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Taxonomic Status of the Fishes of *Acrossocheilus formosanus* and *A. labiatus* (Cyprinidae: Barbinae) from Taiwan Based on Isozyme Electrophoresis[1]

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Wuu-Shiou Sung, Sin-Che Lee and Ming-Jenn Yu (1993) Taxonomic statuts of the fishes of *Acrossocheilus formosanus* and *A. labiatus* (Cyprinidae: Barbinae) from Taiwan based on isozyme electrophoresis. *Bull. Inst. Zool., Academia Sinica* **32**(2): 127-139. A total of 362 specimens of *Acrossocheilus formosanus* (AF) and *A. labiatus* (AL) from five collection sites in Taiwan were analyzed using horizontal starch-gel electrophoresis to clarify their taxonomic status and investigate their genetic variation. A total of 23 loci with 35 alleles were scored for 14 enzymes. The short genetic distance between AF and AL ($D \leq 0.0005$) indicates that they are identical species, therefore enabling these two morhpological types to be merged as the single species *Acrossocheilus paradox* (Steindachner 1892) due to its seniority in the nomenclature. The analyzed samples have genetic distances (D) ranging from 0.002-0.046, polymorphic loci (P) from 0.087-0.261, and mean heterozygosity ($\overline{H}$) from 0.005-0.037, indicating that the genetic relationships among them are very close. We offer a possible explanation that the small geographic size of Taiwan and similar water conditions of its rivers may have diminished allozyme variation.

Key words: *Acrossocheilus*, Cyprinidae, Taxonomic status, Taiwan, Electrophoresis.

The genus *Acrossocheilus*, nominated originally by Oshima (1919) on the basis of the type *Gymnostomus formosanus* Regan (1908), is characterized by having three series of pharyngeal teeth, five branched anal rays, and no more than nine branched dorsal rays. The snout is rounded with traces of small lateral tubercles and four barbels along the fringe of the lips. *Acrossocheilus* mouths are inferior and transverse with the upper jaw projecting beyond the lower; the lower lip is thick and discontinuous (Chu 1931, Oshima 1919, Tchang 1933, Wuu 1977). At least 22 species in the genus *Acrossocheilus* (Cyprinidae: Barbinae) occur around the world. The distribution of these fishes is restricted to the East Asian continent, in-

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cluding the waters of India, Burma, Thailand, Indo-China, Mainland China, and Malaya and its adjoining island, Sumatra (Smith 1945, Weber and de Beaufort 1916, Wuu 1977).

In Taiwan, a recent revision by Chen and Yu (1986) who recognized three nominal species: *Acrossocheilus formosanus* (Regan 1908), *A. labiatus* (Regan 1908) and *A. fasciatus* (Steindachner 1892). The first is restricted to Taiwan while the latter two are extensive in waters off the mainland China. *A. formosanus* and *A. labiatus* were previously placed in separate subgenera, *Acrosschelus* and *Lissochilichthys*, respectively (Wuu 1977).

The main differences among the three previously-recognized *Acrosscheilus* species have been described as follows: *A. formosanus* is characterized by having a horn-like structure on the lower jaw, lower lips that are separated anteriorly by an interspace about 1/3 the width of the mouth, and the absence of any serration on its third unbranched dorsal ray (Regan 1908a). According to the early literature, *A. labiatus* and *A. formosanus* are very closely related in that they share the same dorsal fin features and color patterns, but are also distinguishable by the former lacking a horn-like structure on the lower jaw as well as by having two thick lip lobes which are connected anteriorly plus a slightly protruding snout (Regan 1908b). The third species, *A. fasciatus* (Steindachner 1892), with a single specimen reported as a new record in Taiwan by Herre and Myers (1931) has cross bands similar to those in *A. labiatus*; this, plus an intermediary labial shape between the above two species, suggests that it is not a valid species. The horn-like matter on the lower jaw which was described by the above authors is not reliable since it disappears upon preservation.

After a year of intensive collecting, all of our specimens contain those characters which fit descriptions of the above two species; however fish fitting previously-reported descriptions of *A. fasciatus* were not found. Thus, the present study is confined to the former two species. Since the morphological differences between *A. formosanus* and *A. labiatus* are not well defined, a biochemical approach is required to clarify their taxonomic status.

Because of the species specificity of some proteins, electrophoresis can be used to clarify the taxonomic status of fishes (Shaklee et al. 1982). In cyprinids, electrophoretic analysis of isozymes has already been successfully applied to verify the validity of some doubtful taxonomic designations, for example, in the genus *Notropis* (Buth and Mayden 1981) and *Tribolodon* (Sakai and Hamada 1985). Encouraged by the above examples, We decided to reexamine, based on comparisons of isozyme and morphometric characters in *A. formosanus* and *A. labiatus*, the taxonomic status of *Acrossocheilus* specimens in Taiwan in order to determine if they are valid species. In addition, We will discuss differences in our description and the original description of *Acrossocheilus paradox* (Gunther 1868) in order to clarify the species nomenclature.

