Japan. J. Ichthyol.
40(1): 61-64, 1993

魚類学雑誌
40(1): 61-64, 1993

Genetic Evidence Supporting the Existence of Three Distinct Species in the Genus *Odontobutis* (Gobiidae) from Japan and Korea

Harumi Sakai,[1] Akihisa Iwata[2] and Sang-Rin Jeon[3]

[1] Shimonoseki University of Fisheries, 2-7-1 Nagata-honmachi, Shimonoseki, Yamaguchi 759-65, Japan
[2] Akasaka Imperial Palace, 2-1-8 Motoakasaka, Minato-ku, Tokyo 107, Japan
[3] Sang-Myung Women's University, Seoul 110-743, Korea

(*Received September 18, 1992; in revised form December 24, 1992; accepted March 18, 1993*)

Abstract Three taxa of the genus *Odontobutis* (Gobiidae), *O. obscura obscura* from Japan, and *O. o. interrupta* and *O. platycephala* from Korea were analyzed electrophoretically at 21 isozyme coding loci. They were very distantly related genetically (Nei's genetic distance = 1.053 − 1.230), alleles of 9 loci being displaced and diagnostic of the three taxa. Accordingly, the two subspecies of *O. obscura* were judged to be distinct species, for which the names *O. obscura* (for *O. o. obscura*) and *O. interrupta* (for *O. o. interrupta*) are proposed.

According to Iwata et al. (1985), the gobiid genus *Odontobutis* includes two species with three subspecies; *O. obscura obscura* (Temminck et Schlegel) from Japan, *O. o. interrupta* Iwata et Jeon from Korea, *O. o. potamophila* (Günther) from China and *O. platycephala* Iwata et Jeon from Korea, which are distinguished mainly by their cephalic lateral line systems. A sensory canal is absent in *O. o. obscura*, but present on the postocular part on the supraorbital pit line in *O. o. interrupta* and *O. o. potamophila*. Two canals are present on the preoperculomandibular pit line and three on the supraorbital pit line in *O. platycephala* (Iwata et al., 1985). Three subspecies of *O. obscura* were recognized because of the subtler differences between each than between *O. obscura* and *O. platycephala*, and because of their allopatric distribution (Iwata et al., 1985). Iwata et al. (1988) compared the larval development of *O. o. obscura*, *O. o. interrupta* and *O. platycephala*, reporting that the former two subspecies were more paedomorphic and similar to each other in their developmental processes, than to *O. platycephala*. However, to date the above taxonomy has not been tested by genetic studies. This paper presents an isozyme electrophoretic analysis of *O. o. obscura*, *O. o. interrupta* and *O. platycephala*, with a brief discussion of their taxonomic status based on genetic distance.

Materials and Methods

Two samples of *Odontobutis obscura obscura* from Japan, and three of *O. o. interrupta* and two of *O. platycephala* from Korea, were collected during 1991-1992 (Fig. 1). The fish were frozen immediately after collection and stored at −70°C until pro-

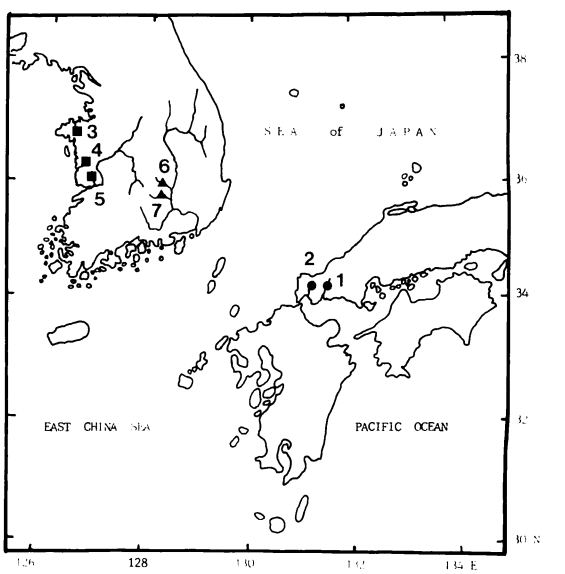

Fig. 1. Map of Japan and the Korean Peninsula showing collection localities of *Odontobutis obscura* (●), *O. interrupta* (■) and *O. platycephala* (▲). *1*—Kotou River; *2*—Koya River; *3*—Yok River; *4*—Ungchon River; *5*—a tributary of Kum River; *6*—Taega River of Naktong River system; *7*—Miryang River of Naktong River system.

