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Genetic Divergence in Lizardfishes of the Genus *Saurida* from Southern Japan

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Abstract Genetic divergence in four taxa of three species of *Saurida*, *S. undosquamis* south (S) and north (N) types, *S. wanieso* and *S. elongata* caught from the East China Sea, Sea of Hyuga and Tosa Bay, was studied based on allelic frequencies at 23 genetic loci surveyed electrophoretically. Fixed allele substitution was observed at eight loci between the S and N types of *S. undosquamis* and their genetic distance was 0.5582, within a range of differentiation at the species level. The S type of *S. undosquamis* was found to inhabit the Sea of Hyuga and off Cape Ashizuri along the Kuroshio Current, in addition to the East China Sea. The low level of genetic variation found for this type was discussed in relation to its restricted habitat at the edge of the continental shelf. These factors, along with some morphological characters, indicate that the two types of *S. undosquamis* should be recognized as distinct species.

Three species of the lizardfish genus *Saurida*, *S. undosquamis* (Richardson), *S. wanieso* Shindo et Yamada and *S. elongata* (Temminck et Schlegel), are common on the continental shelf in the southern waters of Japan (Tatara, 1965; Masuda et al., 1984; Yamada, 1986). The classification of the members of this genus present around Japan has been studied based on morphological characters (Matsubara and Iwai, 1951; Hanabuchi, 1971; Yamada and Ikemoto, 1979) and electrophoretic data (Taniguchi, 1969). Recently, two morphologically and ecologically distinct types (south and north) of *S. undosquamis* have been recognized from the East China Sea, one of the main fishery grounds of the lizardfish (Yamada and Ikemoto, 1979; Yamada, 1986). The boundary of geographical distribution between these two types is at about 28°N (Yamada, 1986). However, it is unclear whether they constitute distinct species or are merely conspecific populations. The specimens which Mizobuchi and Hirata (1982) reported from off Cape Ashizuri at the western end of Tosa Bay are very similar to those of the south type of *S. undosquamis* found in the East China Sea.

In this study, we aim to clarify the genetic relationships of the three species, including the two types of *S. undosquamis*, by applying a population genetic analysis using electrophoretic techniques.

Materials and methods

Materials used in this study were caught in 1987 and 1988 in the East China Sea, the Sea of Hyuga, off Cape Ashizuri, and in Tosa Bay off Kochi City (Fig. 1), as listed in Table 1. All the samples were identified on the basis of external morphology and stored at −20°C until use.

Isozyme and protein markers from the tissue of skeletal muscle, liver, eye and heart were detected by the starch gel electrophoretic method given in Taniguchi and Numachi (1978) and Taniguchi et al. (1978). The analyzed enzyme, their presumed loci, tissue source, and buffer systems used are shown in Table 2. The terminology of loci and alleles are as in Taniguchi et al. (1983). Allelic frequencies were calculated directly from the observed genotype. The distribution of the observed genotypes was compared with that expected from the Hardy-Weinberg equilibrium using a Chi-square test. The genetic differences were quantified by Nei's (1972) genetic distance.

For the individuals of *S. undosquamis* used for electrophoresis and 30 other specimens collected from off Kochi City (Table 1), the following three meristic characters were counted after fixing in 10% formalin solution: the vertebrae including the urostyle, dorsal fin rays and anal fin rays. The vertebral number was counted from radiographs. The following six morphometric characters were also measured by the method described by Matsu-

Table 1. Sampling locations and numbers of samples examined. * For morphological analysis.

Species	Location	No. of sample
S. undosquamis (south type)	East China Sea	53
	Ashizuri	10
	Sea of Hyuga	30
S. undosquamis (north type)	East China Sea	55
	Tosa Bay	44
		30*
	Sea of Hyuga	39
S. wanieso	East China Sea	48
	Tosa Bay	28
	Sea of Hyuga	3
S. elongata	East China Sea	32
	Tosa Bay	25

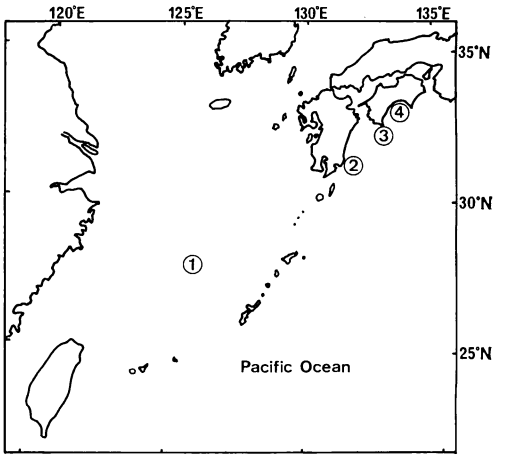

Fig. 1. Map of southern Japan showing sampling locations. 1, East China Sea; 2, Sea of Hyuga; 3, off Cape Ashizuri; 4, Tosa Bay.

Table 2. List of enzymes and proteins examined, locus detected, tissue assayed and buffer systems used. C-APM: Citric acid-aminopropyl morpholine (pH 6.0), gel density of 12%, T-C: Tris-citric acid (pH 8.0), gel density of 12% (as for buffers, see Taniguchi et al., 1978).