MATERIALS AND METHODS

Sample collection

Relevant collection data for two previously-recognized *Acrossocheilus* species from Taiwan, *A. formosanus* (AF) and *A. labiatus* (AL) are presented in Table 1; sampling locations are shown in Fig. 3.

Electrophoresis

Tissues from the skeletal muscle, heart, eyes, and liver of individual specimens were homogenized in 2-3 volumes of extraction buffer (0.1 mM Tris-HCl pH 7.0, with 1 mM

Table 1. Collection data for the genus *Acrossocheilus*. AF, *A. formosanus*; AL, *A. labiatus*.

Collection Site	Species	Temp.(°C) (Mean)	Sampling date	Sampling method	No. specimens	Standard length (Mean) (mm)
Neishuang R.	AF	not available	Nov./'91	angling	54	60.3-164.7 (103.6)
Tunghou R.	AF	11-12 (16)	Nov.-Dec./'91	angling	50	51.8-131.6 (70.7)
	AL				40	48.0-109.7 (63.0)
Fengshan R.	AF	not available	Mar.-May/'91	electric fishing	50	70.1-133.0 (88.9)
Tachia R.	AF	18-25 (21)	Jul./'91	gill netting	44	81.3-147.2 (109.2)
	AL				48	74.8-143.6 (129.3)
Shuili R.	AF	15-26 (22)	Aug./'91	electric fishing	50	75.4-157.0 (95.9)
	AL				26	66.7-160.0 (96.0)

EDTA and 0.05 mM NADP). Homogenates were centrifuged at 13,500 r.p.m. for 40 minutes at 4°C, and supernatants were then stored at −70°C until use. Body remains were preserved in 70% ethanol for later morphological observations.

Tissue extracts were added to sample wells at the cathodal end of the 12% starch gel. Electrophoresis was performed horizontally at 180-350 V under a 40-75 mA current for 5-19 hours (depending on the buffer system used).

After electrophoresis, the gel was sliced into several horizontal pieces and stained with specific recipes described by Murphy *et al.* (1990), Pasteur *et al.* (1988), Redfield and Salini (1980), Shaklee and Keenan (1986), Shaw and Prasad (1970), and Siciliano and Shaw (1976). Stained gels were then fixed with an acetic acid/ethanol solution (Siciliano and Shaw 1976) and photographed.

Treatment of electrophoretic data

Selection of enzymes and their respective expression in different tissues:

Thirty-four enzymes were tested for the skeletal muscles, hearts, eyes, and livers of specimens under five buffer systems: LIOH (Redfield and Salini 1980), TC 7.0 and TC 8.0 (Siciliano and Shaw 1976), T-HCL (Redfield and Salini 1980) and TVB (Siciliano and Shaw 1976). The fourteen enzymes listed in Table 2 were selected for comparing the genetic differences between AF and AL types in both from the same collection site among the five collection sites. Loci and alleles appearing on the gel were designated according to relative mobility (nominated according to Shaklee *et al.* 1989).

Comparison between the two taxa:

Using the Numercial Taxonomy System of Multivariate Statistical Programs (NTSYS),

Table 2. Enzyme systems examined and electrophoretic buffer employed.

Enzyme name (abbreviation)	E.C. number	Locus	Number of alleles resolved	Tissue examined	Buffer system
Lactate dehydrogenase (LDH)	1.1.1.27	*LDH-A**	1	Heart	TC 7.0
		*LDH-B**	3	Heart	TC 7.0
		*LDH-C**	1	Liver	TVB
Malate dehydrogenase (MDH)	1.1.1.37	*sMDH-A**	1	Heart	TC 7.0
		*sMDH-B**	1	Heart	TC 7.0
		*mMDH-A**	1	Heart	TC 7.0
Malic enzyme (NADP+) (ME)	1.1.1.40	*mMEP**	1	Muscle	LIOH
6-phosphogluconate dehydrogenase (6PGD)	1.1.1.43	*6PGD**	2	Liver	TC 8.0
NAD-Glucose dehydrogenase (GLC)	1.1.1.47	*GLC**	2	Liver	TVB
Superoxide dismutase (SOD)	1.15.1.1	*SOD**	1	Liver	TVB
Xanthine dehydrogenase. (XDH)	1.2.1.37	*XDH**	1	Liver	TVB
Aspartate aminotransferase (AAT)	2.6.1.1	*sAAT-1**	2	Liver	TC 8.0
		*mAAT-1**	1	Liver	TC 8.0
Creatine kinase (CK)	2.7.3.2	*CK-A**	2	Eye	LIOH
		*CK-B**	1	Eye	LIOH
		*CK-C**	2	Eye	LIOH
Phosphoglucomutase (PGM)	5.4.2.2	*PGM-1**	1	Muscle	LIOH
		*PGM-2**	1	Muscle	LIOH
3-hydroxybutyrate dehydrogenase (HBDH)	1.1.1.30	*HBDH**	1	Liver	TVB
Fumarase (FH)	4.2.1.2	*FH**	1	Heart	TC 8.0
Mannose-6-phosphate isomerase (MPI)	5.3.1.8	*MPI**	2	Eye	LIOH
Glucosephosphate isomerase (GPI)	5.3.1.9	*GPI-A**	3	Eye	LIOH
		*GPI-B**	3	Eye	LIOH

