Genetic Structure and Phylogeny in Seven Japanese Species of Cynoglossidae (Pisces: Pleuronectiformes)

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Abstract

Genetic structure of seven Japanese species of cynoglossid fish (*Cynoglossus abbreviatus*, *C. interruptus*, *C. itinus*, *C. joyneri*, *C. nigropinnatus*, *C. ochiaii*, *C. robustus*) were examined by using horizontal starch gel electrophoresis, and phylogeny was considered from their genetic relationships. The cynoglossid species generally exhibited high genetic variability, in particular average heterozygosity values of *C. interruptus* and *C. robustus* exceeded 0.2. These values were greater than the maximum value previously recorded for fish. The genetic distances (*D* values) between the species were calculated and a dendrogram based on the distances was created. In the dendrogram, *C. interruptus*, *C. ochiaii*, *C. joyneri* and *C. itinus* (the *kopsii* group) formed a monophyletic clade, which is consistent with the morphological similarity among these species. *Cynoglossus nigropinnatus*, which is placed within the *kopsii* group based on morphological characteristics, belonged to another cluster. *Cynoglossus abbreviatus* differed considerably from the other species in genetic distance.

(accepted December 11, 2008)

Introduction

The cynoglossid fish (Cynoglossidae: Pleuronectiformes) are bottom-dwellers and are commonly known as soles or tonguefish. They include many valuable species for fisheries, which are commercially exploited worldwide. About 20 Japanese cynoglossid species, which also includes some valuable species, are known[1].

Ecological information is available for some species of Japanese cynoglossid fish[2]. However, their genetic information is largely unknown. For fisheries science and breeding, it is important to understand the genetic structure of valuable species. In addition, it is important to evaluate the genetic structure of species systematically, and such systematic evaluations using isozymic genetic variability have been undertaken for many marine fish[3, 4]. For such systematic studies, genetic information of the cynoglossid fish can enrich the data accumulation. Genetic information could be used to examine the phylogeny of the Japanese cynoglossid fish, which is deeply related to their taxonomy and evolution. This in turn would provide basic information for fisheries and breeding science.

The present study therefore examined the genetic characteristics of seven common species of the Japanese cynoglossid fish by using isozymes, and revealed their genetic structure and phylogeny.

Materials and Methods

The seven species studied (Table 1) were collected from the Seto Inland Sea or Tosa Bay by trawling, and were stored at either −30℃ or −80℃ prior to analysis. Species were identified using the morphological characteristics described by Ochiai[5]. *Cynoglossus ochiaii* was identified following the description of Yokogawa *et al.*[6].

Electrophoresis to examine isozymes was performed using the methodologies of previous studies[7, 8]. In addition to the 33 loci identified in these publications, the following 2 loci were detected in the present study; malic enzyme ($NADP^+$), E. C.

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Table 1. Data of Japanese cynoglossid fish

Scientific name	Japanese name	Locality of collection	*n*
Cynoglossus abbreviatus	Korai-aka-shita-birame	Off Kagawa, Seto Inland Sea	12
Cynoglossus interruptus	Genko	Off Kagawa, Seto Inland Sea	54
Cynoglossus itinus	Minami-aka-shita-birame	Off Kochi, Tosa Bay	4
Cynoglossus joyneri	Aka-shita-birame	Off Kagawa, Seto Inland Sea	12
Cynoglossus nigropinnatus	Hire-guro-genko	Off Kochi, Tosa Bay	12
Cynoglossus ochiaii	Oki-genko	Off Kochi, Tosa Bay	53
Cynoglossus robustus	Inu-no-shita	Off Kagawa, Seto Inland Sea	12

