00	1.00	1.00	1.00	1.00	1.00							
*EST**	**a*														
	**b*														
	**c*		1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.97	1.00	1.00	1.00	0.96
	**d*										0.03				0.04
*FH**	**a*														
	**b*		1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
*GPI**	**a*														
	**b*		0.44	0.29	0.46	0.17	0.83	0.20	1.00	0.75	1.00	0.97	0.80	1.00	0.81
	**c*		0.56	0.71	0.54	0.83	0.17	0.80		0.21		0.03	0.20		0.19
	**d*									0.04					
*LDH-1**	**a*		1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.97	1.00	1.00	1.00
	**b*											0.03			
*LDH-2**	**a*														
	**b*		1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
*LDH-3**	**a*		1.00	1.00	1.00	1.00	1.00	1.00							
	**b*								1.00	1.00	1.00	1.00	1.00	1.00	1.00
*MEP**	**a*														
	**b*		0.21	0.58		0.08			1.00	1.00	1.00	1.00	1.00	1.00	1.00
	**c*		0.79	0.42	1.00	0.92	1.00	1.00							
*ODH**	**a*								1.00	1.00	1.00	1.00	1.00	1.00	1.00
	**b*		1.00	1.00	0.83	1.00	1.00	1.00							
	**c*				0.17										
*SDH**	**a*								1.00	1.00	1.00	1.00	0.81	0.81	0.92
	**b*		1.00	0.96	1.00	1.00	1.00	1.00							
	**c*			0.04									0.19	0.19	0.08
*SOD**	**a*		1.00	1.00	1.00	1.00	1.00	1.00							
	**b*														
	**c*								1.00	1.00	1.00	1.00	1.00	1.00	0.88
	**d*														0.12
*XDH**	**a*														
	**b*		1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
*PROT**	**a*														
	**b*		1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
P			14.3	21.4	14.3	14.3	7.1	7.1	7.1	7.1	7.1	21.4	14.3	7.1	28.6
H			0.059	0.070	0.056	0.031	0.020	0.023	0.013	0.028	0.004	0.044	0.045	0.022	0.053

Population numbers refer to those in Figure 1. N; number of specimen. P; percentage of polymorphic loci (%) (criterion : major allele frequency < 0.99). H; average heterozygosity (expected).

UPGMA.

DISCUSSION

In the present study, we analyzed 14 loci of allozymes and estimated genetic differentiation among 7 taxa of *Tridentiger.* In other gobiid fishes, the average heterozygosity has been reported to be 0.006 to 0.062 (expected) for *Caenogobius* (Aizawa *et al.,* 1994), 0.0276 to 0.0642 (expected) for *Periophthalmus* (Chan and Lee, 1994), 0.021 to 0.092 (expected) for *Pomatoschistus* (Wallis and Beardmore, 1984) and 0.025 to 0.054 (observed) for *Rhinogobius* (Katoh and Nishida, 1994). In the present study, the expected average heterozygosity of *Tridentiger* ranged from 0.000 to 0.070 (average 0.034) and these values were close to those of the other gobiid fishes.

In marine and diadromous fishes, genetic distance (D) values among populations have been reported to be 0.0004 to 0.0035 for the Pacific herring *Clupea pallasi* in northern Japan (Kobayashi *et al.,* 1990), 0.001 to 0.172 for the Japanese dace *Tribolodon hakonensis* in the Japanese Archipelago (Hanzawa *et al.,* 1988), and 0.001 to 0.007 for the mudskipper *Periophthalmus cantonensis* in Taiwan (Chan and Lee, 1994). In the land-locked species of the freshwater goby *Rhinogobius flumineus,* the D values among populations are as large as 0.00 to 0.35 in western Japan (Shimizu *et al.,* 1993). The 7 taxa of *Tridentiger* are marine or amphidromous fishes (Akihito, 1987; Akihito *et al.,* 1988; Akihito and Sakamoto, 1989). The D values among the populations ranged from 0.000 to 0.047 (average 0.010) which were close to the above values for marine and