allelic frequencies were applied to obtain Nei's (1978) genetic similarity (I) and genetic distance (D) between taxa (Rohlf *et al.* 1983). Phenetic dendrograms were constructed with unweighted pair-group arithmetic averages (UPGMA) by using the "SAHN Clustering" of NTSYS based on Nei's genetic distance (D). Electrophoretic data for two specimens collected from the same collection site were tested for goodness of fit according to the Hardy-Weinberg hypothesis by x^2 values. The proportion of polymorphic loci (polymorphic loci/total loci) and heterozygosity of individual populations were also compared (Ferguson 1980).

Treatment of morphological characters

Lateral line scale, fin ray, gill raker, and pharyngeal teeth counts were made. The horn-like structures on the lower jaws and the serrations on the last unbranched dorsal rays of specimens were also examined. Color patterns on the bodies and fins of specimens were also examined and compared with those reported in earlier studies.

RESULTS

Allozyme patterns

A total of 23 loci coded for 14 enzymes was consistently scored in *Acrossocheilus* (Table 2). Since there are no fixed allele (loci) differences in either morphological taxa, AF (*A. formosanus*) and AL (*A. labiatus*) isozyme patterns were almost identical. Thus isozyme variability between the two *Acrossocheilus* species is only a matter of slight differences in allelic frequencies.

Among the 23 loci scored, nine are polymorphic: the *sAAT-1** locus has two resolved alleles; the *sAAT-1* 100* allele predominated in most samples while the *sAAT-1* 115* variant was found to be restricted to samples from the Tachia and Shuili Rivers (Table 3). The *CK-A** locus, with three banded heterozygotes, was observed only in Fengshan River samples. The *CK-C** locus, with two banded heterozygotes, was observed only in the Tachia and Shuili River samples (Table 3). The *GLC** locus with two alleles (Table 3) was present with a two-banded heterozygote in the Tunghou, Tachia and Shuili River samples.

Three zones of activity in GPI were observed corresponding to products of the *GPI-A** and *GPI-B** loci and the heteropolymer zone which is supposed to reflect the interaction of peptide chains produced by *GPI-A* and *GPI-B* genes (Benson and Smith 1989; Pasteur *et al.* 1988) (Fig. 1). The latter was not used for data analysis. The *GPI-A** locus was found to be essentially monomorphic, with the exceptions of a heterozygous *GPI-A* –100/–80* genotype in the Fengshan River samples and two heterozygous *GPI-A* –100/–50* genotypes in the Shuili River samples (Table 3). Three *GPI-B** alleles were scored in the Tachia River samples (Fig. 3); the allelic frequencies was listed in Table 3. The heterozygotic states of this dimeric enzyme were observed at *GPI-*A_2* and *GPI-*B_2*, as well as at a site between them. Fig. 2 indicates three *LDH* loci: A, B, and C from two *Acrossocheilus* species, plus some additional heteropolymer bands which are supposed to reflect both interactions between loci and between alleles. As with the GPI, these were not used for data analysis. Among the three *LDH** loci resolved only the *LDH-B** locus was polymorphic with three alleles, indicating differences between northern and southern samples. The *LDH-B* 65* allele was found mostly in northern samples collected from the Tunghou and Fengshan Rivers while the *LDH-B* 75* allele was found in southern samples from the Tachia and Shuili Rivers (Fig. 4).

Table 3. Allelic frequencies of 23 loci scored in combined *Acrossocheilus* samples. FS, Fengshan R; NS, Neishuang R; SL, Shuili R; TC, Tachia R; TH, Tunghou R.

