

## Genetic Differentiation and Reproductive Isolation among Three Types of the Floating Goby (*Chaenogobius annularis*) in Korea

Ho Young Suk, Jong Bum Kim, Mi Sook Min, and Suh Yung Yang\*

Department of Biology, College of Natural Sciences, Inha University, Incheon 402-751, Korea

Since the floating goby, *Chaenogobius annularis*, has intricate and diverse morphological variations, allozymic analysis at 25 loci was carried out for their populations in Korea to clarify its taxonomic status. A genetic assay carried out revealed that the floating gobies were clearly divided into three genetic groups (Type-A, Type-B and Type-C) in Korea. Alternative alleles were fixed at six loci (*Aco*, *Gp*, *Ldh-1*, *Got-1*, *Gp-2*, *Gp-3*). Some loci had considerable heterogeneity among three types and no evidence of gene exchange in sympatric populations was found from statistical analyses. The genetic similarity (Rogers' S) among three types was lower than 0.80 and divergent time estimate indicates that they were speciated during 1.2-1.8 million year before present (MYBP). Also, these three types of *C. annularis* were distinguished morphologically from each other by several characters such as band ornamentations. In conclusion, the evidences presented here support recognition of three types of *Chaenogobius annularis* as typical discrete species.

**KEY WORDS:** Electrophoresis, Cryptic Species, Gobiidae, *Chaenogobius annularis*, Speciation

Scrutinies of taxonomic status in species level, in particular cryptic species, have become a major topic of concern or debate in systematic biology and are essential to establishment of phylogenetic framework among taxa. Without information on this no other biological studies can successfully be carried out in a rational manner (Ferguson, 1980). Nevertheless a number of investigators have utilized only a portion of specific characters which were based largely on morphological variations to identify species. Therefore in many groups, a number of species still remain to be classified unaccurately due to the subjection of the classification criteria, geographic variation, shortage of ecological data and sibling species and

so forth. Especially, because morphological patterns of variation can have a nongenetic component (James, 1983), systematic and evolutionary inferences might be compromised. An example of such a problem in species recognition exists in the floating goby, *Chaenogobius annularis* (Gill) in Korea. *C. annularis*, a small freshwater fish belonging to family Gobiidae, occurs widely throughout eastern Asia, including China, Korea, Japan and southeastern Siberia, and has an outstanding feature which is a black spot on the basal region of the first dorsal fin (Akihito *et al.*, 1984; Akihito, 1986; Cheng and Zheng, 1987). Mori and Uchida (1934) collected specimens of this species from Wonsan and Chyungjin in Korea for the first time and reported as *Rhinogobius macrognotus* (Bleeker). Thereafter, Mori (1952) revised the

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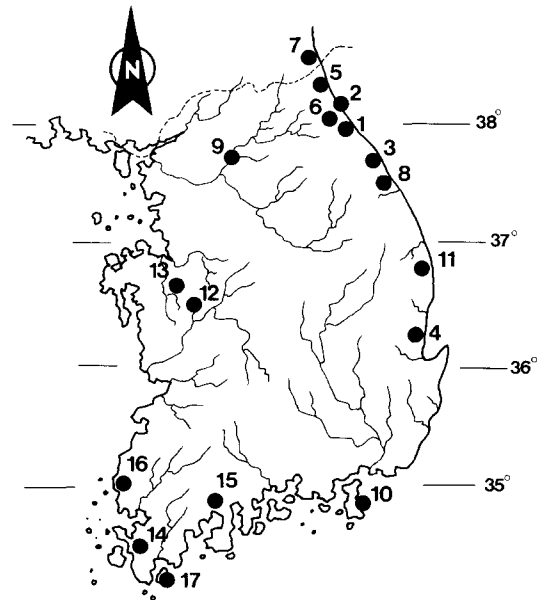
\*To whom correspondence should be addressed.

species name to *Chaenogobius annularis*. But the taxonomy of the various morphotypes assigned to *C. annularis* is in considerable disarray (Kim *et al.*, 1987) and has long been a matter of dispute. As currently recognized, *C. annularis* of Japan is divided into three groups of species level according to habitat specialization and morphology such as cross band ornamentations, coloration during breeding season and called *Chaenogobius urotaenia*, *Chaenogobius* sp. 1 and *Chaenogobius* sp. 2 (Takagi, 1966a, b; Nakanishi, 1978; Akihito *et al.*, 1984; Akihito, 1986), but questions regarding species status frequently has arisen. In recent years, a number of researchers have utilized biochemical technique to identify morphologically cryptic species (Hillis, 1987; Rohlf and Wooten, 1988). Among this approach, isozyme electrophoresis has generated a massive comparative data base and is currently the most widely used approach in molecular systematics. Since proteins are direct gene products, resolution of electromorphs are generally considered to be very useful for estimation of divergence between populations (Taniguchi *et al.*, 1985). Actually it has been well documented in numerous reports that isozyme analysis settles a taxonomic problem, what we call, incipient species, ecophenotype and so on (Dowling and Moore, 1984; Yang and Min, 1988).

In this paper, we report the result of an electrophoretic and morphological survey designed to calibrate the extent of divergence among *C. annularis* populations to elucidate their taxonomic status in Korea.