Table 2. continued

T. kuroiwae	*T. trigonocephalus*			*T. bifasciatus*		*T. nudicervicus*	*T. barbatus*
14 (15)	15 (7)	16 (9)	17 (4)	18 (4)	19 (4)	20 (3)	21 (5)
						1.00	1.00
1.00	1.00	1.00	1.00	1.00	1.00		
	1.00	1.00	1.00	1.00	1.00	1.00	1.00
1.00							
						1.00	1.00
0.40				0.12	0.30		
0.60				0.88	0.70		
	1.00	1.00	1.00				
	1.00	1.00	1.00	1.00	1.00		1.00
1.00						1.00	
						0.83	1.00
	0.93	0.89	0.87		0.10	0.17	
1.00	0.07	0.11	0.13	1.00	0.90		
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
						1.00	1.00
1.00	1.00	1.00	1.00	1.00	1.00		
						1.00	1.00
1.00	1.00	1.00	1.00	1.00	1.00		
	1.00	1.00	1.00	1.00	1.00		
1.00						1.00	1.00
1.00	1.00	1.00	1.00	1.00	1.00		
						1.00	1.00
1.00				1.00	1.00		
	0.93	0.83	1.00				
	0.07	0.17				1.00	1.00
	0.71	0.83	0.87	1.00	1.00	1.00	
							1.00
1.00	0.29	0.17	0.13				
	1.00	1.00	1.00				
1.00				1.00	1.00	1.00	1.00
							1.00
1.00	1.00	1.00	1.00	1.00	1.00	1.00	
7.1	21.4	21.4	14.3	7.1	14.3	7.1	0.0
0.034	0.048	0.054	0.032	0.015	0.043	0.020	0.000

diadromous fishes. The D values among the 7 taxa were larger than those among intraspecific populations.

It was reported that *T. trigonocephalus* and *T. bifasciatus* were morphologically distinct though they were superficially similar (Akihito and Sakamoto, 1989) and that *T. nudicervicus* and *T. barbatus* were morphologically different from each other and the other five taxa of *Tridentiger* (Akihito *et al.,* 1988, 1993a). In the present study, the D values among these 4 taxa were 0.246 to 1.526 and several loci were found where these taxa did not share alleles. These results suggest that they are genetically different from each other in addition to their morphological divergence. Therefore, these 4 taxa are considered to be biological species.

The remaining three taxa *"T. obscurus", "T. brevispinis"* and *"T. kuroiwae"* are distinguished by their color patterns (Akihito, 1987), but several classifications have been proposed (Kawanabe and Mizuno, 1989; Akihito *et al.,* 1993a). In the present study, the D values between *"T. obscurus"* and *"T. brevispinis"* ranged from 0.501 to 0.707 (average 0.564) and the interpopulational genetic differentiation within each taxon was very low (Fig. 3). In addition to this, population Nos. 5 (*T. obscurus*) and 11 (*T. brevispinis*) were collected exactly in the same place (Lake Hinuma) and the sampling locations of population Nos. 2 (*T. obscurus*) and 8 (*T. brevispinis*) are within the same water system (Lakes Nakaumi and Shinji). These two pairs of sympatric populations also showed high genetic divergences (the D values were 0.567 and 0.516, respectively). These results suggest that there exists reproductive isolation between the two taxa.