Enzyme and protein	Enzyme Commission number	Locus	Tissue	Buffer
Aspartate aminotransferase (AAT)	2.6.1.1.	*Aat-1*	liver	C-APM
		Aat-2	liver	C-APM
Alcohol dehydrogenase (ADH)	1.1.1.1.	*Adh*	liver	C-APM
α-Glycerophosphate dehydrogenase (α-GPD)	1.1.1.8.	*α-Gpd*	muscle	C-APM
Fumarate hydratase (FM)	4.2.1.2.	*Fm-1*	liver	C-APM
		Fm-2	liver	C-APM
Glucosephosphate isomerase (GPI)	5.3.1.9.	*Gpi-1*	liver	C-APM
		Gpi-2	muscle	C-APM
Isocitrate dehydrogenase (IDH)	1.1.1.42.	*Idh-1*	liver	C-APM
		Idh-2	muscle	C-APM
Lactate dehydrogenase (LDH)	1.1.1.27.	*Ldh-1*	eye	C-APM
		Ldh-2	muscle, heart	C-APM
		Ldh-3	muscle	C-APM
Malate dehydrogenase (MDH)	1.1.1.37.	*Mdh-1*	muscle	C-APM
		Mdh-2	liver, muscle	C-APM
		Mdh-3	liver, muscle	C-APM
Malic enzyme (ME)	1.1.1.40.	*Me-1*	muscle	C-APM
		Me-2	muscle	C-APM
6-Phosphogluconate dehydrogenase (6-PGD)	1.1.1.44.	*6-Pgd*	liver, muscle	C-APM
Phosphoglucomutase (PGM)	2.7.5.1.	*Pgm*	muscle	C-APM
Superoxide dismutase (SOD)	1.15.1.1.	*Sod*	liver	C-APM
Sarcoplasmic protein (SP)		*Sp-1*	muscle	C-APM
		Sp-2	muscle	C-APM, T-C

Table 3. Allele frequencies at 23 loci in genus *Saurida*. S, south type; N, north type; ECS, East China Sea; AZR, Ashizuri; SH, Sea of Hyuga; TSA, Tosa Bay.

Locus	Allele	*S. undosquamis*-S			*S. undosquamis*-N			*S. wanieso*			*S. elongata*	
		ECS	AZR	SH	ECS	TSA	SH	TSA	ECS	SH	TSA	ECS
Aat-1	113	0.000	0.000	0.000	0.000	0.012	0.026	0.018	0.000	0.000	0.000	0.000
	100	0.649	0.600	0.781	0.991	0.976	0.974	0.964	0.989	0.833	0.000	0.000
	83	0.351	0.350	0.219	0.009	0.000	0.000	0.018	0.000	0.000	0.979	1.000
	71	0.000	0.050	0.000	0.000	0.012	0.000	0.000	0.011	0.167	0.000	0.000
	64	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.021	0.000
Aat-2	−36	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.020	0.000
	−63	1.000	1.000	1.000	0.000	0.000	0.000	1.000	1.000	1.000	0.000	0.000
	−75	0.000	0.000	0.000	0.151	0.125	0.192	0.000	0.000	0.000	0.000	0.000
	−100	0.000	0.000	0.000	0.849	0.875	0.808	0.000	0.000	0.000	0.980	0.969
	−140	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.031
Adh	140	0.000	0.000	0.000	0.000	0.000	0.000	0.018	0.010	0.000	0.000	0.000
	40	0.000	0.000	0.000	0.000	0.000	0.000	0.089	0.010	0.000	0.000	0.000
	−50	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.800	0.781
	−140	0.000	0.000	0.000	0.855	0.898	0.885	0.000	0.000	0.000	0.000	0.000
	−250	1.000	1.000	1.000	0.000	0.000	0.000	0.893	0.969	1.000	0.000	0.000
	−350	0.000	0.000	0.000	0.145	0.102	0.115	0.000	0.000	0.000	0.000	0.000
	−400	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.200	0.200	0.219
	−600	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.000	0.000	0.000
α-Gpd	−100	0.991	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.000
	−160	0.009	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	−200	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000
Fm-1	100	0.000	0.000	0.000	1.000	1.000	1.000	1.000	1.000	1.000	0.980	0.984
	46	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.020	0.016
	90	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Fm-2	123	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.016
	100	0.000	0.000	0.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.984
	90	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Gpi-1	120	0.000	0.000	0.000	0.000	0.012	0.000	0.000	0.000	0.000	0.000	0.000
	105	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.920	0.984
	100	0.951	0.950	0.983	0.962	0.942	0.908	0.982	1.000	0.833	0.040	0.000
	84	0.049	0.050	0.017	0.038	0.035	0.092	0.018	0.000	0.167	0.040	0.016
	60	0.000	0.000	0.000	0.000	0.012	0.000	0.000	0.000	0.000	0.000	0.000
Gpi-2	200	0.000	0.000	0.000	0.000	0.000	0.000	0.409	0.448	0.500	0.000	0.000
	125	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.031
	50	0.000	0.000	0.000	0.000	0.000	0.000	0.523	0.521	0.500	0.000	0.000
	−50	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	−100	0.000	0.000	0.000	0.991	1.000	1.000	0.000	0.000	0.000	0.000	0.000
	−167	0.000	0.000	0.000	0.009	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	−250	0.000	0.000	0.000	0.000	0.000	0.000	0.068	0.031	0.000	1.000	0.969
Idh-1	124	0.000	0.000	0.000	0.009	0.000	0.026	0.000	0.000	0.000	0.022	0.016
	100	0.009	0.000	0.033	0.964	1.000	0.974	0.000	0.000	0.000	0.978	0.984
	75	0.991	1.000	0.967	0.018	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	67	0.000	0.000	0.000	0.000	0.000	0.000	0.685	0.615	1.000	0.000	0.000
	46	0.000	0.000	0.000	0.000	0.000	0.000	0.315	0.385	0.000	0.000	0.000
	42	0.000	0.000	0.000	0.009	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Idh-2	100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000

(Table 3, continued)