Table 2. Genetic features of Japanese cynoglossid fish

		C. abbreviatus	*C. interruptus*	*C. itinus*	*C. joyneri*	*C. nigropinnatus*	*C. ochiaii*	*C. robustus*	Average
Allelic richness		1.303	1.672	1.235	1.523	1.239	1.545	1.680	1.457
Percentage of polymorphic loci	P*	0.229	0.543	0.182	0.455	0.273	0.400	0.471	0.364
	P	0.171	0.171	0.000	0.121	0.061	0.229	0.118	0.124
	P+P*	0.400	0.714	0.182	0.576	0.333	0.629	0.588	0.489
Average heterozygosity	*Ho*	0.081	0.226	0.086	0.179	0.089	0.171	0.219	0.150
	He	0.092	0.230	0.094	0.163	0.083	0.178	0.222	0.152
	ASE	0.003	0.001	0.005	0.006	0.004	0.001	0.004	0.003
	Ho/He	0.881	0.985	1.925	1.099	1.077	0.957	0.983	1.130

P* : Polymorphism less than 0.95. *Ho* : Observed heterozygosity. ASE : Average standard error for *He*.
P : Polymorphism greater than 0.95. *He* : Expected heterozygosity (unbiased value).

1. 1. 1. 40 (*MEP-2**, from muscle tissue), phosphoglucomutase, E. C. 5. 4. 2. 2 (*PGM**, from muscle tissue). The *AH-2** locus used in previous studies[7,8] was omitted from the genetic analysis, because it was not detected, or its electrophoretic bands were too thin to read in some species. Consequently, 34 loci were used in the present study.

The observed heterozygosity (*Ho*) per locus was given from the proportion of heterozygotes to individual number examined, and average values of *Ho* were calculated per species. The expected heterozygosity (*He*) (unbiased value) per locus and its standard error value were calculated after Nei[9], and they were averaged per species. For polymorphic loci, correspondence to the Hardy-Weinberg equilibrium was tested with GENEPOP software (ver. 4.0.7)[10]. Allelic richness per locus was calculated with FSTAT software (ver. 2.9.3.2)[11], and it was averaged per species.

Genetic distances (*D* values) following Nei[12], were calculated using the allelic frequencies of the seven species. A dendrogram of genetic relationships between the seven species was constructed from the genetic distances by using the Neighbor-joining (NJ) method with MEGA software (ver. 4.0)[13], and bootstrap estimates to test reliability of the dendrogram were performed with the Populations genetic software (ver. 1.2.30)[14].

Results

Concerning the correspondence to Hardy-Weinberg equilibrium, because no significance was recognized at any loci of any species, all were regarded to correspond to the equilibrium.

Average allele numbers per locus, rate of polymorphic loci and average heterozygosity (*Ho*, *He*), of the seven species are summarized in Table 2. Because the cynoglossid species exhibited higher values in the genetic indices, they could be regarded as having high genetic variability. In particular, the observed heterozygosity (*Ho*) exceeded 0.2 in *Cynoglossus interruptus* and *C. robustus*, showing considerable high genetic polymorphism in these species. Also, *Ho* approached 0.2 in *C. joyneri* and *C. ochiaii*. While *Ho* was less than 0.1 in *C. abbreviatus*, *C. itinus*, and *C. nigropinnatus*, there was comparatively less polymorphism in this family.

Allelic frequencies at the 34 loci of the seven species are shown in Table 3. Allelic compositions at any loci were unique to each species and alleles were different between species at many loci (Table 3).

The genetic distances (*D* values) between the seven species are shown in Table 4, and a dendrogram based on the genetic distances is presented in Fig. 1. The distance between *C. interruptus* and *C. ochiaii* was the closest (*D*=0.4143) (Table 4).