Table 1. Enzymes, enzyme numbers, loci, tissue distribution and buffer systems used

Enzyme	Enzyme number	Locus	Tissue	Buffer
Aspartate aminotransferase	2.6.1.1	*AAT-1**	L, M	TC
Alcohol dehydrogenase	1.1.1.1	*ADH**	L	AC
Creatine kinase	2.7.3.2	*CK**	M	RW
Glycero-3-phosphate dehydrogenase	1.1.1.8	*G3PDH**	M	TC
Glutamate dehydrogenase (NADP+)	1.4.1.4	*GLUDHP**	L	AC
Glucose-6-phosphate isomerase	5.3.1.9	*GPI-1**	M	RW
		*GPI-2**	L, M	RW
Isocitrate dehydrogenase (NADP+)	1.1.1.42	*IDHP-1**	E, M	TC
		*IDHP-2**	E, L	TC
L-Lactate dehydrogenase	1.1.1.27	*LDH-1**	E, H	TC
		*LDH-2**	M	AC
		*LDH-3**	E	TC
Malate dehydrogenase	1.1.1.37	*MDH-1**	E, H, L, M	TC
		*MDH-2**	E, H, L, M	TC
		*MDH-3**	M	TC
Malic enzyme (NADP+)	1.1.1.40	*MEP**	M	AC
Octanol dehydrogenase	1.1.1.73	*ODH**	L	RW
Phosphogluconate dehydrogenase	1.1.1.44	*PGDH**	E, L	TC
Phosphoglucomutase	5.4.2.2	*PGM**	M	RW
Superoxide dismutase	1.15.1.1	*SOD**	L	TC
Xanthine dehydrogenase	1.1.1.204	*XDH**	L	RW

Tissue: E=eye; H=heart; L=liver; M=muscle. Buffer: TC=Tris-citrate Buffer (pH 8.0, diluted 1:9 for the gel) described by Shaw and Prasad (1970), 4 mA/cm^2 for 4 hours; AC=amine (N-(3-Aminopropyl)-morpholine) citrate buffer (pH 6.0) described by Clayton and Tretiak (1972), 4 mA/cm^2 for 3 hours; RW=discontinuous Tris-citric acid (gel pH 8.5), lithium hydroxide-boric acid (tray pH 8.5) buffer system described by Ridgway et al. (1970), 4 mA/cm^2 for 2 hours.

cessed for horizontal starch-gel electrophoresis. The 15 enzymes analyzed, 21 loci recognized and the buffer systems used are given in Table 1. Locus and gene nomenclature follows Shaklee et al. (1990) and Okazaki et al. (1991). The most common allele at a locus of *O. o. obscura* was designated as **100*. Staining procedures followed Murphy et al. (1990).

Results

The proportion of polymorphic loci and average heterozygosity are shown in Table 2. Pooling of samples of the same species or subspecies showed 5 loci in *Odontobutis obscura obscura*, 2 loci in *O. o. interrupta* and 5 loci in *O. platycephala* to be polymorphic (a locus was judged as polymorphic when the most common allele did not exceed 0.95).

Allelic frequencies of 21 loci are presented in Table 3. At all of the polymorphic loci, five populations (except for two of *O. o. interrupta* from which the sample was very small) were accepted as the Mendelian population. Alleles of 9 loci were notably displaced, being diagnostic of the three taxa, *O. o. obscura*, *O. o. interrupta*, and *O. platycephala*. Two loci of the two subspecies of *O. obscura* vs. *O. platycephala*, 3 loci of *O. o. obscura* vs. *O. o. interrupta* and *O. platycephala*, and 2 loci of *O. o. interrupta* vs. *O. o. obscura* and *O. platycephala* also showed allelic dis-

Table 2. Proportion of polymorphic loci and average heterozygosity in 7 populations of *Odontobutis* from Japan and Korea (see text). Sample numbers correspond to location numbers in Figure 1

Population	Polymorphic loci	Average heterozygosity	
		Observed	Expected
O. obscura			
1	0.286	0.073	0.087
2	0.143	0.059	0.059
O. interrupta			
3	0.095	0.040	0.044
4	0.048	0.038	0.023
5	0.143	0.058	0.054
O. platycephala			
6	0.143	0.054	0.056
7	0.190	0.069	0.060