The D values between *"T. obscurus"* and *"T. kuroiwae"*

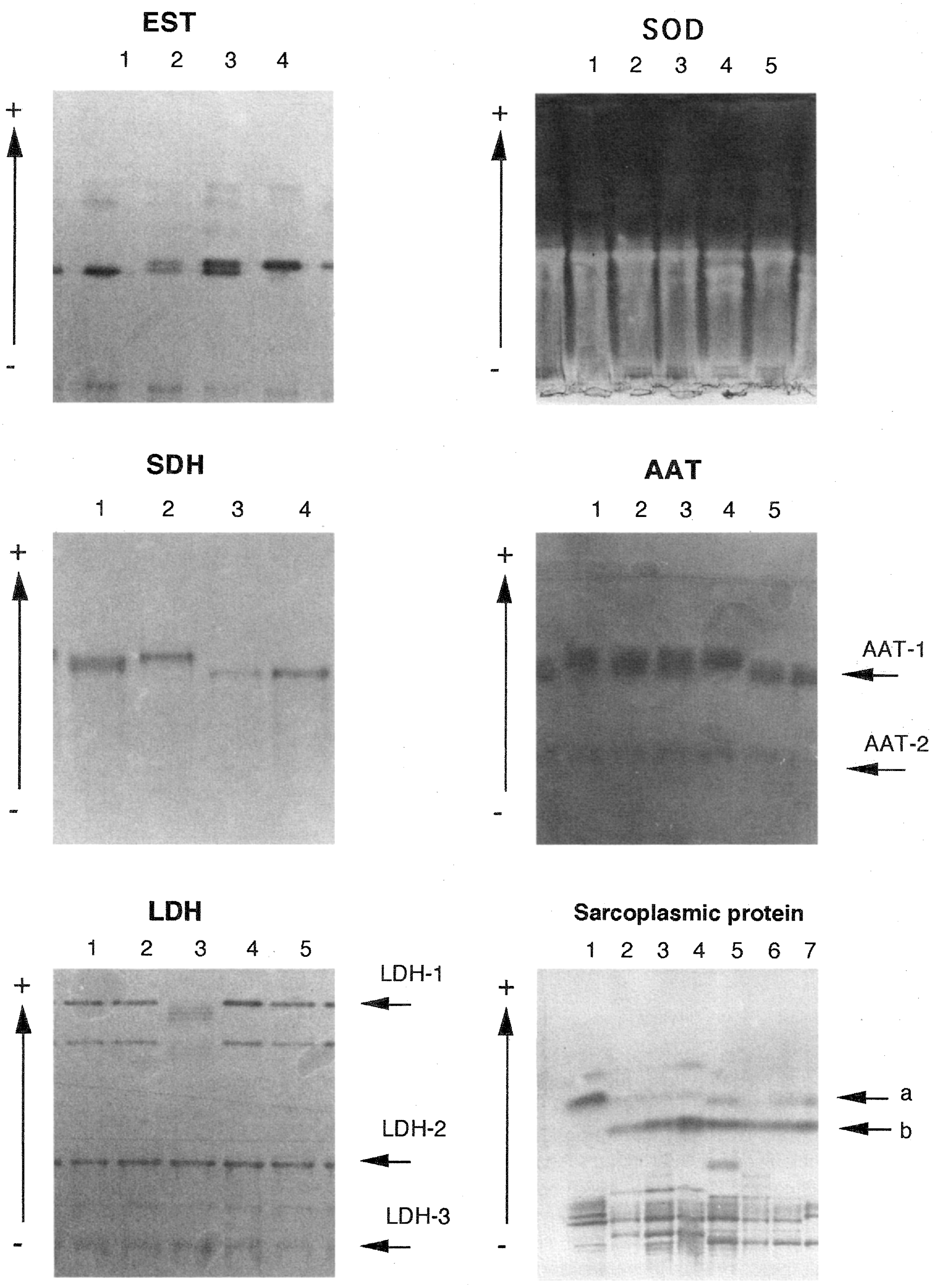

Fig. 2. Electrophoretic patterns of some of the allozymes studied. EST is monomeric, SOD is dimeric, and SDH is tetrameric. AAT shows independent polymorphism of two loci. LDH shows heterotetramers among the products of three loci. In the sarcoplasmic protein, one strongly stained band was scored as the product of one locus *PROT**. Presumed genotypes of each lane are as follows. Locus names are shown in the parentheses. EST (*EST**) lane 1, **c / *c* ; lanes 2 and 3, **b / *c* ; lane 4, **b / *b* . SOD (*SOD**) lanes 1, 2, 3 and 5, **a / *a* ; lane 4, **a / *c*. SDH (*SDH**) lane 1, **b / *c* ; lane 2, **b / *b* ; lanes 3 and 4, **c / *c*. AAT (*AAT-1**) lanes 1 and 4, **a / *a* ; lanes 2 and 3, **a / *b* ; lane 5, **b / *b*. (*AAT-2**) lanes 1, 2, 3, 4 and 5, **a / *a*. LDH (*LDH-1**) lanes 1, 2, 4 and 5, **a / *a* ; lane 3, **a / *b*. (*LDH-2*, 3**) lanes 1, 2, 3, 4 and 5, **b / *b*. Sarcoplasmic protein (*PROT**) lane 1, **a / *a* ; lanes 2, 3, 4, 5, 6 and 7, **b / *b*. Abbreviations refer to those in the text.