Table 3. Allelic frequencies of Japanese cynoglossid fish

Locus	Allele	*C. abbreviatus*	*C. interruptus*	*C. itinus*	*C. joyneri*	*C. nigropinnatus*	*C. ochiaii*	*C. robustus*
*AAT-3**	**-20*	0	0.173	0	0	0	0	0
	**-100*	1.000	0.337	0	0	0	0.019	0
	**-150*	0	0.288	0	0	0	0.028	0
	**-160*	0	0	0	0	0	0	0.042
	**-170*	0	0.135	0	0	1.000	0.925	0.958
	**-175*	0	0	0	1.000	0	0	0
	**-200*	0	0.067	0	0	0	0.028	0
	**-230*	0	0	1.000	0	0	0	0
*ACP**	**145*	0	0.010	0	0.042	0	0.009	0
	**120*	0	0.154	0	0.125	0	0.198	0
	**100*	0.917	0.519	1.000	0.833	0	0.755	0
	**80*	0.083	0.250	0	0	0	0.038	0
	**70*	0	0.058	0	0	0.042	0	0
	**50*	0	0.010	0	0	0.958	0	0.250
	**25*	0	0	0	0	0	0	0.375
	**0*	0	0	0	0	0	0	0.333
	**-20*	0	0	0	0	0	0	0.042
*AH-1**	**120*	0	0	0	0.083	0	0	0
	**115*	0	0	0	0.583	0	0	0
	**110*	0.333	0.040	0	0.333	0	0.980	0
	**100*	0.542	0.930	0.625	0	1.000	0.020	0
	**90*	0.083	0.030	0.375	0	0	0	0
	**80*	0.042	0	0	0	0	0	0.500
	**75*	0	0	0	0	0	0	0.292
	**70*	0	0	0	0	0	0	0.208
*CAT-1**	**125*	0	0	0	0	1.000	0	0
	**100*	0	1.000	0	0.083	0	1.000	0.917
	**90*	0	0	0	0	0	0	0.083
	**80*	1.000	0	0	0	0	0	0
	**75*	0	0	1.000	0.917	0	0	0
*CAT-2**	**-50*	1.000	0	0	0	0	0	0
	**-90*	0	0	0	0	0	0	1.000
	**-100*	0	0.991	1.000	0.063	1.000	1.000	0
	**-110*	0	0	0	0.938	0	0	0
*CK**	**-70*	0	0.075	0	0	0	0.019	0
	**-100*	0.083	0.783	0	1.000	1.000	0.981	1.000
	**-155*	0.917	0.132	0	0	0	0	0
	**-165*	0	0.009	1.000	0	0	0	0
*EST-3**	**100*	0	0.830	1.000	0.958	0	0.028	0
	**90*	0	0.113	0	0.042	0.125	0.123	0
	**80*	1.000	0.057	0	0	0.250	0.736	1.000
	**65*	0	0	0	0	0.625	0.113	0
*FBALD-1**	**0*	0	0	1.000	0	0	0	0
	**-60*	0	0	0	0	0	0	0.182
	**-100*	0	1.000	0	0.083	1.000	1.000	0.773
	**-150*	1.000	0	0	0.917	0	0	0
	**-175*	0	0	0	0	0	0	0.045
*FBALD-2**	**-55*	0	0	0	0	0	0.906	0
	**-65*	0	0	0	0	0	0.094	0
	**-80*	0	0	1.000	0	0	0	0
	**-100*	0	0.704	0	0.136	0	0	0
	**-135*	0	0	0	0.773	1.000	0	0
	**-140*	0	0.296	0	0.091	0	0	0
	**-200*	1.000	0	0	0	0	0	1.000
*FH-2**	**250*	0	0.010	1.000	0	0	0	0
	**200*	0	0	0	0	0	0	1.000
	**100*	1.000	0.981	0	1.000	1.000	1.000	0
	**50*	0	0.010	0	0	0	0	0
*G3PDH-1**	**140*	0	0	0	0	0	0.013	0
	**110*	0.042	0	0	0.042	0.125	0	0.958
	**105*	0.958	0	0	0.875	0	0	0
	**100*	0	1.000	0	0.083	0.875	0.987	0.042
	**55*	0	0	1.000	0	0	0	0
*G3PDH-2**	**120*	0	0	0	0.909	0	0	0.875
	**100*	0	0.991	0	0.091	1.000	1.000	0
	**70*	0.042	0	0	0	0	0	0.042
	**50*	0.917	0	0	0	0	0	0
	**40*	0.042	0.009	0	0	0	0	0.083
	**10*	0	0	1.000	0	0	0	0