Locus	Allele	*S. undosquamis*-S			*S. undosquamis*-N			*S. wanieso*			*S. elongata*	
		ECS	AZR	SH	ECS	TSA	SH	TSA	ECS	SH	TSA	ECS
Ldh-1	125	0.000	0.000	0.000	0.000	0.000	0.013	0.000	0.000	0.000	0.000	0.000
	100	1.000	1.000	1.000	1.000	1.000	0.987	1.000	1.000	1.000	1.000	1.000
Ldh-2	100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Ldh-3	325	0.000	0.000	0.000	0.145	0.091	0.103	0.000	0.000	0.000	0.000	0.000
	300	0.019	0.000	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	100	0.981	1.000	0.983	0.855	0.909	0.897	1.000	1.000	1.000	1.000	1.000
Mdh-1	138	0.000	0.000	0.000	0.009	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	100	1.000	1.000	1.000	0.991	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Mdh-2	138	0.000	0.000	0.000	0.000	0.011	0.000	0.000	0.000	0.000	0.000	0.000
	100	1.000	1.000	1.000	1.000	0.989	1.000	0.982	1.000	1.000	1.000	1.000
	60	0.000	0.000	0.000	0.000	0.000	0.000	0.018	0.000	0.000	0.000	0.000
Mdh-3	−100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.000
	−125	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000
Me-1	112	0.000	0.000	0.000	0.000	0.000	0.000	0.946	0.990	1.000	0.000	0.000
	100	1.000	1.000	1.000	0.964	0.966	0.962	0.054	0.010	0.000	1.000	1.000
	90	0.000	0.000	0.000	0.036	0.034	0.038	0.000	0.000	0.000	0.000	0.000
Me-2	150	0.000	0.000	0.000	0.009	0.000	0.000	0.000	0.000	0.000	1.000	1.000
	100	0.991	0.900	0.983	0.991	1.000	0.974	1.000	1.000	1.000	0.000	0.000
	64	0.009	0.000	0.017	0.000	0.000	0.026	0.000	0.000	0.000	0.000	0.000
	5	0.000	0.100	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
6-Pgd	120	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.021	0.000
	110	0.969	1.000	0.966	0.000	0.013	0.000	0.857	0.969	1.000	0.979	0.969
	100	0.000	0.000	0.000	0.845	0.988	0.868	0.000	0.000	0.000	0.000	0.000
	90	0.031	0.000	0.036	0.131	0.000	0.132	0.054	0.031	0.000	0.000	0.031
	80	0.000	0.000	0.000	0.024	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Pgm	−40	0.000	0.000	0.000	0.027	0.045	0.013	0.000	0.010	0.000	0.000	0.000
	−100	0.000	0.000	0.000	0.700	0.602	0.654	0.464	0.500	0.333	0.000	0.000
	−200	0.000	0.000	0.000	0.273	0.352	0.333	0.536	0.490	0.667	0.000	0.000
	−270	1.000	1.000	0.983	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	−320	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000
	−380	0.000	0.000	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Sod	100	0.000	0.000	0.000	0.655	0.716	0.692	0.000	0.000	0.000	0.000	0.000
	88	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	67	0.000	0.000	0.000	0.345	0.284	0.308	0.000	0.000	0.000	0.000	0.000
	58	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000	0.000	0.000
	35	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.457	0.703
	30	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.543	0.297
Sp-1	100	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000
	90	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000
	80	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000	0.000	0.000
Sp-2	100	0.000	0.000	0.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000
	−40	1.000	1.000	1.000	0.000	0.000	0.000	1.000	1.000	1.000	0.000	0.000
	−120	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000

bara (1955): standard length, head length, snout length, lower jaw length, eye diameter and interorbital width.

Results

Electrophoretic analysis. Twenty-three genetic loci presumed by 12 enzymes and one non-enzymatic protein were scored in each of the four taxa of the three species of *Saurida* studied here. The following genetic variants were observed in the 23 loci in each taxon: S type of *S. undosquamis*, 8 loci; N type of *S. undosquamis*, 15 loci; *S. wanieso*, 9 loci; and *S. elongata*, 10 loci. Allele frequencies for each locus of the 11 lots are given in Table 3. Genetic interpretation of banding patterns are described below.

Aspartate aminotransferase (*Aat-1* and *Aat-2*): The activity of this enzyme appeared in three zones in the liver, two migrating to the anode and the other to the cathode. However, one locus of the anode band, the mobility of which was smaller, could not be reliably interpreted due to the instability of the band. Five alleles, designated as $Aat\text{-}1^{64}$, $Aat\text{-}1^{71}$, $Aat\text{-}1^{83}$, $Aat\text{-}1^{100}$ and $Aat\text{-}1^{113}$, were estimated at the anodal locus. All four taxa were polymorphic at this locus and shared allele $Aat\text{-}1^{83}$. The gene frequencies for $Aat\text{-}1^{83}$ in *S. elongata* were predominant, while those in the other three taxa were low. Allele $Aat\text{-}1^{64}$ was observed exclusively in *S. elongata*, whereas the $Aat\text{-}1^{113}$ allele was not observed in the S type of *S. undosquamis*. Five alleles, designated as $Aat\text{-}2^{-36}$, $Aat\text{-}2^{-63}$, $Aat\text{-}2^{-75}$, $Aat\text{-}2^{-100}$ and $Aat\text{-}2^{-140}$, were estimated at the cathodal locus. The S type of *S. undosquamis* and *S. wanieso* were fixed for allele $Aat\text{-}2^{-63}$. This allele was not observed in the N type of *S. undosquamis* and *S. elongata*, which were polymorphic at this locus and shared allele $Aat\text{-}2^{-100}$. The gene frequencies for $Aat\text{-}2^{-100}$ in the N type of *S. undosquamis* were predominant. The two types of *S. undosquamis* shared no alleles at the *Aat-2* locus.

Alcohol dehydrogenase (*Adh*; Fig. 2A): One locus was scored in this enzyme system near the origin. Eight alleles, designated as Adh^{40}, Adh^{140}, Adh^{-50}, Adh^{-140} Adh^{-250}, Adh^{-350}, Adh^{-400} and Adh^{-600}, were estimated. The S type of *S. undosquamis* was fixed for allele Adh^{-250}; the three other taxa were polymorphic and did not share any allele with each other. The two types of *S. undosquamis* shared no alleles at this locus.

α-Glycerophosphate dehydrogenase (*α-Gpd*): One *α-Gpd* locus was scored in the muscle extract. Three alleles, designated as $\alpha\text{-}Gpd^{-100}$, $\alpha\text{-}Gpd^{-160}$ and $\alpha\text{-}Gpd^{-200}$, were estimated. The N type of *S. undosquamis* and *S. wanieso* were fixed for the $\alpha\text{-}Gpd^{-100}$ allele, and *S. elongata* for allele $\alpha\text{-}Gpd^{-200}$. The S type of *S. undosquamis* was polymorphic and had two alleles ($\alpha\text{-}Gpd^{-100}$ and $\alpha\text{-}Gpd^{-160}$). *S. elongata* shared no alleles with the three other taxa.