Locus	Allele	NS (N = 54)	TH (N = 90)	FS (N = 50)	TC (N = 92)	SL (N = 76)
*sAAT-1**	*115*	0.000	0.000	0.000	0.033	0.039
	100	1.000	1.000	1.000	0.967	0.961
*mAAT-1**	*−100*	1.000	1.000	1.000	1.000	1.000
*Ck-A**	*115*	0.000	0.000	0.030	0.000	0.000
	100	1.000	1.000	0.970	1.000	1.000
*Ck-B**	*100*	1.000	1.000	1.000	1.000	1.000
*Ck-C**	*100*	1.000	1.000	1.000	0.989	0.987
	95	0.000	0.000	0.000	0.011	0.013
*FH**	*100*	1.000	1.000	1.000	1.000	1.000
*GLC**	*140*	0.000	0.017	0.000	0.082	0.086
	100	1.000	0.983	1.000	0.918	0.914
*GPI-A**	*−100*	1.000	1.000	0.990	1.000	0.986
	−80	0.000	0.000	0.010	0.000	0.000
	−50	0.000	0.000	0.000	0.000	0.014
*GPI-B**	*115*	0.000	0.005	0.000	0.038	0.000
	100	1.000	0.995	1.000	0.842	0.895
	80	0.000	0.000	0.000	0.120	0.105
*HBDH**	*100*	1.000	1.000	1.000	1.000	1.000
*LDH-A**	*100*	1.000	1.000	1.000	1.000	1.000
*LDH-B**	*100*	0.991	0.956	0.740	0.989	0.796
	75	0.009	0.000	0.000	0.011	0.204
	65	0.000	0.044	0.260	0.000	0.000
*LDH-C**	*−100*	1.000	1.000	1.000	1.000	1.000
*sMDH-A**	*100*	1.000	1.000	1.000	1.000	1.000
*sMDH-B**	*100*	1.000	1.000	1.000	1.000	1.000
*mMDH-A**	*100*	1.000	1.000	1.000	1.000	1.000
*mMEP**	*100*	1.000	1.000	1.000	1.000	1.000
*MPI**	*100*	0.991	1.000	0.960	1.000	0.993
	95	0.009	0.000	0.040	0.000	0.007
*6PGD**	*115*	0.991	0.367	0.000	0.000	0.000
	100	0.009	0.633	1.000	1.000	1.000
*PGM-1**	*100*	1.000	1.000	1.000	1.000	1.000
*PGM-2**	*100*	1.000	1.000	1.000	1.000	1.000
*SOD**	*100*	1.000	1.000	1.000	1.000	1.000
*XDH**	*100*	1.000	1.000	1.000	1.000	1.000

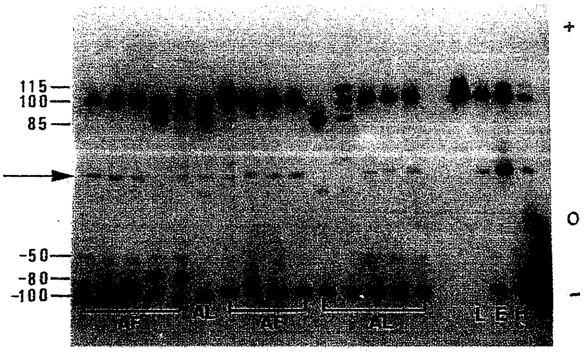

Fig. 1. Tissue expression of GPI activity in skeletal muscle (M), heart (H), eye (E), and liver (L), with comparison of *GPI* loci scored from eyes among individuals of each morphological type. AF, *Acrossocheilus formosanus*; AL, *A. labiatus.*

Table 4. Comparison of Nei's (1978) genetic similarity coefficients (above diagonal) and genetic distance (below diagonal) between *A. formosanus* and *A. labiatus* from three collection sites. Abbreviations refer to Fig. 5.

	THAF	THAL	TCAF	TCAL	SLAF	SLAL
THAF	—	1.000	0.993	0.992	0.991	0.991
THAL	0.000	—	0.993	0.993	0.992	0.992
TCAF	0.007	0.007	—	1.000	0.999	0.998
TCAL	0.008	0.007	0.000	—	0.998	0.997
SLAF	0.009	0.008	0.001	0.002	—	1.000
SLAL	0.009	0.008	0.002	0.003	0.000	—

The *MPI-A** locus has two alleles: the *MPI* 100* allele is distributed throughout all individuals with the exception of two heterozygotes, from the Neishuang and Fengshan Rivers (Table 3). The *6PGD** locus with the *6PGD* 100* allele was predominant in most populations except for Neishuang River samples. The three-banded heterozygotes were mainly found in the Tunghou River samples (Table 3).

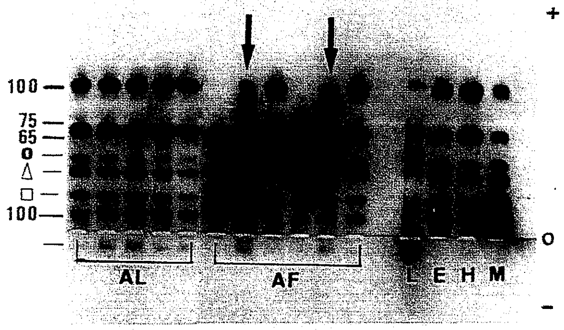

Fig. 2. Tissue expression of LDH activity in skeletal muscle (M), heart (H), eye (E), and liver (L) (TC 7.0 buffer), with comparison of *LDH* loci scored from hearts among individuals of each morphological type. AF, *Acrossocheilus formosanus*; AL, *A. labiatus*. The mark ○ indicates a heteropolymer band produced by *LDH* B^{100} and *LDH* A^{100}; △ indicates a heteropolymer band produced by *LDH* B^{65} and *LDH* A^{100}; □ indicates a heteropolymer band produced by interaction between △ and *LDH* A^{100}. Two lines with arrows indicate polymorphism.