Table 3. Nei's genetic identity (above diagonal) and genetic distance (below diagonal) between pair from 21 populations of *Tridentiger* based on allele frequencies at 14 loci

	obs						bre							kur	tri			bif		nud	bar
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1	–	0.988	0.994	0.994	0.986	0.993	0.560	0.564	0.563	0.532	0.576	0.572	0.570	0.627	0.460	0.463	0.467	0.552	0.546	0.542	0.310
2	0.012	–	0.970	0.981	0.954	0.975	0.580	0.597	0.581	0.555	0.606	0.590	0.602	0.670	0.450	0.454	0.468	0.574	0.558	0.574	0.342
3	0.006	0.030	–	0.991	0.989	0.993	0.545	0.556	0.547	0.519	0.562	0.555	0.561	0.608	0.460	0.463	0.474	0.550	0.544	0.513	0.282
4	0.006	0.019	0.009	–	0.969	1.000	0.523	0.543	0.525	0.498	0.548	0.532	0.547	0.628	0.435	0.441	0.452	0.564	0.553	0.521	0.296
5	0.014	0.047	0.011	0.031	–	0.972	0.562	0.561	0.565	0.536	0.567	0.572	0.568	0.571	0.476	0.476	0.485	0.514	0.512	0.448	0.289
6	0.007	0.025	0.007	0.000	0.028	–	0.517	0.537	0.520	0.493	0.540	0.526	0.541	0.618	0.435	0.440	0.451	0.560	0.549	0.513	0.289
7	0.580	0.545	0.607	0.648	0.576	0.660	–	0.996	0.999	0.987	1.000	1.000	0.996	0.841	0.599	0.589	0.577	0.709	0.713	0.455	0.295
8	0.573	0.516	0.587	0.611	0.578	0.622	0.004	–	0.995	0.976	1.000	0.999	0.999	0.871	0.595	0.585	0.575	0.737	0.738	0.448	0.290
9	0.575	0.542	0.603	0.644	0.571	0.655	0.001	0.005	–	0.979	1.000	1.000	0.996	0.843	0.607	0.598	0.587	0.711	0.715	0.446	0.288
10	0.631	0.589	0.656	0.697	0.624	0.707	0.013	0.024	0.021	–	0.987	0.981	0.975	0.823	0.571	0.562	0.551	0.688	0.692	0.492	0.328
11	0.548	0.501	0.576	0.601	0.567	0.615	0.000	0.000	0.000	0.012	–	0.997	0.998	0.863	0.604	0.597	0.583	0.728	0.730	0.393	0.233
12	0.559	0.528	0.589	0.631	0.559	0.642	0.000	0.001	0.000	0.019	0.003	–	1.000	0.838	0.613	0.601	0.587	0.710	0.716	0.464	0.288
13	0.562	0.507	0.578	0.603	0.566	0.614	0.004	0.001	0.004	0.025	0.002	0.000	–	0.864	0.607	0.600	0.588	0.736	0.738	0.461	0.299
14	0.467	0.400	0.498	0.465	0.560	0.481	0.173	0.138	0.171	0.195	0.147	0.177	0.146	–	0.473	0.470	0.462	0.701	0.701	0.367	0.218
15	0.777	0.799	0.777	0.832	0.742	0.832	0.512	0.519	0.499	0.560	0.504	0.489	0.499	0.749	–	0.998	0.997	0.722	0.739	0.291	0.224
16	0.770	0.789	0.769	0.819	0.742	0.821	0.529	0.536	0.514	0.576	0.517	0.509	0.511	0.756	0.002	–	0.997	0.736	0.753	0.308	0.232
17	0.747	0.759	0.747	0.794	0.723	0.795	0.550	0.553	0.533	0.596	0.539	0.533	0.531	0.772	0.003	0.003	–	0.742	0.748	0.295	0.218
18	0.594	0.556	0.599	0.573	0.666	0.580	0.344	0.305	0.342	0.374	0.317	0.349	0.307	0.355	0.326	0.307	0.299	–	0.997	0.363	0.288
19	0.605	0.584	0.609	0.592	0.670	0.599	0.338	0.304	0.335	0.368	0.315	0.344	0.304	0.355	0.302	0.284	0.290	0.003	–	0.370	0.292
20	0.612	0.555	0.667	0.652	0.802	0.667	0.787	0.803	0.807	0.709	0.934	0.768	0.774	1.002	1.234	1.178	1.221	1.013	0.994	–	0.782
21	1.171	1.072	1.266	1.219	1.241	1.240	1.221	1.238	1.245	1.115	1.455	1.196	1.207	1.524	1.496	1.459	1.526	1.244	1.230	0.246	–