Fumarate hydratase (*Fm-1* and *Fm-2*): Two loci were scored at the anode. Only *S. elongata* was polymorphic, the remaining three taxa being monomorphic. The two types of *S. undosquamis* did not share any alleles at these loci.

Glucosephosphate isomerase (*Gpi-1* and *Gpi-2*): The activity of this enzyme appeared in two zones, one migrating to the anode in the liver and the other near the origin in the muscle extract. Five alleles, designated as $Gpi\text{-}1^{60}$, $Gpi\text{-}1^{84}$, $Gpi\text{-}1^{100}$, $Gpi\text{-}1^{105}$ and $Gpi\text{-}1^{120}$, were estimated at the anodal locus, where all four taxa were polymorphic and shared alleles $Gpi\text{-}1^{84}$ and $Gpi\text{-}1^{100}$. Allele $Gpi\text{-}1^{105}$ was observed exclusively in *S. elongata*. Seven alleles, designated as $Gpi\text{-}2^{50}$, $Gpi\text{-}2^{125}$, $Gpi\text{-}2^{200}$, $Gpi\text{-}2^{-50}$, $Gpi\text{-}2^{-100}$, $Gpi\text{-}2^{-167}$ and $Gpi\text{-}2^{-250}$, were estimated near the origin. At this locus the S type of *S. undosquamis* was monomorphic, whereas the other three taxa were polymorphic. The gene frequencies for $Gpi\text{-}2^{-100}$ and $Gpi\text{-}2^{-250}$ were predominant in the N type of *S. undosquamis* and *S. elongata*, respectively. The two types of *S. undosquamis* shared no alleles with each other at the *Gpi-2* locus.

Isocitrate dehydrogenase (*Idh-1* and *Idh-2*; Fig. 2B): Two loci were scored at the anode. The fast locus had a high activity in the liver (*Idh-1*) and the slow one in the muscle extract (*Idh-2*). Six alleles, designated as $Idh\text{-}1^{42}$, $Idh\text{-}1^{46}$, $Idh\text{-}1^{67}$, $Idh\text{-}1^{75}$, $Idh\text{-}1^{100}$ and $Idh\text{-}1^{124}$, were estimated at the locus. All four taxa were polymorphic. The N type of *S. undosquamis*, had four alleles, and the S type, *S. wanieso* and *S. elongata* had two alleles at this locus. The *Idh-2* locus was monomorphic for all taxa studied. The gene frequencies for alleles at *Idh-1* between the two types of *S. undosquamis* differed clearly from each other.

Lactate dehydrogenase (*Ldh-1*, *Ldh-2* and *Ldh-3*): Three loci were scored at the anode. The fast locus, specific for the eye, was designated as *Ldh-*

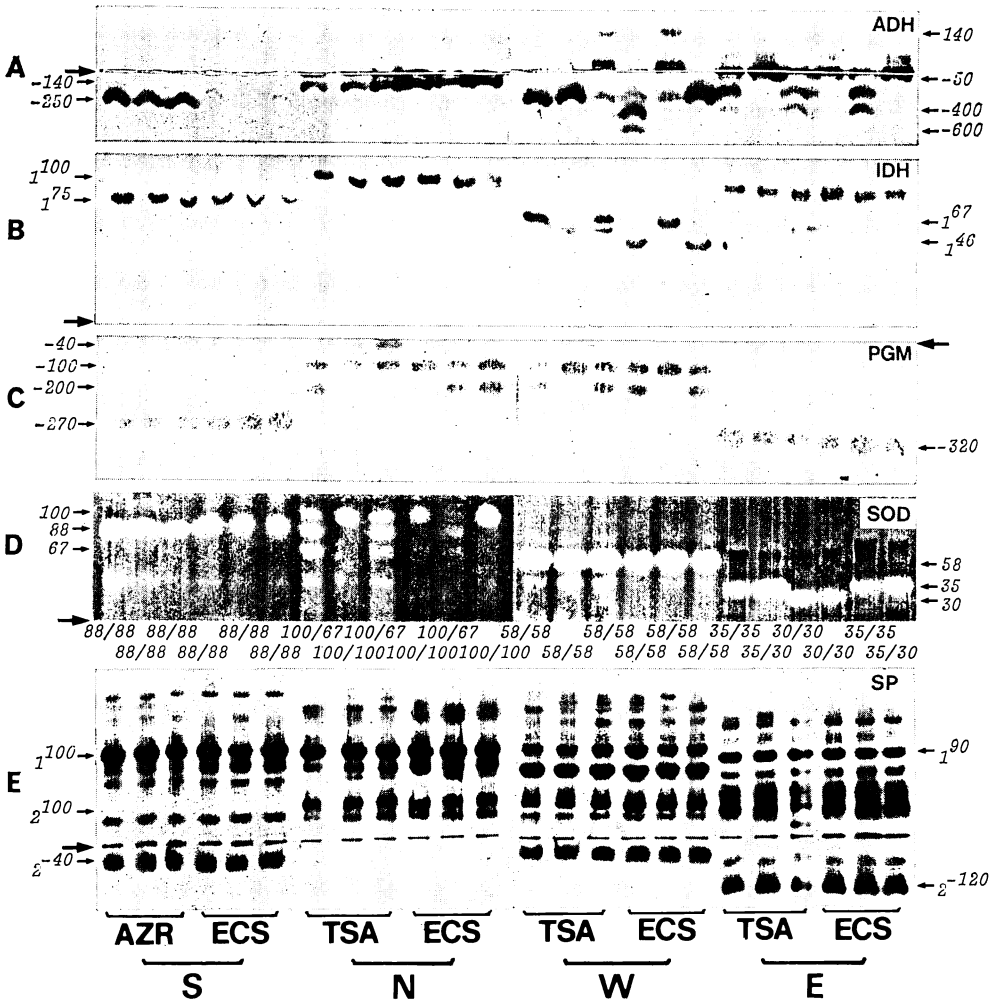

Fig. 2. Examples of electropherograms of ADH, IDH, PGM, SOD and SP. Locations of assumed homopolymeric bands are designated by italic number. Large arrow-head marks show the origin. A, alcohol dehydrogenase; B, isocitrate dehydrogenase; C, phosphoglucomutase; D, superoxide dismutase; E, sarcoplasmic protein. AZR, Ashizuri; ECS, East China Sea; TSA, Tosa Bay. S, south type of *Saurida undosquamis*; N, north type of *S. undosquamis*; W, *S. wanieso*; E, *S. elongata*.