Allelic variation

Comparison between sexes:

We found no difference in Acrossocheilus gene expression between sexes. Data for the two sexes from identical localities were combined in order to analyze allelic variation between taxa and localities.

Comparison between taxa:

Nei's (1978) data matrix of genetic distance (D) and genetic similarity coefficients (I) for all paired comparisons of *Acrossocheilus* species is shown in Table 4; it was derived from the allelic frequencies of 23 loci scored in *A. formosanus* and *A. labiatus* specimens collected from three sites. A phenetic dendrogram based on genetic distance illustrates the relationships between two *Acrossocheilus* species from three collection sites (Fig. 5). The shorter genetic distance ($D \leq 0.0005$) between the

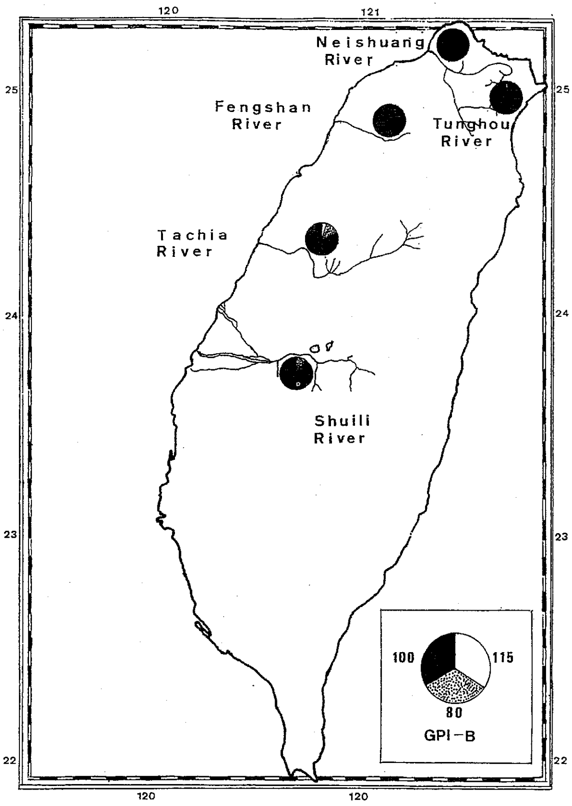

Fig. 3. Geographic variability in *GPI-B* allele frequencies in *Acrossocheilus* from five collection sites. Symbols depict proportion of alleles in each locality.

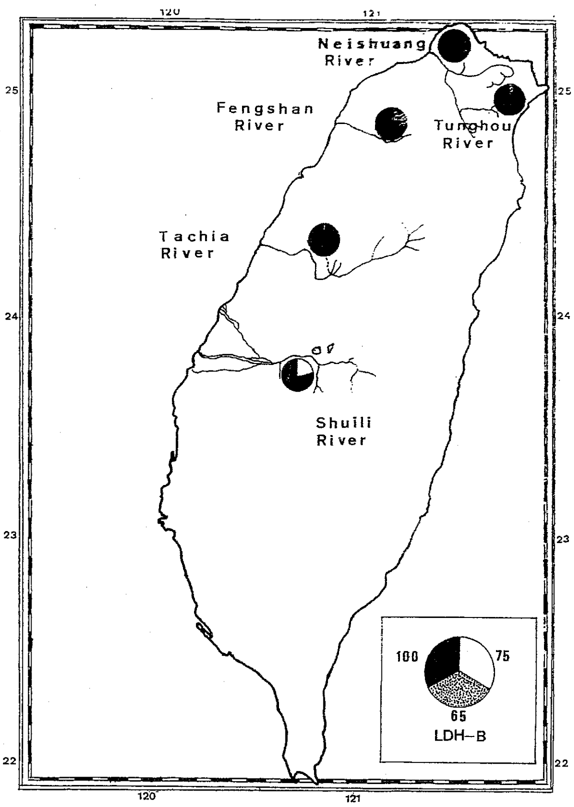

Fig. 4. Geographic variability in *LDH-B* allele frequencies in *Acrossocheilus* from five collection sites. Symbols depict proportion of alleles in each locality.

two species was insufficient for separating them into two species.