## Materials and Methods

A total of 428 individuals of 24 population samples collected in 17 localities in Korea were examined for genetic analysis (Fig. 1, Table 1). Alive samples were transported to the laboratory, then were frozen and stored at  $-70^{\circ}\text{C}$ . Skeletal muscle was excised and grinded individually by glass homogenizer in equal volume of distilled water (small individuals were ground whole) and was centrifugated at 18,000 rpm for 30 min. to



**Fig. 1.** Collection locales for floating goby, *Chaenogobius annularis* in Korea. Population numbers refer to Table 1.

obtain the supernatant for electrophoresis. After the muscle excision each specimen's band characters introduced by Akihito (1986) were recorded and tagged for preservation. Water soluble general proteins and enzymes were resolved by horizontal starch gel (12%) electrophoresis following the methods of Yang *et al.* (1991). Staining protocols followed the method of Buth (1986) and three buffer systems were used; (1) Tris-Citrate II gel and tray buffer, pH 8.0; (2) Tris-Citrate gel buffer and Lithium Hydroxide-boric acid tray buffer, pH 8.1; and (3) Tris malate EDTA gel and tray buffer, pH 7.4. Genetic data were analyzed with BIOSYS-I (Swofford and Selander, 1981). Loci were numbered sequentially with integers beginning with one for the most anodal form, and alleles were designated alphabetically. Allele frequencies between populations were compared and the degree of genetic variability within populations was estimated. Matrices of genetic distance coefficients (Nei, 1972) and genetic similarity coefficients (Rogers, 1972) were calculated for paired combinations of all populations. Rogers' (1972) similarity values

**Table 1.** Collection localities, sample sizes and collection dates of *Chaenogobius annularis* examined in this study.

Collection localities	Sample size	Date
1. Kangreung: Jibyun-dong, Kangreung-city, Kangwon-do	20	Apr. 28, 1992
2. Samcheog: Geundeog-myon, Samcheog-gun, Kangwon-do	22	Jun. 30, 1992
3. Sokcho: Seolak-dong, Sokcho-city, Kangwon-do	65	Aug. 10, 1993
4. Yeongdog: Chooksan-myon, Yeongdog-gun, Kyeongsangbuk-do	10	Jun. 27, 1992
5. Songjiho: Jookwang-myon, Koseong-gun, Kangwon-do	20	Aug. 12, 1993
6. Yeongok: Yungok-ri, Myeongjoo-gun, Kangwon-do	20	Aug. 13, 1993
7. Myeongpa: Myeongpa-ri, Hyeonne-myon, Koseong-gun, Kangwon-do	62	Aug. 9, 1993
8. Hosan: Hosan-ri, Wondeog-eup, Samcheog-gun, Kangwon-do	45	Sep. 12, 1993
9. Kapyeong: Kapyeong-eup, Kapyeong-gun, Kyunggi-do	10	Sep. 25, 1993
10. Geoje: Sinhyeon-eup, Geoje-gun, Kyeongsangnam-do	43	Oct. 3, 1993
11. Uljin: Noeum-ri, Keunam-myon, Uljin-gun, Kyeongsangbuk-do	20	Jun. 29, 1992
12. Gwangchun: Gwangchun-eup, Boryung-gun, Choongchungnam-do	20	Jun. 14, 1993
13. Jinjook: Jinjookmyon, Hongseong-gun, Choongchungnam-do	20	Jun. 14, 1993
14. Haemam: Gyegok-myon, Haenam-gun, Cheollanam-do	20	Jul. 11, 1994
15. Gwangyang: Gwangyang-eup, Gwangyang-gun, Cheollanam-do	20	Jun. 25, 1992
16. Mooan: Cheonggye-myon, Mooan-gun, Cheollanam-do	4	Jun. 25, 1992
17. Wando: Yongsojeong, Wando-gun, Cheollanam-do	7	May. 25, 1991

between pairs of sample were further summarized by cluster analysis derived from unweighted pair-group method using arithmetic averages linkage (UPGMA, Sneath and Sokal, 1973). In sympatric areas to test for departures from expected Hardy-Weinberg proportions of genotypes within population and the degree of reproductive isolation, we employed chi-square test, Wright's (1965) fixation index and exact probability which were used in evaluating the chi-square statistics (Vithayasai, 1973).

## Results

Observed allelic frequencies for the variable loci are given in Table 2. All populations shared a single common allele for ten (40%) of the 25 presumptive loci scored, *Alat-1*, *Alat-2*, *Pgi-2*, *Got-2*, *Ldh-2*, *Mdh-1*, *Mdh-3*, *Gp-4*, *Gp-5*, *Gp-6*, whereas the remaining 15 loci exhibited varying degrees of electrophoretic variation. As we have represented in Table 2, 3 and Fig. 2 genetic assay revealed that *Chaenogobius annularis* species were clearly divided into three genetic groups (Type-A, Type-B, Type-C) in Korea. These types were consistently fixed for the alternative electromorphs, Type-A distinguished from Type-B on