Table 3. Alleles (allelic frequencies in parentheses) at 21 loci in 7 populations of *Odontobutis* from Japan and Korea. Sample numbers correspond to location numbers in Figure 1, with sample size in parentheses

Locus	*O. obscura*		*O. interrupta*			*O. platycephala*	
	1 (N=15)	2 (18)	3 (54)	4 (5)	5 (5)	6 (39)	7 (43)
*AAT-1**	*100	*100	*100	*100	*100	*100	*100
*ADH**	*−100	*−100	*−185	*−185	*−185	*−190	*−190
*CK**	*100	*100	*95	*95	*95	*100	*100
*G3PDH**	*100 (.967)	*100	*130	*130	*130	*80	*80
	*125 (.033)						
*GLUDHP**	*100	*100	*80	*80	*80	*85	*85
*GPI-1**	*100 (.900)	*100 (.556)	*95	*95	*95 (.900)		
	*75 (.100)	*115 (.444)			*70 (.100)	*70	*70
*GPI-2**	*100	*100	*75	*75	*75	*85 (.628)	*85 (.849)
						*70 (.372)	*70 (.151)
*IDHP-1**	*130 (.467)	*130 (.111)	*100	*100	*100	*100	*100
	*100 (.533)	*100 (.889)					
*IDHP-2**	*100	*100	*90	*90	*90	*87	*87
*LDH-1**	*100	*100	*100	*100	*100	*100	*100
*LDH-2**	*100 (.900)	*100	*100	*100	*100	*100 (.897)	*100
	*45 (.100)					*20 (.103)	
*LDH-3**	*100	*100	*102	*102	*102	*102	*102
*MDH-1**	*100	*100	*100	*100	*100	*100	*100
*MDH-2**	*130 (.333)	*130 (.333)	*160	*160	*160	*140 (.372)	*140 (.649)
	*100 (.667)	*100 (.667)				*97 (.628)	*97 (.326)
*MDH-3**	*100	*100	*90	*90	*90	*75	*75
*MEP**	*100	*100	*160	*160	*160	*0	*0
*ODH**	*100	*100	*110	*110	*110	*100	*100
*PGDH**	*100	*100 (.972)	*100 (.731)	*100 (.400)	*100 (.800)	*140	*140
			*95 (.269)	*95 (.600)	*95 (.100)		
		*85 (.028)			*85 (.100)		
*PGM**	*100 (.900)	*100	*98 (.537)		*98 (.500)	*98	*98 (.802)
	*90 (.100)		*85 (.426)		*85 (.300)		*110 (.198)
			*70 (.037)	*70	*70 (.200)		
*SOD**	*100 (.833)	*100 (.972)	*155	*155	*155	*35 (.974)	*35 (.849)
	*65 (.167)	*65 (.028)				*−5 (.026)	*−5 (.151)
*XDH**	*100	*100	*100	*100	*100	*95	*95

Table 4. Nei's (1972) genetic distance (above diagonal) and number of diagnostic loci (below diagonal) among 7 populations of *Odontobutis* from Japan and Korea. Sample numbers correspond to location numbers in Figure 1

Population	1	2	3	4	5	6	7
O. obscura							
1		0.017	1.158	1.224	1.141	1.123	1.106
2	0		1.104	1.165	1.089	1.070	1.053
O. interrupta							
3	14	14		0.041	0.003	1.132	1.130
4	14	14	0		0.036	1.230	1.210
5	14	14	0	0		1.117	1.113
O. platycephala							
6	14	14	13	13	13		0.010
7	14	14	13	13	13	0	

placement.

Nei's (1972) genetic distance (D) and the numbers of diagnostic loci among the 7 populations are presented in Table 4. The D measurements were 1.089–1.224 between *O. o. obscura* and *O. o. interrupta*, 1.053–1.123 between *O. o. obscura* and *O. platycephala*, and 1.113–1.230 between *O. o. interrupta* and *O. platycephala.*

Discussion

In contrast to the morphological and developmental similarity between the two subspecies (Iwata et al., 1985, 1988), *Odontobutis obscura obscura* and *O. o. interrupta* were very distantly related genetically (mean D=1.147), such distance being a little larger than that between the two species, *O. obscura* and *O. platycephala* (mean D=1.128). In fact, D measurements among these three (1.053–1.230) corresponded to the intergeneric level applied to fish genera by other workers (Shaklee et al., 1982; Buth, 1984).