Comparison among localities:

Since *A. formosanus* and *A. labiatus* data were apparently identical, data from the two species from the same site were combined (Table 3). Thus, our data matrix for Nei's (1978) genetic distance (D) and genetic similarity coefficients (I) for all paired comparisons of samples from five localities were calculated from the allelic frequencies presented in Table 3; our results are shown in Table 4. A phenetic dendrogram was constructed in order to illustrate the relationships among samples from the five collection sites (Figs. 3, 4 and 6); this dendrogram indicates slight differences between northern and southern specimens. The calculated genetic distances among samples from five localities ranged between 0.046 and 0.002. A goodness-of-fit test for a Hardy-Weinberg hypothesis of nine polymorphic loci showed a significant difference only in the Tunghou River samples at the *6PGD** locus. The overall mean heterozygosities ($\overline{H}$) at nine polymorphic loci (*sAAT-1**, *Ck-A**, *Ck-C**, *GLC**, *GPI-A**, *GPI-B**, *LDH-B**, *MPI** and *6PGD**) at the Neishuang, Tunghou, Fengshan, Tachia and Shuili Rivers were 0.005, 0.037, 0.026, 0.023 and 0.035, respectively. In addition, the proportion of polymorphic loci (P) in samples from the above five localities were, respectively, 0,

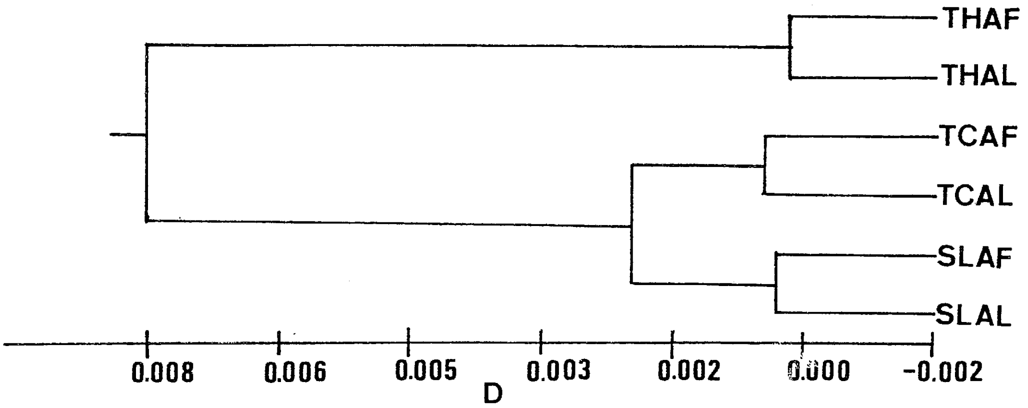

Fig. 5. Phenetic dendrogram of *A. formosanus* (AF) and *A. labiatus* (AL) from three collection sites based on genetic data from UPGMA method (Nei's D value). THAF, AF of Tunghou River; THAL, AL of Tunghou River; TCAF, AF of Tachia River; TCAL, AL of Tachia River; SLAF, AF of Shuili River; SLAL: AL of Shuili River.

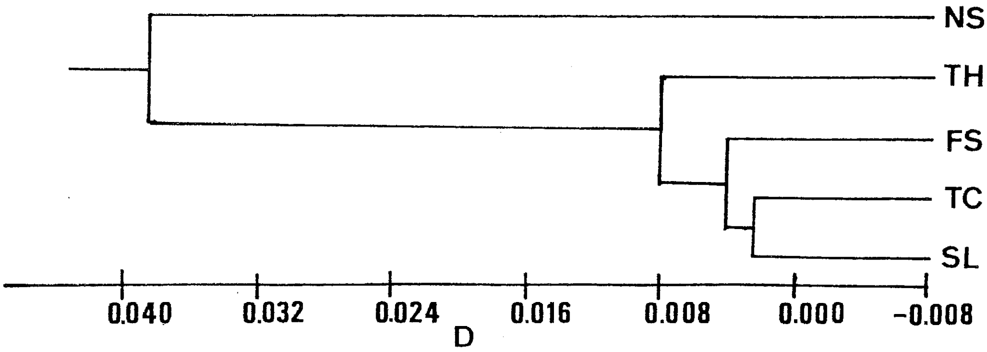

Fig. 6. Phenetic dendrogram of combined samples from five collection sites based on genetic data from UPGMA method (Nei's D value). NS, Neishuang River; TH, Tunghou River; FS, Fengshan River; TC, Tachia River; SL, Shuili River.

0.044, 0.044, 0.087 and 0.130 at the 5% level and 0.087, 0.130, 0.174, 0.217 and 0.261 at the 1% level.

Morphological comparisons

The external features of the two studied *Acrossocheilus* species are almost identical (Table 5). The few exceptions include: the lower lip lobes of AL are thick and more tightly connected to each other, while those of AF are widely separated anteriorly, greater than 1/5 the mouth width; the AL snout projects slightly beyond the upper lip, while the AF snout does not; and a horn-like structure on the lower jaw is usually found in AF, but only occasionally found in AL. The pharyngeal tooth formula for both species is arranged in a series of 5(4), 3, 2(1)-2(1), 3, and 5(4), from which the outermost tooth is deciduous. The color pattern for both species is variable, usually with 6-8 black vertical streaks on each side. These streaks may fade and be replaced by uniformly whitish

Table 5. Comparison of morphological characters measured for AF and AL types with those from previous reports.