Table 3. continued

Locus	Allele	*C. abbreviatus*	*C. interruptus*	*C. itinus*	*C. joyneri*	*C. nigropinnatus*	*C. ochiaii*	*C. robustus*
*G3PDH-3**	**125*	0	0.009	0	0.792	0	0	0
	**90*	1.000	0	0	0	0.818	0	0.875
	**0*	0	0	0	0	0	0	0.125
	**-75*	0	0.037	0	0	0.182	0.962	0
	**-100*	0	0.898	1.000	0.208	0	0.028	0
	**-220*	0	0.056	0	0	0	0.009	0
*GPI-1**	**110*	0	0	0	0	0.833	0	0
	**100*	0	0.546	1.000	0.042	0	0.160	0.917
	**90*	0.125	0	0	0.958	0	0.604	0.083
	**80*	0.667	0.306	0	0	0.167	0.189	0
	**75*	0.000	0.083	0	0	0	0.019	0
	**65*	0.167	0.065	0	0	0	0.019	0
	**50*	0.042	0	0	0	0	0.009	0
*GPI-2**	**-25*	0	0	0	0	0	0.038	0
	**-60*	0.042	0.028	0	1.000	0	0.887	0
	**-100*	0	0.954	1.000	0	0	0.066	0
	**-145*	0.958	0	0	0	1.000	0	0.958
	**-160*	0	0.019	0	0	0	0.009	0.042
*IDDH**	**215*	0	0.061	0	0	0.136	0.031	0
	**160*	0	0.207	0	0.208	0.864	0.031	0.500
	**100*	0	0.732	0	0.750	0	0.719	0.375
	**85*	0	0	1.000	0	0	0	0
	**30*	0.042	0	0	0	0	0	0
	**0*	0.958	0	0	0.042	0	0.219	0.125
*IDHP-1**	**140*	0	0.010	0	0	0.042	0	0
	**135*	0	0.196	0	0	0.083	0	0
	**105*	0	0	0	0	0.875	0	0
	**100*	0	0.745	0	0.958	0	0.010	0.833
	**95*	0	0	0.125	0	0	0	0
	**90*	0	0.049	0	0.042	0	0	0.083
	**80*	0	0	0	0	0	0	0.083
	**65*	1.000	0	0.875	0	0	0.990	0
*IDHP-2**	**300*	1.000	0	0	0	0	0	0
	**100*	0	1.000	1.000	1.000	1.000	1.000	1.000
*LDH-1**	**200*	0	0	0	0.875	0	0	0
	**100*	0	0	0	0	1.000	0	1.000
	**0*	0	0	0	0.125	0	0	0
	**-100*	0	1.000	1.000	0	0	0.811	0
	**-170*	1.000	0	0	0	0	0	0
	**-220*	0	0	0	0	0	0.113	0
	**-300*	0	0	0	0	0	0.028	0
	**-400*	0	0	0	0	0	0.047	0
*LDH-2**	**-10*	0	0	1.000	0	1.000	0	1.000
	**-100*	0	1.000	0	1.000	0	1.000	0
	**-130*	1.000	0	0	0	0	0	0
*MDH-1**	**130*	0	0	0	0.125	0	0	0
	**120*	0	0.009	0	0.750	0	0	0
	**110*	0	0	0	0.125	0	0	0.833
	**100*	0	0.962	0	0	1.000	0.019	0.167
	**70*	1.000	0	1.000	0	0	0.981	0
	**60*	0	0.028	0	0	0	0	0
*MDH-2**	**200*	0	0	0	0	0	0	1.000
	**150*	1.000	0	0	0	0	0	0
	**130*	0	0.019	0	0	0	0.020	0
	**100*	0	0.896	1.000	0	0	0.980	0
	**80*	0	0.085	0	0	0	0	0
	**70*	0	0	0	0	1.000	0	0
	**35*	0	0	0	1.000	0	0	0
*MDH-3**	**0*	0	0	0	1.000	0	0	0
	**-30*	0	0	1.000	0	0	0	0
	**-70*	0	0.009	0	0	0	0	0
	**-100*	0	0.972	0	0	0	0	0
	**-135*	0.958	0	0	0	1.000	0	0
	**-140*	0	0	0	0	0	0	0.958
	**-160*	0.042	0.019	0	0	0	1.000	0.042