*Aco*, *Est-2* and *6Pgd* loci, from Type-C on *Ldh-1*, *6Pgd*, *Aco*, *Got-1*, *Gp-2* and *Gp-3* and allelic disparity at *Aco*, *Ldh-1*, *Got-1*, *Est-2*, *Gp-2* and *Gp-3* were found in comparison between Type-B and Type-C. Since each type shared no same electromorph at these loci mentioned above we convinced that these loci are genetic markers to discriminate *C. annularis* types. Also, significant heterogeneity among three types for dominant allele frequencies in three loci, *Pept-1*,  $\alpha$ -*Gpd* and *Mdh-2* were detected (Table 2). Two alleles generally occurred at  $\alpha$ -*Gpd* locus. Allele  $\alpha$ -*Gpd*<sup>c</sup> was fixed in Type-C and had a frequency of 0.953 in Type-A, on the other hand allele  $\alpha$ -*Gpd*<sup>b</sup> had a frequency of 0.893 in Type-B. At the *Mdh-2* locus, there were two alleles, *Mdh-2*<sup>a</sup> and *Mdh-2*<sup>b</sup> in *C. annularis*. The *Mdh-2*<sup>b</sup> allele was fixed in Type-C but the *Mdh-2*<sup>a</sup> allele is near fixation in Type-A and Type-B at the frequency of 0.897, 0.985 respectively. Five alleles occurred at the *Pept-1*. The *Pept-1*<sup>a</sup> allele is found mainly in Type-A (0.867), *Pept-1*<sup>c</sup> allele is near fixation in Type-B and *Pept-1*<sup>b</sup> allele is fixed in Type-C. One of three types, Type-A, occupies the eastern part of Korea, Gapyeong in western part and Geoje in southern part, Type-B inhabits in all parts of the country and Type-C is confined in north-east coastal region.

**Table 2.** Allele frequencies of 15 polymorphic loci for 24 populations of *Chaenogobius annularis*. See Table 1 for key to locality numbers.

Locus Allele	<i>Chaenogobius annularis</i> Type-A										Type-B		
	1	2	3	4	5	6	7	8	9	10	2	3	
<i>Est-2</i>	a	1.000	0.952	0.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.053	
	b		0.032									0.947	1.000
	c		0.016										
<i>Aco</i>	a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000		
	b												
	c											1.000	1.000
$\alpha$ - <i>Gpd</i>	a												
	b	0.025		0.133			0.075		0.059	0.250		0.882	0.500
	c	0.075	1.000	0.887	1.000	1.000	0.925	1.000	0.941	0.750	1.000	0.118	0.500
	d												
<i>Pept-1</i>	a	1.000	0.790	1.000	1.000	0.775	0.700	0.818	0.618	1.000	1.000		
	b												
	c		0.210			0.225	0.300	0.182	0.382			1.000	1.000
	d												
	e												
<i>Me-1</i>	a												
	b	1.000	1.000	0.906	1.000	1.000	1.000	0.909	1.000	1.000	1.000	1.000	1.000
	c			0.094				0.091					
<i>Idh</i>	a			0.026								0.027	
	b	1.000	1.000	0.974	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.947	1.000
	c												
<i>Pgm</i>	a	0.225	0.290	0.221	0.500	0.100	0.250	0.068		0.500			
	b	0.290	0.710	0.789	0.500	0.875	0.750	0.941	1.000	0.500	1.000	0.961	1.000
	c					0.025						0.039	
<i>Mdh-2</i>	a	0.975	0.919	0.947	0.929	0.950	0.900	0.909	0.853	0.250	0.350	0.947	1.000
	b	0.025	0.081	0.053	0.071	0.050	0.100	0.091	0.147	0.750	0.650	0.026	
<i>Pgi-1</i>	a			0.263	0.071	0.175	0.175	0.200		0.050	0.100		
	b	1.000	0.883	0.737	0.929	0.929	0.825	0.800	1.000	0.050	0.400	0.816	1.000
	c		0.117								0.500	0.184	
<i>Got-1</i>	a												
	b												
	c	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
<i>Ldh-1</i>	a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.947	1.000
	b											0.028	
<i>Gp-1</i>	a										1.000		
	b	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000		1.000	1.000
<i>Gp-2</i>	a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	b												
<i>Gp-3</i>	a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	b												
<i>6pgd</i>	a												
	b	1.000	1.000	0.968	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.039	
	c			0.032								0.961	1.000

Table 2. Continued.