1, the intermediate one, specific for the heart, as *Ldh-2*, and the slow one near the origin, specific for the muscle extract, as *Ldh-3*. Two alleles, designated as $Ldh\text{-}1^{100}$ and $Ldh\text{-}1^{125}$, were estimated at the locus. Only the N type was polymorphic, the other three taxa being monomorphic. All four taxa were monomorphic at the *Ldh-2* locus. Three alleles, $Ldh\text{-}3^{100}$, $Ldh\text{-}3^{300}$ and $Ldh\text{-}3^{325}$, were estimated at this locus, where the two types of *S. undosquamis* were polymorphic and the other two taxa were monomorphic.

Malate dehydrogenase (*Mdh-1*, *Mdh-2* and *Mdh-3*): Three loci were observed for this enzyme, two at the anode and one at the cathode. The fast locus showing a high activity in the muscle extract was designated as *Mdh-1*, and the other locus at the anode as *Mdh-2*. The locus at the cathode showing considerable activity in the liver was *Mdh-3*. Two alleles, designated as $Mdh\text{-}1^{100}$ and $Mdh\text{-}1^{138}$, were estimated at the *Mdh-1* locus. The N type of *S. undosquamis* was polymorphic and the other three taxa were monomorphic, fixed for the $Mdh\text{-}1^{100}$ allele. Three alleles, $Mdh\text{-}2^{00}$, $Mdh\text{-}2^{100}$ and $Mdh\text{-}2^{138}$, were estimated at the

Mdh-2 locus. The N type of *S. undosquamis* and *S. wanieso* were polymorphic, and the S type of *S. undosquamis* and *S. elongata* were monomorphic, fixed for the *Mdh-2*^{100} allele. All four taxa were monomorphic at the *Mdh-3* locus. The two types of *S. undosquamis* and *S. wanieso* were fixed for the *Mdh-3*$^{-100}$ allele, whereas *S. elongata* was fixed for the *Mdh-3*$^{-125}$ allele.

Malic enzyme (*Me-1* and *Me-2*): Two loci were observed at the anode for this enzyme. Three alleles, *Me-1*^{90}, *Me-1*^{100} and *Me-1*^{112}, were estimated at the *Me-1* locus. The N type of *S. undosquamis* and *S. wanieso* were polymorphic, and the S type of *S. undosquamis* and *S. elongata* were monomorphic, fixed for the *Me-1*^{100} allele. Four alleles, designated as *Me-2*^{5}, *Me-2*^{64}, *Me-2*^{100} and *Me-2*^{150}, were estimated at the *Me-2* locus. The two types of *S. undosquamis* were polymorphic, each having three alleles. *S. wanieso* and *S. elongata* were monomorphic, fixed for the *Me-2*^{100} and *Me-2*^{150}, respectively.

6-Phosphogluconate dehydrogenase (*6-Pgd*): A single *6-Pgd* locus was scored at the anode. Five alleles, *6-Pgd*^{80}, *6-Pgd*^{90}, *6-Pgd*^{100}, *6-Pgd*^{110} and *6-*

Table 4. Genetic variability in the genus *Saurida* based on 23 isozyme loci. S, south type; N, north type; ECS, East China Sea; AZR, Ashizuri; SH, Sea of Hyuga, TSA, Tosa Bay. P*: Criterion for polymorphism is lower than 0.95 in major allele frequency. P: Polymorphic ($p<0.99$). M: Monomorphic.

Locus	*S. undosquamis*-S		*S. undosquamis*-N			*S. wanieso*		*S. elongata*	
	ECS (n=53)	SH (30)	ECS (55)	TSA (44)	SH (39)	TSA (28)	ECS (48)	TSA (25)	ECS (32)
Aat-2	P*	P*	P	P	P	P	P	P	P
Aat-2	M	M	P*	P*	P*	P*	P	P	P
Adh	M	M	P*	P*	P*	P*	P	P*	P*
α-Gpd	P	M	M	M	M	M	M	M	M
Fm-1	M	M	M	M	M	M	M	P	P
Fm-2	M	M	M	M	M	M	M	M	P
Gpi-1	P	P	P	P*	P*	P	M	P*	P
Gpi-2	M	M	P	M	M	P*	P*	M	P
Idh-1	P	P	P	M	P	P*	P*	P	P
Idh-2	M	M	M	M	M	M	M	M	M
Ldh-1	M	M	M	M	P	M	M	M	M
Ldh-2	M	M	M	M	M	M	M	M	M
Ldh-3	P	P	P*	P*	P*	M	M	M	M
Mdh-1	M	M	P	M	M	M	M	M	M
Mdh-2	M	M	M	P	M	P	M	M	M
Mdh-3	M	M	M	M	M	M	M	M	M
Me-1	M	M	P	P	P	P*	P	M	M
Me-2	P	P	P	M	P	M	M	M	M
6-Pgd	P	P	P*	P	P*	P*	P	P	P
Pgm	M	P	P*	P*	P*	P*	P*	M	M
Sod	M	M	P*	P*	P*	M	M	P*	P*
Sp-1	M	M	M	M	M	M	M	M	M
Sp-2	M	M	M	M	M	M	M	M	M
No. of loci	23	23	23	23	23	23	23	23	23
No. P+P*	7	7	13	10	12	10	8	8	9
No. of P*	1	1	6	6	7	7	3	3	2
Proportion									
of P+P*	0.304	0.304	0.565	0.435	0.522	0.435	0.348	0.348	0.391
of P*	0.043	0.043	0.261	0.261	0.304	0.304	0.130	0.130	0.087
No. of allele	30	30	40	37	36	36	34	32	32
Mean No. of allele	1.304	1.304	1.739	1.609	1.565	1.565	1.478	1.391	1.391
H(ob)	0.025	0.025	0.094	0.083	0.108	0.097	0.066	0.051	0.048
H(exp)	0.030	0.026	0.095	0.076	0.097	0.095	0.073	0.051	0.046
(Hob)/H(exp)	0.833	0.962	0.989	1.092	1.113	1.021	0.904	1.000	1.043