Table 3. continued

Locus	Allele	*C. abbreviatus*	*C. interruptus*	*C. itinus*	*C. joyneri*	*C. nigropinnatus*	*C. ochiaii*	*C. robustus*
*MEP-1**	**145*	0	0	0	0	0	0	1.000
	**130*	0.958	0	0	0	1.000	0	0
	**120*	0	0.019	0	0	0	0.019	0
	**110*	0.042	0.167	0	0	0	0.094	0
	**100*	0	0.704	0	0.083	0	0.642	0
	**90*	0	0	0	0.750	0	0	0
	**80*	0	0.093	0	0.125	0	0.198	0
	**65*	0	0.019	0	0.042	0	0.047	0
	**50*	0	0	0.750	0	0	0	0
	**40*	0	0	0.250	0	0	0	0
*MEP-2**	**240*	0	0	0.333	0	0	0	0
	**140*	0	0	0.667	0	0	0	0
	**125*	0	0.029	0	0	0	0	0
	**100*	0.958	0.519	0	0.125	0	0.010	0
	**70*	0	0.452	0	0.333	0	0	0
	**40*	0.042	0	0	0	0.833	0.220	0.042
	**25*	0	0	0	0	0.125	0.490	0.500
	**0*	0	0	0	0.542	0.042	0.280	0.458
*MPI**	**120*	0	0	0	0	1.000	0	0
	**110*	1.000	0	0	0	0	0	0.625
	**100*	0	1.000	1.000	0.955	0	1.000	0.042
	**90*	0	0	0	0.045	0	0	0.250
	**75*	0	0	0	0	0	0	0.083
*ODH**	**165*	0	0	0	0	0.167	0	1.000
	**155*	0	0	0	0	0.833	0	0
	**130*	0	0.108	0	0	0	0.010	0
	**100*	0	0.882	0.625	1.000	0	0.990	0
	**85*	1.000	0	0	0	0	0	0
	**70*	0	0	0.375	0	0	0	0
	**55*	0	0.010	0	0	0	0	0
*PGDH**	**200*	0	0	0	0	1.000	0	0
	**160*	0.042	0.009	0	0	0	0.375	0
	**150*	0.250	0.333	0.875	0.083	0	0.106	0.708
	**100*	0.417	0.630	0.125	0.167	0	0.385	0.167
	**80*	0.292	0.028	0	0.750	0	0.135	0.042
*PGM**	**700*	0	0	0	0.875	0	0	0
	**520*	0	0	0	0.042	0	0	0
	**250*	0	0.048	0.375	0	0.875	0.048	0
	**100*	0.833	0.567	0.500	0.083	0	0.538	0.042
	**-100*	0.167	0.385	0	0	0.125	0.375	0.583
	**-250*	0	0	0.125	0	0	0.038	0.375
*PROT-1**	**200*	0	0	0	1.000	1.000	0	0
	**160*	0	0	0	0	0	1.000	0
	**150*	0	0	0	0	0	0	1.000
	**140*	0	0	1.000	0	0	0	0
	**100*	0	1.000	0	0	0	0	0
	**40*	1.000	0	0	0	0	0	0
*PROT-2**	**120*	0	0	0	1.000	0	0	0
	**105*	0	0	0	0	0	0	1.000
	**100*	0	1.000	1.000	0	1.000	1.000	0
	**25*	1.000	0	0	0	0	0	0
*PROT-3**	**120*	0	0	0	0	1.000	0	0
	**100*	0	0.944	0	0	0	1.000	0.542
	**95*	0	0	0	0	0	0	0.458
	**85*	1.000	0.056	0	0	0	0	0
	**70*	0	0	1.000	1.000	0	0	0
*SOD-1**	**140*	0	0	0	1.000	0	0.563	0.292
	**130*	0	0	0	0	1.000	0.438	0.208
	**115*	0	0.286	0	0	0	0	0.125
	**100*	0	0.663	0	0	0	0	0.167
	**80*	1.000	0.051	0	0	0	0	0.208
	**60*	0	0	1.000	0	0	0	0
*SOD-2**	**100*	0	1.000	1.000	1.000	1.000	1.000	1.000
	**-100*	1.000	0	0	0	0	0	0