Locus Allele	<i>Chaenogobius annularis</i> Type-B										Type-C		
	4	8	10	11	12	13	14	15	16	17	2	7	
<i>Est-2</i>	a	0.053										1.000	1.000
	b	1.000	0.964	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000		
	c												
<i>Aco</i>	a												
	b											1.000	1.000
	c	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000		
<i>α-Gpd</i>	a											0.025	
	b	1.000	0.929	0.875	0.975	0.975	0.875	0.900	0.875	1.000	0.929		
	c	-0.071	0.125	0.025	0.025	0.125	0.075	0.125			0.071	1.000	1.000
	d												
<i>Pept-1</i>	a												
	b											1.000	1.000
	c	1.000	0.964	0.975		1.000	1.000	1.000	1.000	1.000	1.000		
	d	0.036		0.025									
	e	1.000											
<i>Me-1</i>	a											0.071	0.029
	b	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.929	0.971
	c												
<i>Idh</i>	a											0.014	
	b	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.957
	c												
<i>Pgm</i>	a												
	b	1.000	0.964	0.825	1.000	0.950	0.875	1.000	1.000	1.000	0.857	1.000	1.000
	c	0.039		0.175	0.050		0.125					0.143	
<i>Mdh-2</i>	a	1.000	1.000	1.000	1.000	1.000	1.000	0.875	1.000	1.000	1.000		
	b											0.125	1.000
<i>Pgi-1</i>	a	0.107		0.125	0.025	0.150	0.150	0.175	0.200	0.333	0.143	0.500	0.029
	b	1.000	0.839	0.875	0.900	0.850	0.850	0.825	0.800	0.667	0.857	0.500	0.971
	c	0.054		0.075									
<i>Got-1</i>	a											1.000	1.000
	b											0.053	
	c	1.000	1.000	1.000	1.000	1.000	1.000	0.947	1.000	1.000	1.000		
<i>Ldh-1</i>	a	1.000	1.000	1.000	1.000	1.000	1.000	0.974	1.000	1.000	1.000		
	b											0.026	1.000
<i>Gp-1</i>	a	1.000			1.000	1.000	1.000	1.000	1.000	1.000	1.000		
	b	1.000	1.000	1.000									
<i>Gp-2</i>	a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000		
	b											1.000	1.000
<i>Gp-3</i>	a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000		
	b											1.000	
<i>6pgd</i>	a											0.036	
	b	0.036		0.036		0.039						1.000	0.964
	c	1.000	0.964	1.000	0.964	1.000	1.000	0.061	1.000	1.000	1.000		

**Table 3.** Rogers (1972)' genetic similarities(above diagonal) and Nei (1972)' genetic distances (below diagonal) for 24 populations of *Chaenogobius annularis*.

Population	<i>Chaenogobius annularis</i> Type-A										Type-B	
	1	2	3	4	5	6	7	8	9	10	2	3
<i>C. annularis</i> Type-A												
1. Kangreung	-	.984	.972	.943	.977	.975	.942	.959	.947	.893	.811	.790
2. Samcheog	.003	-	.965	.937	.980	.977	.931	.956	.951	.901	.796	.787
3. Sokcho	.003	.005	-	.929	.975	.979	.936	.969	.931	.884	.813	.806
4. Yeongdog	.045	.048	.048	-	.930	.928	.891	.911	.900	.846	.754	.745
5. Songjiho	.004	.003	.003	.052	-	.986	.947	.974	.947	.895	.815	.797
6. Yeongok	.006	.004	.002	.051	.001	-	.937	.976	.940	.894	.814	.800
7. Myeongpa	.045	.048	.044	.097	.044	.046	-	.938	.906	.855	.822	.791
8. Hosan	.010	.010	.006	.061	.003	.003	.043	-	.935	.892	.832	.807
9. Gapyeong	.030	.024	.027	.076	.027	.027	.071	.027	-	.924	.768	.755
10. Geoje	.081	.072	.079	.131	.075	.074	.123	.075	.056	-	.740	.717
<i>C. annularis</i> Type-B												
2. Samcheog	.192	.198	.170	.256	.175	.170	.175	.150	.232	.282	-	.019
3. Sokcho	.201	.207	.174	.261	.186	.178	.191	.165	.234	.279	.940	-
4. Yeongdog	.176	.184	.153	.240	.161	.153	.161	.141	.216	.249	.052	.037
8. Hosan	.214	.219	.187	.281	.196	.188	.200	.170	.249	.286	.009	.012
10. Geoje	.378	.368	.379	.368	.322	.324	.328	.179	.261	.299	.096	.078
11. Uljin	.224	.231	.197	.291	.207	.199	.208	.367	.398	.389	.010	.012
12. Gwangchun	.267	.271	.240	.336	.248	.240	.252	.222	.306	.234	.049	.053
13. Jinjook	.269	.274	.243	.340	.251	.243	.253	.223	.309	.236	.049	.056
14. Haenam	.278	.284	.250	.349	.260	.251	.262	.232	.319	.243	.052	.055
15. Gwangyang	.274	.278	.246	.344	.254	.246	.257	.227	.313	.238	.050	.055
16. Moonan	.342	.343	.309	.417	.319	.310	.325	.291	.379	.294	.101	.104
17. Wando	.330	.337	.299	.404	.311	.303	.314	.284	.375	.296	.095	.096
<i>C. annularis</i> Type-C												
3. Sokcho	.542	.526	.527	.623	.521	.520	.593	.504	.490	.546	.538	.561
7. Myeongpa	.520	.521	.510	.603	.510	.511	.569	.489	.491	.556	.561	.593

Based on allelic frequencies listed in Table 2 average genetic similarities and distances among populations of *C. annularis* were estimated (Table 3). Intertypal genetic similarities ( $S_{A-B}=0.752$ ,  $S_{A-C}=0.578$ ,  $S_{B-C}=0.531$ ) are notably lower than conspecific populations that is known in many vertebrates species (Patton and Yang, 1977; Avise and Aquadro, 1982). Type-A populations of *C. annularis* was more similar to Type-B populations than to Type-C populations. The UPGMA cluster analysis (Fig. 2) clearly shows the level of dissimilarities among the types as well as the similarities among populations within a given types.