Pgd^{120}, were estimated. All four taxa were polymorphic at this locus, the S and N types of *S. undosquamis*, *S. wanieso* and *S. elongata* having four, two, three and three alleles, respectively. The gene frequencies for alleles at this locus differed clearly between the two types of *S. undosquamis*.

Phosphoglucomutase (*Pgm*; Fig. 2C): Six alleles, Pgm^{-40}, Pgm^{-100}, Pgm^{-200}, Pgm^{-270}, Pgm^{-320} and Pgm^{-380} were estimated at this single locus system. *S. elongata* was monomorphic for allele Pgm^{-320} and the other three taxa were polymorphic. The N type of *S. undosquamis* and *S. wanieso* shared three alleles, Pgm^{-40}, Pgm^{-100} and Pgm^{-200}. Alleles Pgm^{-270} and Pgm^{-380} were observed exclusively in the S type of *S. undosquamis*, and the two types of *S. undosquamis* thus shared no alleles at this locus.

Superoxide dismutase (*Sod*; Fig. 2D): One *Sod* locus was scored at the anode in the liver extract. Six alleles, Sod^{30}, Sod^{35}, Sod^{58}, Sod^{67}, Sod^{88} and Sod^{100}, were estimated at this locus. The N type of *S. undosquamis* and *S. elongata* were polymorphic, whereas the S type of *S. undosquamis* and *S. wanieso* were monomorphic, fixed for the Sod^{88} and Sod^{58} alleles, respectively. The two types of *S. undosquamis* shared no alleles at this locus.

Sarcoplasmic protein (*Sp-1* and *Sp-2*; Fig. 2E): The activity of *Sp* in the muscle extract appeared in two zones, one migrating to the anode and the other to the cathode at the T-C buffer-system, being coded by two loci, *Sp-1* and *Sp-2*. All four taxa were monomorphic at these two loci. The two types of *S. undosquamis* shared no alleles at the *Sp*-2 locus.

The distribution of the observed phenotypes of the 11 lots in the four taxa based on the polymorphic loci presumed in this study generally did not deviate significantly from that expected from the Hardy-Weinberg equilibrium except at *Gpi-1* ($p<0.01$) and *6-Pgd* ($p<0.001$) of the S type of *S. undosquamis* and *Me-1* ($p<0.001$) of the N type of *S. undosquamis* from the East China Sea, *Pgm* ($p<0.05$) of the N type of *S. undosquamis* from Tosa Bay, *Gpi-2* ($p<0.05$) of *S. wanieso* from Tosa Bay and *Idh-1* ($p<0.05$) of *S. wanieso* from the East China Sea. These deviations might be due to sampling error. The allele frequencies for each locus of the 11 lots are given in Table 3.

Genetic variation. Since the number of the specimens of *S. undosquamis* obtained from off Cape Ashizuri, and of *S. wanieso* obtained from the Sea of Hyuga, examined for electrophoretic analysis was small ($n=10$ and 3, respectively), these lots were not used for the analysis of genetic variation (Table 4).

Of the 23 loci examined, five loci (*Idh-2*, *Ldh--2*,

Table 5. The genetic distance (*D*) between every pair of 11 lots of four types (below the diagonal) and its mean values of lots within and between four types (above the diagonal). S, south type; N, north type; ECS, East China Sea; AZR, Ashizuri; SH, Sea of Hyuga; TSA, Tosa Bay.

	S. undosquamis-S			*S. undosquamis*-N			*S. wanieso*			*S. elongata*	
	1. ECS	2. AZR	3. SH	4. ECS	5. TSA	6. SH	7. TSA	8. ECS	9. SH	10. TSA	11. ECS
1.			0.0011								
2.	0.0006				0.5582			0.4145			0.9815
3.	0.0009	0.0017									
4.	0.5555	0.5638	0.5426			0.0013					
5.	0.5642	0.5720	0.5514	0.0018				0.4724			0.7001
6.	0.5595	0.5676	0.5468	0.0007	0.0013						
7.	0.4108	0.4148	0.4020	0.4517	0.4600	0.4534			0.0072		
8.	0.4098	0.4135	0.4007	0.4616	0.4701	0.4638	0.0013				0.8767
9.	0.4279	0.4305	0.4209	0.4955	0.5008	0.4948	0.0092	0.0110			
10.	0.9749	0.9683	0.9901	0.6963	0.6966	0.6964	0.8645	0.8732	0.8786		0.0031
11.	0.9822	0.9757	0.9788	0.7035	0.7040	0.7035	0.8738	0.8825	0.8875	0.0031	

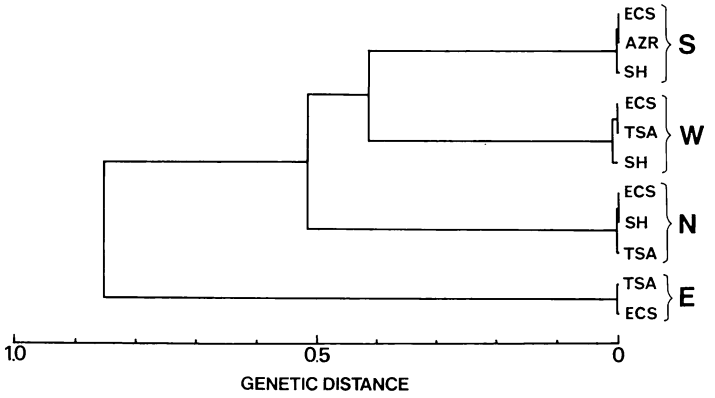

Fig. 3. Dendrogram showing relationships among four taxa in the genus *Saurida* based on values of genetic distance. SH, Sea of Hyuga. Other abbreviations are as in Fig. 2.