Table 4. Genetic distances (*D* values) between Japanese cynoglossid fish

	C. abbreviatus	*C. interruptus*	*C. itinus*	*C. joyneri*	*C. nigropinnatus*	*C. ochiaii*
C. abbreviatus						
C. interruptus	1.8983					
C. itinus	2.0897	0.8358				
C. joyneri	1.9103	0.9714	1.3527			
C. nigropinnatus	1.6922	0.9599	1.6591	1.5610		
C. ochiaii	1.5758	0.4143	1.0147	0.9131	0.9841	
C. robustus	1.6926	1.2448	1.8232	1.5120	1.0421	1.1693

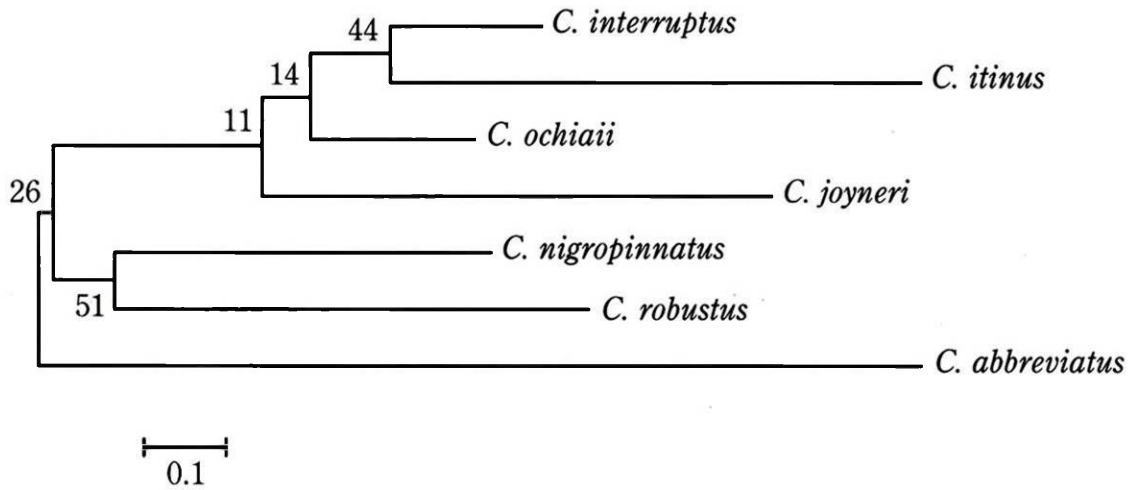

Fig. 1. A dendrogram for seven species of Japanese cynoglossid fish using the Neighbor-joining (NJ) method based on genetic distances (*D* values). Numbers in the dendrogram represent bootstrap probability values based on 1000 replicates.

Cynoglossus abbreviatus was most distant from the other six species (D=1.6922 – 2.0897; average 1.8098) (Table 4).

In the dendrogram, *C. interruptus*, *C. itinus*, *C. ochiaii* and *C. joyneri* formed a single cluster (Fig. 1). Although the distance between *C. nigropinnatus* and *C. robustus* was further, they formed another single cluster, which was connected with the former cluster (Fig. 1).