Three genetic types of *C. annularis* were distinguished morphologically from each other by

cross band ornamentations (1), a black spot on the basal part on the first dorsal fin (2) and the shape of spot on caudal peduncle (3) (Fig. 3).

To examine the level of reproductive isolation among the types at three sympatric areas, Sokcho (Type-A, B, C), Myeongpa (Type-A, C) and Hosan (Type-A, B) were analysed statistically (Table 4). These populations were deviated significantly from expectation under random mating equilibrium for marker loci and heterogeneous loci among three types ( $\chi^2$  test,  $P<0.001$ ). In Wright (1965)'s  $F$ -statistics fixation index ( $F_{IS}$ ) '+' indicates heterozygote deficiency and  $F_{IS}$  value for each sympatric population close to '1' as would be expected for obligate inbreeding ( $F_{IS}$  value : Sokcho=0.938, Myeongpa=0.963, Hosan=

Table 3. Continued.

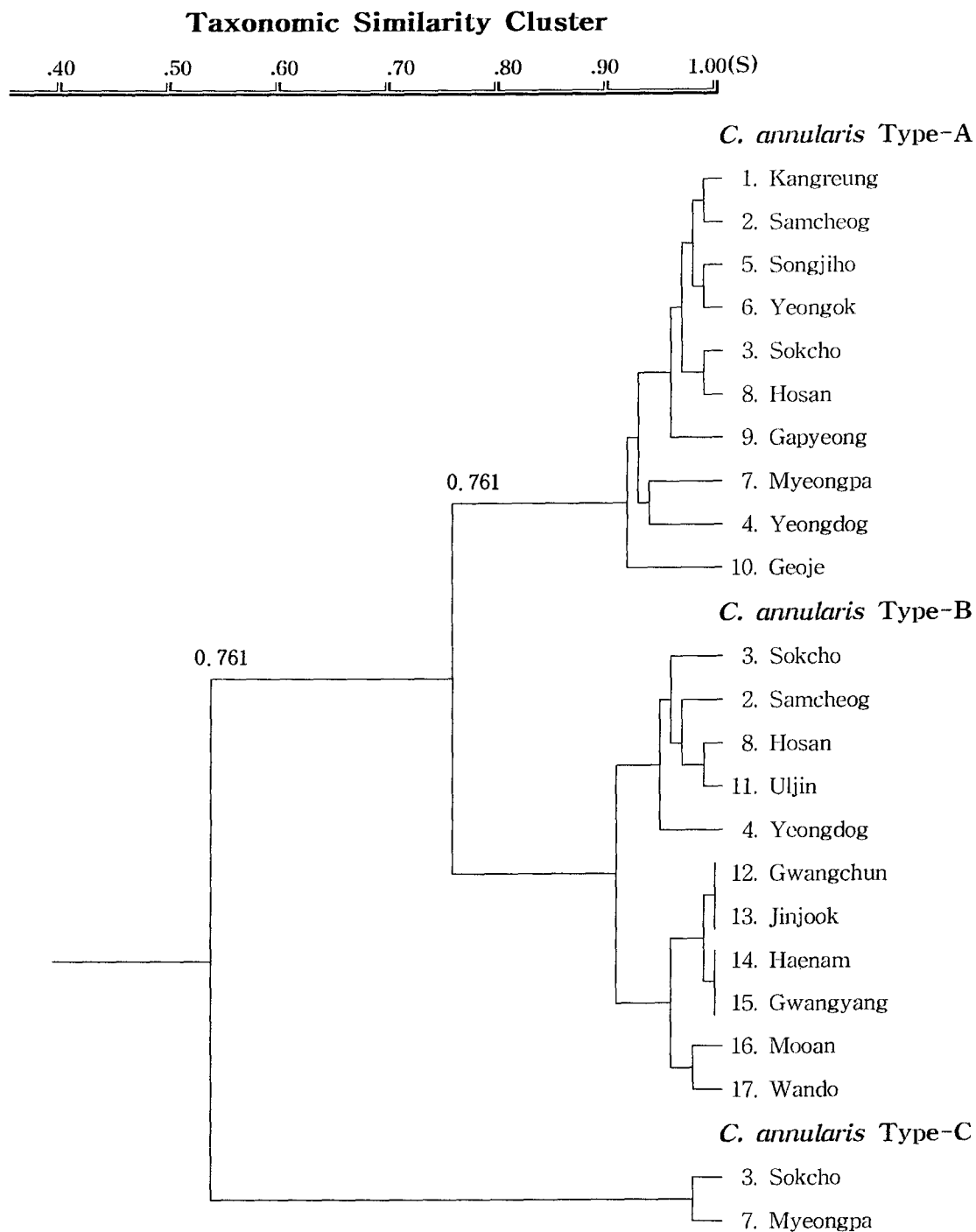
Population	<i>C. annularis</i> Type-A										Type-B	
	1	2	3	4	5	6	7	8	9	10	2	3
<i>C. annularis</i> Type-A												
1. Kangreung	.831	.792	.762	.789	.751	.750	.747	.748	.698	.709	.589	.572
2. Samcheog	.816	.788	.742	.780	.752	.747	.744	.747	.704	.706	.586	.585
3. Sokcho	.830	.801	.789	.798	.760	.759	.755	.757	.711	.722	.588	.576
4. Yeongdog	.774	.740	.712	.736	.701	.699	.696	.697	.647	.659	.540	.527
5. Songjiho	.835	.806	.791	.799	.769	.776	.763	.767	.716	.724	.595	.589
6. Yeongok	.834	.805	.789	.797	.770	.765	.762	.765	.717	.724	.589	.585
7. Myeongpa	.842	.802	.791	.800	.758	.761	.757	.758	.708	.716	.562	.545
8. Hosan	.846	.823	.799	.816	.775	.780	.774	.775	.728	.732	.602	.592
9. Gapyeong	.788	.759	.723	.753	.718	.716	.712	.715	.673	.673	.599	.604
10. Geoje	.760	.732	.691	.724	.770	.771	.765	.768	.733	.723	.558	.578
<i>C. annularis</i> Type-B												
2. Samcheog	.940	.973	.912	.976	.934	.939	.933	.934	.887	.891	.576	.558
3. Sokcho	.927	.957	.904	.954	.917	.914	.913	.915	.867	.880	.538	.527
4. Yeongdog	–	.950	.904	.954	.900	.909	.911	.907	.867	.866	.516	.498
8. Hosan	.039	–	.925	.992	.943	.952	.950	.950	.905	.908	.556	.547
10. Geoje	.099	.123	–	.142	.942	.951	.901	.901	.901	.903	.523	.518
11. Uljin	.041	.000	.067	–	.887	.877	.953	.949	.907	.907	.555	.542
12. Gwangchun	.089	.044	.077	.044	–	.991	.989	.992	.943	.953	.515	.512
13. Jinjook	.086	.042	.035	.042	.001	–	.994	.995	.948	.952	.517	.509
14. Haenam	.085	.042	.075	.042	.001	.001	–	.996	.950	.954	.514	.507
15. Gwangyang	.086	.042	.086	.043	.001	.000	–	.948	.954	.954	.516	.510
16. Mooan	.134	.088	.123	.088	.045	.044	.042	.043	–	.984	.544	.552
17. Wando	.132	.087	.133	.086	.045	.043	.043	.042	.003	–	.553	.545
<i>C. annularis</i> Type-C												
3. Sokcho	.685	.577	.614	.583	.663	.650	.679	.671	.599	.599	–	.980
7. Myeongpa	.658	.592	.607	.603	.652	.665	.663	.658	.598	.595	.009	–