Character	*A. formosanus* (Regan,1908)	*A. labiatus* (Regan,1908)	*A. fasciatus* (Steindachner, 1892)	*Barbus paradox* (Gunther,1868)	AF type (present collection)	AL type
Gap of lower lips	1/5-1/2 mouth width (MW)	connected or with a narrow notch	>1/3 MW	?	>1/5 MW	connected or with a narrow notch
Dorsal rays	3.8	3.8	3.8	3.8	3.8	3.8
Anal rays	3.5	3.5	3.5	3.5	3.5	3.5
Pharyngeal teeth	5,3,2(1)-2(1),3,5(4)	5(4)3,2,-2,3,5(4)	5,3,2,-2,3,5	5,3,2,-2,3,5	5,3,2-2,3,5	5,3,2-2,3,5
L.l.	40-42	39-42	37-41	40	39-42[1]	39-42
L.tr.	5.5-6/3.5-4	6/3-4	5.5/3.5	6/?	5-6/3-4[1]	5-6/3-4
Gill rakers	14-16	9-15	14-16	?	14-16	14-16
Vertical bars	6-7	6-7	6-7	Absent[3]	6-8[2]	6-8[2]
Longitudinal stripe	Absent	Absent	Present	Absent[3]	Occasionally present	Occasionally present
Horny structure on lower jaw	Present	Absent	present	Absent	Present	Occasionally present
Serration on longest unbranched dorsal ray	Absent	Absent	Weak?	Absent	Absent	Absent

1. one exceptional specimen (L.l. 47, L.tr. 8.5/7) from Fengshan River.
2. a few specimens without vertical stripes.
3. type specimen of 8-8 1/2 inch mature female having longitudinal and transverse bands disappeared.

or greyish colors. A few large males from both species had a longitudinal black stripe along the lateral line profile. The longest unbranched doral ray of both fish is not serrated. The lateral line scales of both types are L1.39-42; Ltr.5-6/3-4. One exceptional specimen collected from the Fengshan River had scales that were unusually smaller than other specimens (L1.47; Ltr.8.5/7). However, both morphological and isozyme data were found to be consistent with data for other specimens.

When comparing the morphological characters of our collected specimens with those previously studied and described (*Gymnostomus formosanus*, Regan 1908; *G. labiatus*, Regan 1908; *Crossochilus fasciatus*, Steindachner 1892; *Barbus paradox*, Gunther 1868) (Table 5), we found identical distinctive characters with only a few minor exceptions. These minor differences include variation in both lateral bands and the width of the interspace between the two lower lip lobes. These are not suitable criteria for species validity since they vary with growth and sexual maturity. Thus, the almost identical morphological characters among the above taxa indicate that they belong to the same species.

DISCUSSION

The calculation of genetic distance (D) is primarily based on variability in the allelic frequencies of enzymes; this variability can subsequently be used to interpret relationships between taxa. However, our essential problem was to determine whether enzymes scored for systematic study are species-specific. Since tests of 14 enzymes between two *Zacco* species as well as those between *Acrossocheilus* and its most closely allied *Candidia* show strong differences (Wang, unpublished), it is certain that the 14 enzymes used in this study are reliable characters for confirming species validity.

The D value ($D<0.0005$) between the two species of *Acrossocheilus* we studied is far lower than those found in other cyprinids (the 0.1-0.26 in the genus *Campostoma* (Buth and Burr 1978) and 0.64 in the genus *Zacco* (Okazaki *et al.* 1991)). Genetic distances between samples from different localities for our study ($D=0.002-0.046$) are far below those obtained for other cyprinid species ($D=0.06-0.20$) (Stein *et al.* 1985). The polymorphic loci proportion and mean heterozygosity obtained in this study are also far below the overall levels reported for other fish species (Kirpichnikov 1992). Thus, the low D values among *Acrossocheilus* samples may lead to the assumption that they are highly homogenous both between species and between populations. Since overall genetic divergence is only correlated with large geographic distance (Frati *et al.* 1992), the small geographic size of Taiwan may have diminished allozyme variation.

Creatine kinase is known to be a dimer in birds and mammals (Dawson *et al.* 1965); however, it showed a monomeric structure in this study, as it is also found in other cyprinids (Scopes and Gosselin-Rey 1968; Buth and Burr 1978). The specific expression of the *LDH-C** locus scored in the liver of *Acrossocheilus* is different from that found in more advanced euteleosts (Mok *et al.* 1988) whose expression is restricted to the eye. Our data from five localities indicate a slight geographic change between north and south, as expressed in the *sAAT-1**, *GPI-B**, and *LDH-B** loci. Water conditions in the five river habitats used in this study are nearly identical except for slight temperature differences. Thus changes in allelic frequencies are possibly attributable to thermal differences. This possibility requires further study.

Results from our electrophoretic comparison of these two nominal species indicates that they may be combined into one species-though with minor differences in several highly variable external features. Thus We suggest that *A. formosanus* and *A. labiatus* are identical, and further suggest that the corrected scientific name should be *Acrossocheilus paradoxus* (Gunther 1868) due to seniority in the nomenclature. In addition, the two studied species should be redesignated as types AF and AL.