Population numbers refer to those in Figure 1. obs, *T. obscurus*; bre, *T. brevispinis*; kur, *T. kuroiwae*; tri, *T. trigonocephalus*; bif, *T. bifasciatus*; nud, *T. nudicervicus*; bar, *T.barbatus*

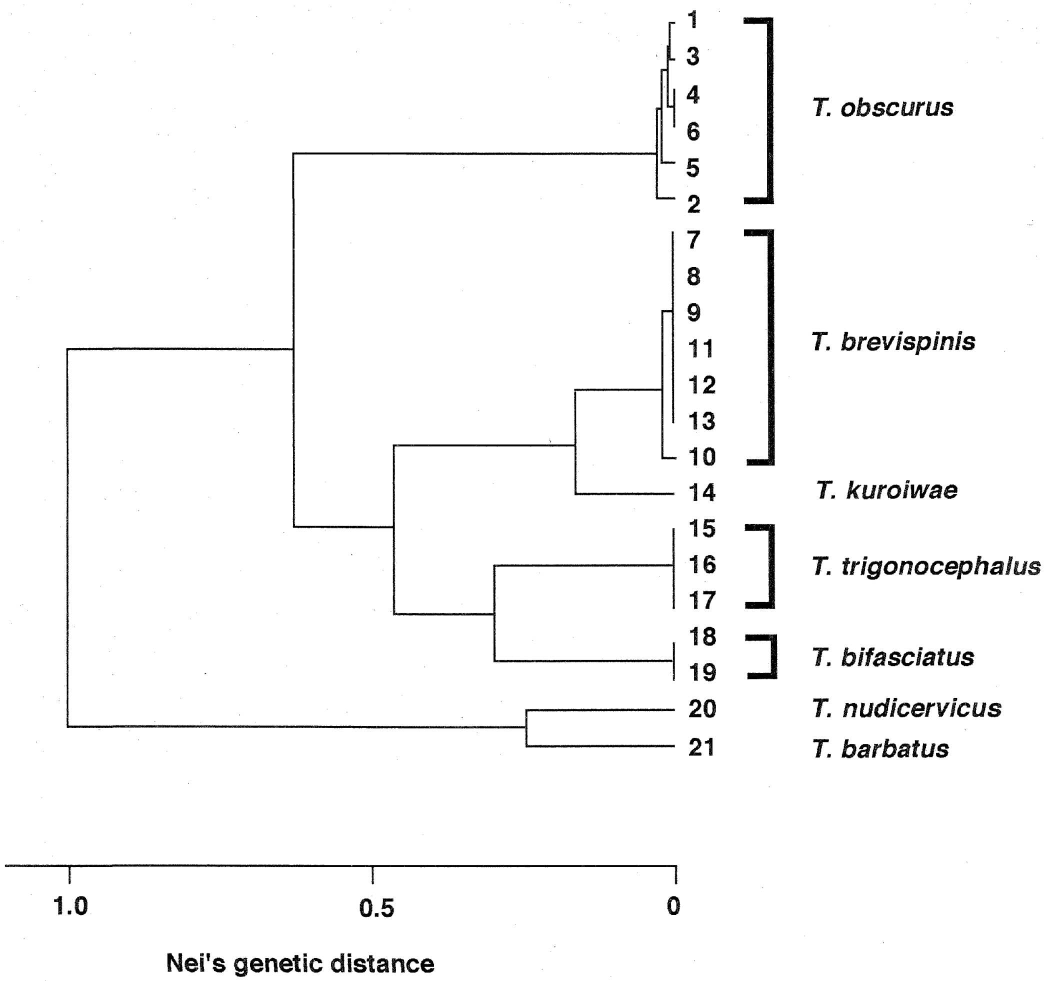

Fig. 3. A dendrogram of 21 populations of *Tridentiger* species constructed by UPGMA based on values of genetic distance. Population numbers refer to those in Figure 1.

were 0.400 to 0.560 (average 0.479), which were close to those between *"T. obscurus"* and *"T. brevispinis"*. On the other hand, the D values between *"T. brevispinis"* and *"T. kuroiwae"* were 0.138 to 0.195 (average 0.164), which were the lowest range of D values among the 7 taxa (Fig. 3). These facts provide us with a difficult taxonomic problem because *"T. kuroiwae"* is allopatric to *"T. obscurus"* and *"T. brevispinis"*.

A similar situation exists between the two subspecies of the ayu *Plecoglossus altivelis altivelis* and *P. a. ryukyuensis*. It has been reported that the local populations of the Ryukyu Islands (*P. a. ryukyuensis*) are distinguished from the populations of the Japanese Archipelago (*P. a. altivelis*) in some morphological features and allozyme polymorphism (the average D value between them based on 27-28 loci is 0.19) (Nishida, 1986, 1988). Their distribution is similar to that of *"T. kuroiwae"* and *"T. brevispinis"* and the D value between the two subspecies of the ayu is close to that between *"T. kuroiwae"* and *"T. brevispinis"*. These facts may suggest that *"T. kuroiwae"* and *"T. brevispinis"* have diverged from each other in subspecies level, supporting the classification of Kawanabe and Mizuno (1989).