0.914). Data in Table 4 imply that three types of *C. annularis* are separate species and have separate gene pools.

Based on allelic frequencies listed in Table 2 the degree of genetic variabilities of each populations was estimated (Table 5). The mean number of allele/locus ( $\bar{A}$ ), mean percentage of polymorphic loci ( $\bar{P}$ ) and heterozygosity (observed, expected,  $\bar{H}_o$ ,  $\bar{H}_e$ ) were more or less low ( $\bar{A}=1.19$ ,  $\bar{P}=14.0$ ,  $\bar{H}_o=0.035$ ,  $\bar{H}_e=0.045$  for Type-A,  $\bar{A}=1.14$ ,  $\bar{P}=8.73$ ,  $\bar{H}_o=0.020$ ,  $\bar{H}_e=0.027$  for Type-B and  $\bar{A}=1.19$ ,  $\bar{P}=14.0$ ,  $\bar{H}_o=0.035$ ,  $\bar{H}_e=0.045$  for Type-C). Low genetic variability may represent a general attribute of freshwater goby when compared to the average for freshwater fish (Yang *et al.*, 1991; Kim *et al.*, 1992; Suk *et al.*, 1993).

## Discussion

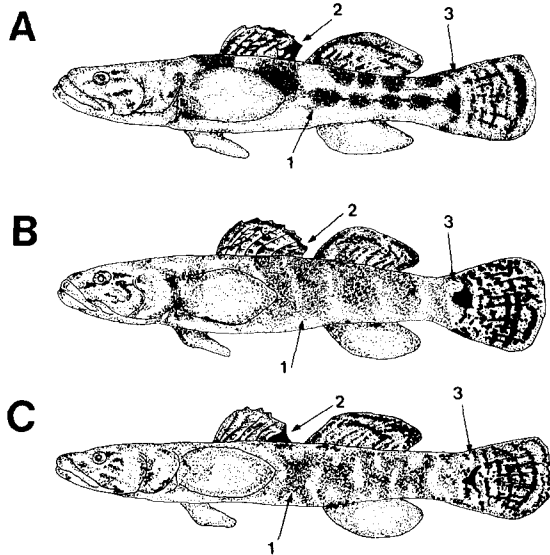
So far *C. annularis* has been known to unique species and the taxonomic status of this species has been the object of spirited debate for a long time owing to the occurrence of various morphotypes in Korea. Kim *et al.* (1987) recognized species based on the pattern of sensory canals and pit organs on its head and mentioned that several morphological types occur in Korea. But *C. annularis* has been only imperfectly understood in systematics, for the dearth of comparative data and taxonomically useful morphological character has made it difficult to execute stable groupings of the populations of this species. To circumvent this obstacles for morphological classification and to perform exact