Both AF and AL types as well as a few types in between-were found among samples collected from the Tunghou River. The in-between specimens had the intermediate character of a much narrower interspace (less than 1/5 of mouth width) between the two lobes of lower lip, while showing the same zymogram patterns as AF and AL.

Both AF and AL inhabit clean flowing water and feed mainly on aquatic insect larvae and algae (Huang 1984). This may further support the presumption that they belong to the same taxon.

A. fasciatus (Steindachner 1892) was

previously recorded as a new addition to Taiwan fauna by Herre and Myers (1931); it was designated on the basis of a single specimen which differed from *A. paradoxus* by having serrations on the third unbranched dorsal ray and a black longitudinal stripe in addition to six black vertical stripes on its side. However, it has been considered a synonym of *A. paradoxus* by several other authors (Chu 1931; Tchang 1933). Chu (1931) stated that the serration of the third unbranched dorsal ray in *A. fasciatus* was very weak and variable. The additional black longitudinal stripe on the body was also occasionally found in our specimens. We therefore suggest that distinctive character described for *A. fasciatus* is identical to that of *A. paradoxus*. In conclusion, we believe that nominal *Acrossocheilus* species in Taiwan are of one species— *A. paradoxus*.

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以電泳法決定台灣兩種光唇屬魚類之分類地位

宋武修　李信徹　于名振

本實驗利用電泳同功酶分析法，分析採自內雙溪、桶後溪、鳳山溪、大甲溪、水里溪等五條溪流中的臺灣石𩼧(AF)及軟䰾(AL)共三百六十二尾：以確定其是否爲同一種魚，並對其遺傳變異作初步的分析。在電泳同功酶分析方面，在十四個同功酶中共得到二十三個基因座(loci)及三十五個等位基因(alleles)。種間的遺傳距離$D \leqslant 0.0005$，顯示臺灣石𩼧和和軟䰾爲同一種魚。在五個不同的採集地點中，遺傳距離的範圍爲$D = 0.002 - 0.046$，多型性基因(polymorphic loci (P))的比例範圍爲$P = 9\% - 26\%$，平均異型結合數(mean heterozygosity (H))的範圍爲$H = 0.005 - 0.037$，顯示不同採集地點的標本的遺傳關係很近，這可能由所棲息各河川之環境相近及台灣島之小範圍地理條件而使異構酶之變異性降低之故。

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Short Note

Addition of Mesenteries during Asexual Reproduction of the Polyglyphic Sea Anemone *Anthopleura dixoniana* (Anthozoa: Actiniaria)[1]

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James Lin, Chang-Po Chen and I-Ming Chen (1993) Addition of mesenteries during asexual reproduction of the polyglyphic sea anemone *Anthopleura dixoniana* (Anthozoa: Actiniaria). *Bull. Inst. Zool., Academia Sinica* **32**(2): 140-147. The mesenterial arrangement of the polyglyphic sea anemone *Anthopleura dixoniana* (Haddon & Shackleton) during asexual reproduction was studied from November, 1987 through December, 1988. Individuals were collected from the upper intertidal zone at Wanlitung (120°41'E, 22°01'N) in southern Taiwan. In exocoels, two mesenteries were added in exocoels and developed into either directives or non-directive mesenteries. In a directive endocoel, two mesenteries with retractor muscles facing one another were added, resulting in two adjacent pairs of directive mesenteries; each pair consists of one new and one old member. In a non-directive endocoel, four mesenteries were added, resulting in a pair of directive mesenteries flanked by two pairs of non-directives; each pair of non-directive mesenteries consists of one new and one old member. *Anthopleura dixoniana* maintained a constant ratio between the number of non-directive and directive mesenteries among dividing, divided, and non-dividing individuals. After division, two daughter *A. dixoniana* did not differ significantly in their numbers of mesenteries.

Key words: Asexual reproduction, Mesentery, *Anthopleura dixoniana*, Actiniaria, Anthozoa.

Asexual reproduction in sea anemones has been proposed to be a kind of growth meant to circumvent environmental restrictions on body size (Sebens 1979 1982, Lin *et al.* 1992). Many aspects of asexual reproduction have been studied, including periodicity, regulative factors, and influences on genotypic structure of populations (Minasian 1976 1979 1982, Smith and Lenhoff 1976, Minasian and Mariscal 1979, Sebens 1980, Ayre 1983 1984, Hunter 1984, Bucklin 1987a 1987b, Lin *et al.* 1992). However, little information is available on the numerical variation of mesenteries associated with asexual reproduction, except for *Haliplanella lineata* (Verrill) (=*Sagartia luciae* =*H. luciae*) (Davis 1919, Atoda 1976) and *Anthopleura elegantissima* (Brandt) (Sebens 1983).

Mesenteries contain most of the important discrete musculature of sea anemones. Each of the several

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