Mdh-3, *Sp-1* and *Sp-2*) were monomorphic. At 12 loci, *Aat-1*, *Aat-2*, *Adh*, *Gpi-1*, *Gpi-2*, *Idh-1*, *Ldh-3*, *Me-1*, *Me-2*, *6-Pgd*, *Pgm* and *Sod*, the frequencies of major alleles were below 0.95 at least in one lot, suggesting high genetic variations. The remaining six loci included rare variants.

The genetic variation in the lots was considerably diversified (Table 4). The S type of *S. undosquamis* from the East China Sea showed genetic variation only at seven loci, whereas the N type of *S. undosquamis* from the East China Sea did at 13 loci. It is noteworthy that the number of polymorphic loci with the criterion lower than 0.95 (P*) was the smallest (1) in the S type from the East China Sea and the Sea of Hyuga. The proportion of the polymorphic loci (P+P*) of *S. undosquamis* from the East China Sea ranged from 0.304 for the S type to 0.565 for the N type. The average allele number of each locus ranged from 1.304 of the S type of *S. undosquamis* from both the East China Sea and the Sea of Hyuga to 1.739 of the N type of *S. undosquamis* from the East China Sea. The range of the observed and expected average heterozygosities of the nine lots were 0.025 to 0.108 and 0.026 to 0.097, respectively, with the values of the average heterozygosities of the N and S types of *S. undosquamis* being large and small, respectively. The mean values of the observed and expected average heterozygosities of the nine lots were 0.066 and 0.065, respectively. Therefore, the number of polymorphic loci, the average allele number of polymorphic loci, the average allele number and the average heterozygosity value of the N type were all larger than those of the S type of *S. undosquamis*, suggesting that the former type has a higher genetic variation than the latter.

Genetic relationships. Estimates of the genetic distance derived from pairwise comparisons of the 11 lots of four taxa, computed according to Nei's (1972) method, are shown in Table 5. The mean values of the genetic distance between the S and N

Table 6. Morphometric data, expressed as mean±standard deviation of percentage of standard length, of *Saurida undosquamis* from each location. ECS, East China Sea; AZR, Ashizuri; SH, Sea of Hyuga; TSA, Tosa Bay.

Character	South type			North type		
	ECS	AZR	SH	TSA	TSA	SH
Standard length (mm)	236.06±31.70	237.67±49.28	253.31±41.79	164.55±25.61	170.92±32.65	207.32±25.28
Head length	25.05± 1.03	24.73± 0.77	24.79± 0.79	24.58± 0.85	24.24± 0.70	24.38± 0.56
Snout length	5.50± 0.29	5.40± 0.23	5.50± 0.23	5.55± 0.36	5.67± 0.28	5.66± 0.25
Lower jaw length	18.85± 0.56	18.45± 0.58	18.31± 0.47	18.97± 0.56	18.47± 0.59	18.64± 0.45
Eye diameter	5.06± 0.35	5.18± 0.29	5.04± 0.32	4.59± 0.29	4.71± 0.34	4.47± 0.27
Interorbital width	4.45± 0.30	4.68± 0.34	4.48± 0.31	4.21± 0.30	4.08± 0.25	4.19± 0.21

types of *S. undosquamis* were large (D=0.5582). By contrast, the genetic distance between every pair of lots within each taxon was very samll.

A dendrogram illustrating the inferred genetic relationships of the four taxa of lizardfishes is presented in Fig. 3, according to the unweighted paired group method (UPGMA) (Sneath and Sokal, 1973). The dendrogram shows that *S. elongata* was distantly related with the other three taxa. Among the latter three taxa, *S. wanieso* was inferred to be more closely related with the S type of *S. undosquamis* than with the N type.

Morphological comparison between south and north types of *S. undosquamis*. Six morphometric characters were measured and proportions to the standard length were compared (Table 6). Clear differences were found for eye diameter and interorbital width between every pair of lots of the two types of *S. undosquamis*, with the S type showing the larger values of the two characters (Table 7).

The frequency distribution of the number of vertebrae in the two types of *S. undosquamis* is given in Fig. 4. A clear difference was seen in the vertebral number between the S (mode=49) and N types (mode=47 or 48) of *S. undosquamis*. Although no clear differences were found for the fin ray number of the dorsal and anal fins (Table 7), the S and N types of *S. undosquamis* did tend to have 11 and 12 dorsal fin rays, respectively, with the former type showing a narrower range of the anal fin ray number (10–11) than the latter type (8–13).

All the specimens of the S type of *S. undosquamis* were dark colored on the ventral half of the body, whereas the specimens of the N type were white in that region.

Table 7. The result of t-test of morphological characters of *Saurida undosquamis* between every pair of location. HL, head length/standard length; SNL, snout length/SL; LJL, lower jaw length/SL; ED, eye diameter/SL; IOW, Interorbital width/SL; DFR, number of dorsal fin rays; AFR, number of anal fin rays. 1, East China Sea (south type); 2, Ashizuri; 3, Sea of Hyuga (south type); 4, East China Sea (North type); 5, Tosa Bay; 6, Sea of Hyuga. *, $P<0.05$; **, $P<0.01$.