**Fig. 2.** UPGMA cluster analysis of Rogers' (1972) genetic similarity among 24 populations of *Chaenogobius annularis* from Korea.



grouping of *C. annularis* populations we employed genetic analysis. Though the use of electrophoretic characters as diagnostic key is presently being rarely utilized, it holds great potential (Avisé, 1974) and certainly be extremely useful in confirming suspected hybrids and



**Fig. 3.** Three morphological types of *Chaenogobius annularis* from Korea. Arrows and numbers (1: cross band ornamentalations, 2: a black spot on the basal part on the first dorsal fin, 3: the shape of spot on caudal peduncle) indicate distinct characters among three types. A: Type-A, B: Type-B, C: Type-C.

identifying juveniles or morphologically anomalous specimen (Buth, 1980). And because the results of evolutionary researches at the morphological and molecular levels are sometimes uncoupled (Hillis, 1987), protein studies often reveal discordance between allozyme divergence and taxonomic boundaries inferred from morphological criteria. As a result of electrophoretic survey we confirmed Korean populations of *C. annularis* comprise three genetically distinct groups which showed considerable differentiation (Fig. 2, Table 2, 3). There were three diagnostic loci between Type-A and Type-B, six diagnostic loci between Type-A and Type-C, and six diagnostic loci between Type-B and Type-C. The amount of genetic divergence between each type ( $S_{A-B}=0.752$ ,  $S_{A-C}=0.578$ ,  $S_{B-C}=0.531$ ) were correspond to the range of values obtained at the interspecific level of differences for the vertebrate species by other researcher (Patton and Yang, 1977; Barrowclough *et al.*, 1981; Avisé and Aquadro, 1982). Divergent time estimation ( $t=5 \times 10^6D$ ; Nei, 1975) of three types indicate that they diverged during 1.2-1.8 million year before present (MYBP). Even if we show the substantial amount of genetic divergence, genetic similarity is not always correspond in compliance with taxa. And also, because according to biological species concept species refer to natural populations that is reproductively isolated from other such groups (Mayr, 1969), genetic similarity is not necessarily

**Table 4.** Observed ( $H_o$ ) and expected ( $H_e$ ) number of heterozygosity, chi-square statistics (Degree of freedom in parenthesis) for deviation from Hardy-Weinberg expectation in all genotypes and Wright (1965)' fixation index ( $F_{IS}$ ) for deficiency of heterozygote on diagnostic loci among each type at three sympatric areas.

Locus	Sokcho (N=152)				Myeongpa (N=170)				Hosan (N=90)			
	$H_o$	$H_e$	$F_{IS}$	$\chi^2$ value	$H_o$	$H_e$	$F_{IS}$	$\chi^2$ value	$H_o$	$H_e$	$F_{IS}$	$\chi^2$ value
<i>Est-1</i>	3	77.23	0.961	149.03(3)	–	–	–	–	0	42.55	1.000	91.14(1)
<i>Aco</i>	0	87.71	1.000	310.34(3)	0	84.83	1.000	171.01(1)	0	42.55	1.000	91.14(1)
<i><math>\alpha</math>-Gpd</i>	8	78.10	0.897	324.18(6)	–	–	–	–	4	43.44	0.907	75.09(1)
<i>Pept-1</i>	10	87.17	0.885	468.89(3)	14	91.52	0.847	297.30(3)	10	35.37	0.716	162.95(3)
<i>Mdh-2</i>	10	30.21	0.668	68.04(1)	9	84.75	0.893	171.02(1)	–	–	–	–
<i>Got-1</i>	0	25.43	1.000	153.12(1)	0	84.83	1.000	171.02(1)	–	–	–	–
<i>Ldh-1</i>	0	25.43	1.000	153.12(1)	1	84.75	0.988	167.00(1)	–	–	–	–
<i>Gp-2</i>	0	25.43	1.000	153.12(1)	0	84.83	1.000	171.02(1)	–	–	–	–
<i>Gp-3</i>	0	25.43	1.000	153.12(1)	0	84.83	1.000	171.02(1)	–	–	–	–
<i>6Pgd</i>	2	73.28	0.973	144.85(1)	1	84.59	0.988	167.00(1)	2	43.28	0.945	91.18(3)