Discussion

Fujio and Kato[3] examined and summarized genetic variation in fish from Japan (8 orders with 41 species, including 15 in the Order Pleuronectiformes [heterosomate species]) using isozyme analysis. They gave average values of 0.194 and 0.059 for the proportion of polymorphic loci (P+P*) and heterozygosity (*Ho*), respectively. For the 15 heterosomate species, the P+P* and Ho values were 0.294 and 0.082, respectively. For the seven cynoglossid species examined in the present study, these values were 0.454 and 0.153, respectively (Table 2), and therefore higher than those of the 41 and 15 heterosomate species.

Smith and Fujio[4] reviewed genetic variation in many marine fish worldwide (9 orders with 106 species, including 29 heterosomate ones). They gave average *Ho* values of 0.055 for all species, and 0.071 for heterosomates, values that are similar to those given by Fujio and Kato[3]. According to their summary, the *Ho* values ranged from 0.000 to 0.180. In contrast, the *Ho* values of the seven cynoglossid species ranged from 0.081 to 0.226, and those of some species (*Cynoglossus interruptus* and *C. robustus*) exceeded the maximum given by Smith and Fujio[4] (Table 2). This also indicates how high the genetic variability of the cynoglossid species is. No species to date have been shown to have a heterozygosity exceeding that of the two species.

Smith and Fujio[4] hypothesized that habitat specialists, including the heterosomate species, tend to have higher *Ho* values. The cynoglossid species may support their hypothesis, as they may be so specialized ecologically that this accounts for their high

genetic variability.

Kijima *et al.*[15] examined and analyzed genetic structures of 31 heterosomate species (including four in the Cynoglossidae) by using isozymic markers. Their results showed that the average *He* values were between 0 and 0.2, and the average *He* was 0.082, being similar to the values given in the former two reports. Although their study included *C. interruptus*, the *He* value did not exceed 0.2. This might be due to differences in the number of loci examined, they examined 15 loci and the present study examined 34. In a species with high genetic polymorphism, the heterozygosity may be higher in proportion to the examined locus number.

The genetic polymorphism of *C. interruptus* was high, i.e. *Ho* resulted in the highest value (0.226) of all (Table 2). In this species, the presence of some local populations, which are genetically divergent from one another, has been revealed[8]. In the case of a species with high genetic polymorphism, such variation may occur. As such, intraspecific variation may be detected from the other cynoglossid species, and further studies are now required.

In terms of genetic distance, that between *C. interruptus* and *C. ochiaii* was the least of all (D=0.4143) (Table 4). It shows genetic "similarity" of the two species, although the distance between the two species was great enough for the specific level[9]. These two species are morphologically so similar that until recently they were regarded as the same species[5, 16, 17]. Therefore, the genetic "similarity" between the two species is consistent with the morphology.

In the dendrogram, *C. interruptus*, *C. itinus*, *C. ochiaii* and *C. joyneri* formed a single cluster (Fig. 1), suggesting monophyly of these four species. Menon[17], who reviewed the taxonomy of the Genus *Cynoglossus* worldwide, designated several groups and complexes in the genus based on morphology. One of his designations of the *kopsii* group included the *kopsii* complex, which consisted of *C. kopsii*, *C. interruptus* and *C. joyneri*. The *kopsii* group also included the *itinus* complex, which consisted of *C. itinus* only. As he regarded *C. ochiaii* as just a "form" of *C. interruptus*, *C. ochiaii* obviously belongs to the *kopsii* complex according to his criteria. Therefore, the fact that *C. interruptus*, *C. ochiaii*, *C. joyneri* and *C. itinus* formed a single cluster (Fig. 1), corresponds well with Menon's opinion.