	Character						
	HL	SNL	LJL	ED	IOW	DFR	AFR
1-2	—	—	*	—	*	—	—
1-3	—	—	**	—	—	—	—
1-4	*	—	—	**	**	**	**
1-5	**	*	**	**	**	—	—
1-6	**	**	—	**	**	**	**
2-3	—	—	—	—	—	—	—
2-4	—	—	**	**	**	*	—
2-5	—	**	—	**	**	—	—
2-6	—	**	—	**	**	—	—
3-4	—	—	**	**	**	**	*
3-5	**	*	—	**	**	—	—
3-6	**	**	**	**	**	**	*
4-5	—	—	**	—	*	**	—
4-6	—	—	**	*	—	—	—
5-6	—	—	—	**	—	*	—

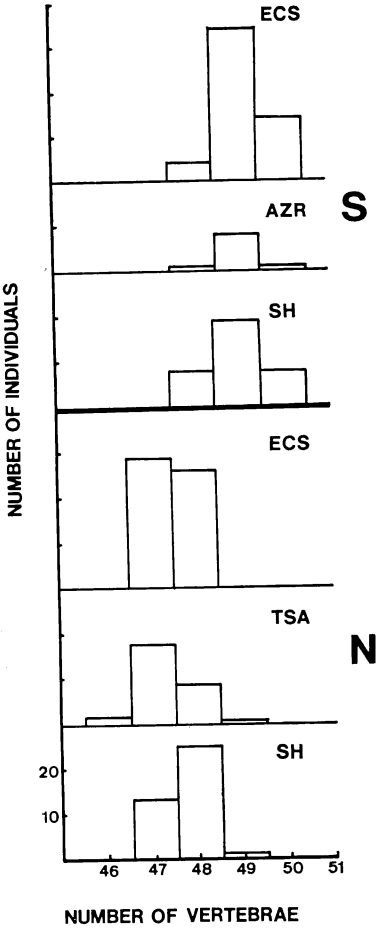

Fig. 4. Frequency distribution of vertebral number in *Saurida undosquamis*. Abbreviations are as in Figs. 2 and 3.

Discussion

Yamada and Ikemoto (1979) and Yamada (1986) recognized the two types of *Saurida undosquamis* from the East China Sea as conspecific populations on the basis of morphological and ecological features. However, the present biochemical-genetic data show that at eight out of the 23 loci, the two types of *S. undosquamis* do not share any common alleles. At two other loci (*Idh-1* and *6-Pgd*), they share alleles but at very different frequencies (Table 3). The genetic distance of 0.5555 found between these two types from the East China Sea falls within the range of the values reported among the species of marine teleost fishes, including Synodontidae (Shaklee et al., 1982). The values of the genetic distance between each species of the genus *Saurida* from Hawaii ranging from 0.42 to 0.75 (Waples, 1981) agree with ours. Furthermore, in bottom-living sparids and sciaenids, the average genetic distance between congeneric species tends to be small (D=0.115 and 0.092, respectively) (Taniguchi et al., 1986; Menezes and Taniguchi, 1988).

These factors indicate that the divergence between the two types of *S. undosquamis*, both bottom fishes, adequately reaches the species level. Fig. 3 seems to suggest that the S type of *S. undosquamis* might be more closely related with *S. wanieso* than the N type. However, precise interpretations of the relationships among these three taxa are not possible until further morphological, ecological and genetical data are available. It is certain that at least three of the four studied taxa have diversified as distinct species. There is a strong agreement for *S. elongata* between the estimate of relationships based on the genetic parameter and that based on morphological similarities (see Yamada and Ikemoto, 1979).

The S type from three lots showed a very small mean genetic distance of 0.0011 indicating that they are conspecific subpopulations. The same interpretation can be drawn for the N type of *S. undosquamis* from the three lots. This supposition is supported by the fact that the genetic distance between conspecific subpopulations of sparids ranges from 0.0007 to 0.0040 (D=0.0020) (Taniguchi et al., 1986), and in *Pennahia argentata*, from 0.00087 to 0.00220 (D=0.0017) (Menezes and Taniguchi, 1988).

The small value of H(ob)/H(exp) found in the S type of *S. undosquamis* from the East China Sea (0.833) could be due to the following reasons: sampling error, inbreeding and isolate breaking. As it is almost impossible that inbreeding occurs in open waters, sampling error and the isolate breaking seem to be the most likely factors contributing to the small observed value.

The mean heterozygosity of the S type of *S. undosquamis* is clearly lower than that of the N type (Table 4). The S type occurs on the continental shelf in the south region of the East China Sea and at its edge, but the distribution has widened northwards along the Kuroshio Current (Yamada, 1986). In the Sea of Hyuga and off Cape Ashizuri where the Kuroshio Current runs, the S type is caught at depths of about 200 m at the edge of the continental shelf (Nishiyama and Yamaoka, unpublished). By contrast, the N type generally inhabits muddy bottoms less than 150 m deep (Tatara, 1965). These features suggest that one of the main habitats of the S type of *S. undosquamis* around southern Japan is the small area at the edge of the continental shelf. This restricted habitat might be correlated with the low value of the heterozygosity seen in the S type, which can be recognized as a taxon of the genus *Saurida* adapted to the deeper habitat. It also suggests that the S type of *S. undosquamis* has diverged as a small population from an ancestral species.

The degree of genetic divergence found between the S and N types of *S. undosquamis* is clearly higher than the degree of the divergence of the external morphology. It must be recognized that slight differences in the external morphology in the populations of a species might be taxonomically significant in marine fishes.

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南日本産マエソ属魚類の遺伝的分化

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東シナ海，日向灘，土佐湾産マエソ属魚類，マエソ南方型，北方型，ワニエソ，トカゲエソの3種4分類群について，12酵素及び1非酵素蛋白の電気泳動像を検出し，それらの間の遺伝的分化の程度を調べた．23遺伝子座の対立遺伝子頻度を推定した．その内の8遺伝子座で，マエソ南方型と北方型の間に完全な遺伝子の置換が認められた．両者間の遺伝的距離（*D*）は0.5582となり，マエソ南方型と北方型は別種であることが強く示唆された．マエソ南方型は東シナ海の他に，黒潮の流れに沿った日向灘，足摺岬沖にも生息することが本研究によって明らかとなった．

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