Consequently, the two subspecies of *O. obscura* recognized by Iwata et al. (1985) are now considered to be distinct species as is *O. platycephala*. Accordingly, *O. o. obscura* should henceforth be referred to as *O. obscura* (Temminck et Schlegel), and *O. o. interrupta* as *O. interrupta* Iwata et Jeon.

Unfortunately, there was no opportunity to examine fresh samples of *O. o. potamophila* from China, which is the form most similar morphologicaly to *O. interrupta* (Iwata et al., 1985). If *O. o. potamophila* is subsequently shown to be conspecific with *O. interrupta*, either by genetic or other means, the name *potamophila* has priority over *interrupta.*

Acknowledgments

We are grateful to Dr. Toshio Okazaki, National Research Institute of Aquaculture, for his kind help in collecting materials and to Dr. Richard C. Goris, Yokohama City University, for reading the manuscript.

Literature Cited

Buth, D. G. 1984. Allozymes of the cyprinind fishes. Pages 561–590 *in* B. T. Turner, ed. Evolutionary genetics of fishes. Plenum Press, New York.

Clayton, J. W. and D. N. Tretiak. 1972. Amine-citrate buffers for pH control in starch gel electrophoresis. J. Fish. Res. Bd. Can., 29: 1169–1172.

Iwata, A., S.-R. Jeon, N. Mizuno and K.-C. Choi. 1985. A revision of the eleotrid goby genus *Odontobutis* in Japan, Korea and China. Japan. J. Ichthyol., 31: 373–388.

Iwata, A., S.-R. Jeon, N. Mizuno and K.-C. Choi. 1988. Larval development of a gobiid fish, *Odontobutis obscura obscura* in comparison with that of *O. o. interrupta* and of *O. platycephala.* Japan. J. Ichthyol., 35: 371–381. (In Japanese with English abstract.)

Murphy, R. W., J. W. Sites, D. G. Buth and C. H. Haufler. 1990. Chapter 4. Proteins I: isozyme electrophoresis. Pages 45–126 *in* D. M. Hillis and C. Moritz, eds. Molecular systematics. Sinauer Associates, Sunderland, Massachusetts.

Nei, M. 1972. Genetic distance between populations. Am. Nat., 106: 283–292.

Okazaki, T., M. Watanabe, K. Mizuguchi and K. Hosoya. 1991. Genetic differentiation between two types of dark chub, *Zacco temmincki*, in Japan. Japan. J. Ichthyol., 38: 133–140.

Ridgway, G. L., S. W. Sherburne and R. D. Lewis. 1970. Polymorphism in the esterases of Atlantic herring. Trans. Am. Fish. Soc., 99: 147–151.

Shaklee, J. B., C. S. Tamura and R. S. Waples. 1990. Speciation and evolution of marine fishes studied by the electrophoretic analysis of proteins. Pacif. Sci., 36: 141–157.

Shaw, C. R. and R. Prasad. 1970. Starch gel electrophoresis of enzymes—a compilation of recipes. Biochem. Genet., 4: 297–320.

日本および韓国産ドンコ属が独立した3種であることを支持する遺伝学的証拠

酒井治己・岩田明久・田　祥麟

日本および韓国産ドンコ属 (*Odontobutis*) ドンコ *O. obscura obscura*, セマダラドンコ *O. o. interrupta* およびコウライドンコ *O. platycephala* の分化の程度を，アイソザイムを支配する21遺伝子座を用いて遺伝学的に検討した．その結果，各々は遺伝的に非常に離れており (D=1.053–1.230)，3者の間で9遺伝子座において対立遺伝子の置換が見られた．それゆえ，ドンコ2亜種ドンコおよびセマダラドンコは，コウライドンコと同様に独立した種とみなすべきと判断された．学名としてドンコに対しては *O. obscura* を，セマダラドンコに対しては *O. interrupta* を使うことを提唱した．

(酒井：〒759-65 下関市永田本町 2-7-1 水産大学校；岩田：〒107 港区元赤坂 2-1-8 赤坂御所；田：110-743 ソウル市　祥明女子大学校，韓国)