The dendrogram shown in Figure 3 indicates that the 7 taxa of the genus *Tridentiger* can be divided into two groups. One group consists of *T. nudicervicus* and *T. barbatus,* and the other group consists of *T. obscurus, T. brevispinis, T. kuroiwae, T. trigonocephalus* and *T. bifasciatus*. Morphologically, distribution pattern of the cephalic sensory organs is considered to be a good key character for the gobiid classification. No apparent differences in the sensory canals and sensory papillae have been reported within the latter group (Akihito *et al.,* 1988, 1993b), which agrees with our genetic results.

Although this dendrogram shows that *T. barbatus* is closely related to *T. nudicervicus, T. barbatus* is morphologically distinct from the other 6 taxa of *Tridentiger* including *T. nudicervicus.* For example, *T. barbatus* has barbels on the lateral sides of the head and the lower jaw while the other taxa do not, and the distribution of sensory canal pores (Akihito *et al.,* 1988) and the egg shape are different from those of the others (Dotu, 1957). If this dendrogram shows the true phylogenetic relationships, it is suggested that the above-mentioned morphological characters of *T. barbatus* are autapomorphic and the classification which includes *T. barbatus* in the genus *Tridentiger* may be natural.

In the present study, only a small number of individuals were examined for *T. bifasciatus* (8 individuals), *T. nudicervicus* (3) and *T. barbatus* (5), and their relationships shown in Figure 3 may need further verification. More studies including morphology, ecology and mitochondrial DNA analysis are needed to make the evolutionary history and taxonomy of this gobiid genus clearer.

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