**Table 5.** Genetic variability measures of *Chaenogobius annularis* three types over 24 populations.

Population	Mean No. of Alleles/Locus	Percentage of Polymorphic Loci	Mean Heterozygosity	
			Direct( $H_o$ )	Expected( $H_e$ )
<i>Chaenogobius annularis</i> Type-A				
1. Kangreung	1.1	4.0	0.018±0.014	0.018±0.014
2. Samcheog	1.2	16.0	0.034±0.018	0.040±0.021
3. Sokcho	1.4	24.0	0.035±0.014	0.067±0.023
4. Yeongdog	1.1	12.0	0.017±0.009	0.033±0.022
5. Songjiho	1.2	16.0	0.038±0.021	0.039±0.020
6. Yeongok	1.2	20.0	0.064±0.029	0.058±0.026
7. Myeongpa	1.1	8.0	0.007±0.005	0.022±0.014
8. Hosan	1.3	24.0	0.033±0.017	0.056±0.024
9. Gapyeong	1.2	8.0	0.028±0.021	0.051±0.031
10. Geoje	1.1	24.0	0.080±0.047	0.067±0.037
Average	1.19	14.0	0.035	0.045
<i>Chaenogobius annularis</i> Type-B				
2. Sokcho	1.5	24.0	0.057±0.039	0.077±0.027
3. Samcheog	1.0	4.0	0.040±0.040	0.040±0.040
4. Yeongdog	1.0	0.0	0.000±0.000	0.000±0.000
8. Hosan	1.2	8.0	0.016±0.013	0.025±0.013
10. Geoje	1.2	7.5	0.022±0.010	0.024±0.010
11. Uljin	1.2	4.0	0.012±0.008	0.027±0.008
12. Gwangcheon	1.1	12.0	0.020±0.014	0.034±0.019
13. Jinjook	1.1	8.0	0.010±0.010	0.019±0.013
14. Haenam	1.1	8.0	0.018±0.013	0.016±0.011
15. Gwangyang	1.2	12.0	0.018±0.010	0.023±0.014
16. Mooan	1.0	4.0	0.000±0.000	0.021±0.021
17. Wando	1.1	12.0	0.029±0.016	0.027±0.015
Average	1.14	8.73	0.020	0.027
<i>Chaenogobius annularis</i> Type-C				
3. Sokcho	1.1	4.0	0.044±0.040	0.025±0.022
7. Myeongpa	1.1	0.0	0.007±0.004	0.009±0.005
Average	1.1	2.0	0.026	0.017

\* A locus is considered polymorphic if the frequency of the most common allele does not exceed 0.95.

credible in recognition of species. Therefore, the test of speciation ideally must involve a determination of the level of reproductive isolation between the taxa. Protein electrophoresis is well suited for such a determination, provided suitable marker genes distinguish taxa at sympatry (Dowling and Moore, 1984). To manifest reproductive isolation among three types of *C. annularis*, intensive reckon on interbreeding was requested at sympatry where sample size large enough to detect significant deviations from Hardy-Weinberg equilibrium. Under regarded as unique species certified genotype frequency at

three sympatric area, Myungpa (N=170), Sokcho (N=152), Hosan (N=90) disclosed severe heterozygote deficiency in marker loci (using  $F_{IS}$  value) and deviation from random mating expectation based on  $\chi^2$  test ( $P < 0.001$ ), imply that these populations have separate gene pools (Table 4). Comparison of genotypic frequencies with Hardy-Weinberg expectations allows an unambiguous inference with regard to the level of reproductive isolations (Dowling and Moore, 1984). Specially consistent deficiency of heterozygotes should be taken as evidence for reproductive isolation either assortative pre-

isolation (Knight and Waller, 1987) or postmating selection against hybrids (Min and Yang, 1989) such as hybrid sterility or inviability. Hereafter, so as to explicate which factors contribute to formation of isolating barrier, it is requisited that we should go abreast of analysis on microenvironmental habitat and other ecology as to spawning and courtship. As mentioned above three genetic types are also distinct from each other in several external characters (Fig. 3). Compared with characters introduced by Akihito (1986) the Type-A, Type-B, Type-C of *C. annularis* fall under *C. urotaenia*, *Chaenogobius* sp.1, *Chaenogobius* sp.2 in Japan respectively, however, since they haven't been compared at first hand we are not convinced whether they are conspecific. We suggest that the issue will be resolved by additional morphological data such as the pattern of sensory canal on the head or meristic character and a first hand information of genetic feature will be essential for correct comparison.

In conclusion, we prefer to recognize the three types of *C. annularis* as distinct species based on our present data, that is genetic differentiation, morphological differences and no evidence of hybridization at sympatry.

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한국산 꼭저구(*Chaenogobius annularis*) 3형의 유전적 분화 및 생식적 격리  
 석호영 · 김종범 · 민미숙 · 양서영 (인하대학교 이과대학 생물학과)

복잡한 형태적 변이로 분류학적 논란이 많은 한국산 꼭저구(*Chaenogobius annularis* Gill)의 분류학적 위치를 명확히 하기 위하여 동위효소 전기영동을 이용한 유전자분석을 실시. 총 14개의 효소 및 단백질에서 25개의 유전자를 검출하였다. 분석 결과 국내에는 유전적으로 뚜렷이 구분되는 3개 type(A-Type, B-Type, C-Type)이 서식하고 있음을 알 수 있었는데 이들은 *Aco. 6pgd*, *Ldh-1*, *Got-1*, *Gp-2*, *Gp-3* 등 6개 유전자에서 중복없이 서로 다른 대립인자로 구성되어 있었고 각 type간의 유전적 근연치(Rogers' S)는 0.8 이하로 일반적인 척추동물의 종간 수준의 값을 보였다. Nei(1975)의 공식을 이용하여 분화연대를 추산해 본 결과 이들 각 type은 120~180만년전에 분화된 것으로 추정되었다. 특히 이들 3 type의 공서지에서 type간 유전자 교환이 이루어지지 않으며 또한 형태적으로도 반문 등의 형질에 있어서 뚜렷한 차이를 보였다. 상기의 결과로 미루어 한국산 꼭저구 3 type은 이미 생식적 격리가 이루어져 종분화가 완성된 별종으로 사료된다.