On the other hand, Menon regarded *C. nigropinnatus* as a synonym of *C. interruptus*. However, these two species are so genetically different from each other (Fig. 1, Table 4), they are predictably distinct species. A recent morphological revision by Yokogawa *et al.*[6] also supported independency of the two species. Although *C. nigropinnatus* may also be categorized into the *kopsii* complex according to Menon's criteria, it was placed far from members of the *kopsii* complex in the dendrogram (Fig. 1). In fact, *C. nigropinnatus* was somewhat closer to *C. interruptus* and *C. ochiaii* in genetic distance (D values less than 1) (Table 4). However, as it is far from *C. joyneri* and *C. itinus* (D values more than 1.5) (Table 4), it is divergent from the cluster of the *kopsii* group members (Fig. 1). More genetic information based on DNA analysis is now needed to help reveal the phylogeny of *C. nigropinnatus*.

In Menon's revision[17], *C. abbreviatus* and *C. robustus* were categorized into the *arel* group (the *arel* complex) and the *heterolepis* group (the *heterolepis* complex), respectively, i.e. the two groups were phylogenetically placed far from the *kopsii* group. In the present study, they were placed separately from the cluster of the *kopsii* group members (Fig. 1), implying phylogenetic independency. This is consistent with Menon's view.

Of the seven cynoglossid species examined, although most have three lateral (dorsolateral, midlateral and ventrolateral) lines on the trunk, *C. interruptus* and *C. robustus* lack the ventrolateral line. Because *C. interruptus* and *C. robustus* are not closely related (Fig. 1), this suggests that loss of the ventrolateral line has occurred more than once during the evolution of the cynoglossids. Furthermore, loss of the ventrolateral line has occurred in species that are phylogenetically close to each other, e.g. *C. interruptus* and *C. ochiaii*. Thus the loss of the ventrolateral line may not be a very significant change for the cynoglossids. Because they usually have many lateral line systems, which are cutaneous sensory organs[2], it may not matter when some systems disappear.

The genetic information of the cynoglossids given by the present study and their morphological similarity were mostly congruent. However, there are still some incongruencies. In order to reveal the

phylogeny of this taxon, further studies will require the use of another genetic marker such as DNA. In addition it will be important to genetically examine additional cynoglossid species, which are distributed worldwide.

Acknowledgments

The author is grateful to Mr. Chomatsu Nishikawa, Marugame, Kagawa Pref., who assisted in collecting specimens of *Cynoglossus interruptus*. Dr. Hiromitsu Endo, Faculty of Science, Kochi University, and Mr. Hideo Sakaji, National Research Institute of Fisheries Science, Fisheries Research Agency, provided some specimens of *Cynoglossus ochiaii*. Dr. Nobuhiko Taniguchi, Faculty of Life Science and Biotechnology, Fukuyama University, gave available information on tools of statistical computation. Dr. Shingo Seki, Faculty of Agriculture, Kochi University, provided some literature. Finally, I wish to thank Ms. Dawn L. McKendry, Middleburg, Florida, US, for checking the manuscript.

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日本産シタビラメ科（カレイ目）魚類７種の遺伝構造と系統

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日本産シタビラメ科魚類７種、コウライアカシタビラメ *Cynoglossus abbreviatus*、ゲンコ *C. interruptus*、ミナミアカシタビラメ *C. itinus*、アカシタビラメ *C. joyneri*、ヒレグロゲンコ *C. nigropinnatus*、オキゲンコ *C. ochiaii*、イヌノシタ *C. robustus* のアイソザイム分析により遺伝構造を調べ、またこれらの系統類縁関係について考察した。本科魚類は全般に高い遺伝的多様性を示したが、特にゲンコとイヌノシタでは平均ヘテロ接合体率の値が0.2を超え、魚類でこれまでに知られている最高値を上回った。各種の遺伝子頻度から種間の遺伝的距離（*D*値）を計算し、それに基づいて分岐図を作成した。分岐図では、ゲンコ、オキゲンコ、アカシタビラメ、ミナミアカシタビラメがひとつのクラスターを形成し、形態による類縁関係とよく一致した。一方、形態的特徴からこのグループに含まれるヒレグロゲンコはこれらの種とは別のクラスターに含まれた。また、コウライアカシタビラメと他種との遺伝的距離がかなり大きかった。