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Genetic Diversity of Featherback Fish in Thailand

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ABSTRACT

Four featherback fish of the Family Notopteridae in Thailand, *Notopterus notopterus, Chitala ornata, Chitala blanci* and *Chitala lopis,* were studied using allozyme electrophoresis to obtain their genetic diversity informations.

With a total of 38 enzyme loci screened, 28 variable loci were found of being allelically different between the four species. Of the 28 variable loci, 11 loci (*ADA*, ADH*, FH-2*, G3PDH-1*, G3PDH-2*, IDHP*, IDDH*, LDH-2*, LDH-3*, MPI** and *SOD-2**) were found to be polymorphic in *N. notopterus*, two loci (*IDHP** and *SOD-2**) in *C. ornata* and also two loci (*IDHP** and *MPI**) in *C. blanci*, while no polymorphic loci were found in *C. lopis*. The estimated genetic variabilities ($P_{0.99}$, H_e) ranged from $P_{0.99}$ = 0 and H_e = 0 in *C. lopis* to $P_{0.99}$ = 0.289 and H_e = 0.049±0.017 in *N. notopterus*. The observed genotypes in any species were not significantly deviated from Hardy-Weinberg equilibrium ($\chi^2_{p>0.05}$), suggesting no significant differences between the samples within a species, while the F_{ST} values (above 0.25) and the heterogeneity contingency chi-square analyses (p<0.001) suggested rather large genetic divergence between these species. Genetic structure and relationships of the four species were phylogenetically presented as two separate clades, the *N. notopterus* clade and the clade of three *Chitala* species, revealing that the three *Chitala* species were genetically related more closely to each other than to *N. notopterus* and consequently supporting the present nomenclature of the fish being used.

Key words : genetic diversity, Thai featherback fish, Notopteridae, allozyme electrophoresis

INTRODUCTION

A group of fish commonly called 'featherbacks' is one of the economically important freshwater fishes of Thailand. The featherbacks found in Thailand are composed of four species in two genera of the Family Notopteridae (Roberts, 1992), *Notopterus notopterus* (Pallas), *Chitala ornata* (Gray), *Chitala blanci* (D' Aubenton) and *Chitala lopis* (Bleeker), most of which are similar to each other. The only one *Notopterus* species, *N. notopterus*, can morphologically be separated from the group, while the other three cannot clearly be separated from each other, neither with morphometric nor meristic characters, except different patterns of body spots and bands on the adult fish. Roberts (1992), therefore, has retained this only one species as *Notopterus notopterus*, and gathered the others into the same genus of *Chitala* as being congenerically close species. Based on genetic characterization of the species, Kanasuta and Sodsuk (1998) have also supported this idea.

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Other evidences of being close to each other of the fish are that they all appear to have similar banding patterns on bodies of the young or immature fish (Sodsuk *et al*., 1983; Roberts, 1992), and have equal chromosome numbers of *2n = 42* with the equal number of arms, *42* (Donsakul and Magtoon, 1990). Due to these appearances of their closeness, the informations on their genetic diversity concerning the variation, differentiation and structure, that are very useful not only to the breeding and culture development programmes but also to the wild populations utilization of the species, are particularly interesting and should be known.

This study aimed to acquire all genetic diversity informations of the four featherback species (Family Notopteridae) found in Thailand. A molecular genetic technique called allozyme electrophoresis was utilized to achieve this goal.

MATERIALS AND METHODS

Specimens and collection

Specimens of *N. notopterus, C. ornata, C. blanci* and *C. lopis*, were collected from natural water resources in Thailand. The sources and number of specimens are shown in Table 1. The specimens of each species were unequally employed depending upon the commonness or rareness of each species in nature. Live specimens or pieces of different dissected tissue samples placed either on dry ice or in liquid nitrogen were transported to the Population Genetics Laboratory of the National Aquaculture Genetics Research Institute (NAGRI), where the samples were kept at -70°C for allozyme electrophoretic analysis.

Allozyme electrophoresis

The samples were electrophoretically analysed on the potato starch gelled media for 24 different enzymes (Table 2) to obtain genetic data called allozymes. Various steps of the analytical procedure were performed following those of Richardson *et al*. (1986), Aebersold *et al*. (1987), Morizot and Schmidt (1990), Murphy *et al*. (1990) and Sodsuk (1993).

Enzyme names and nomenclature of the enzymes-coding genes interpreted as loci and alleles follow the system of Shaklee *et al*. (1990). Loci were designated numerically beginning from the cathodal end of a gel; the locus with the least anodal migration was designated one, the next two, and so on. Alleles were designated according to their mobilities relative to the most common allele in all species, which was designated **100*; allelic variants were given numbers that indicated the mobility of their products in percentage faster or slower than that of the common allele. Alleles migrating cathodally from the origin were given a minus sign.

Table 1 Species, number and sources of the Notopterid specimens used in this study.

Species	Number	Sources
Notopterus notopterus (Pallas)	67	Natural freshwater bodies over the country, including three main rivers in three parts of Thailand, Chao Phraya River, Tapi River and Mekhong River
Chitala ornata (Gray)	50	Chao Phraya River, Tapi River, Mekhong River
Chitala blanci (D' Aubenton)	9	Mekhong River (Nakhon Phanom, Nong Khai)
Chitala lopis (Bleeker)	6	Tapi River, Cheowlaan Reservoir

Calculations and data analyses

All calculations and data analyses performed in this study were done using the software BIOSYS-1 release 1.7, a special computer program for the studies in population genetics and biochemical systemetics (Swofford and Selander, 1989).

Allozymically genetic data obtained from the electrophoretic work were calculated into genetic variation informations as gene or allele frequencies, loci polymorphism (*P*) and heterozygosity (*H*). The interpreted allozyme data as heterozygous and homozygous genotypes of each polymorphic locus observed in each species were tested for the Hardy-Weinberg equilibrium hypothesis, so that each of the species could be assured of being sampled from single panmictic (large random mating) populations (Richardson *et al*., 1986). The chi-square goodness-of-fit test was therefore performed using Yates correction for small sample size (Siegel, 1956).

A number of calculations of inbreeding coefficients (or the fixation index, F_{ST}, of Wright, 1978) and contingency chi-square analyses of the heterogeneity (Workman and Niswander, 1970) were performed to all four species, the three *Chitala* species and each pair of species to see their hierarchically genetic differentiation.

Genetic structure and relationships of the four species were studied by reconstructing a phylogenetic tree (a dendrogram) from the genetic distances (*D*) calculated for each pair of species. The distance Wagner algorithm of Farris (1972) was the method used for the tree reconstruction in this study, because of its effectively heuristic procedure that can build an additive representation of a phylogenetic tree by sequential additional taxa (as reviewed by Sodsuk, 1993). The Cavalli-Sforza and Edwards (1967) distances were used in this study instead of the commonly used distances of Nei (1972, 1978) and Rogers (1972). This is because the arc and chord distances of Cavalli-Sforza and Edwards (1967) incorporate some realistic assumptions on the nature of evolutionary change in gene frequencies without the undesirable properties of the Nei (1972, 1978) and Rogers (1972) distances (Swofford and Olsen, 1990) which are not appropriate for the distance Wagner algorithm of Farris (1972).

RESULTS AND DISCUSSIONS

Allozymes and genetic variabilities measured as allele frequencies, loci polymorphisms (*P*) and heterozygosities (*H*)

Thirty-eigth enzyme loci were detected from the electrophoretic analyses at 24 different enzymes (Table 2). All electrophoretic conditions and number of loci detected in each enzyme were the same as those formerly performed by Kanasuta and Sodsuk (1998).The allozyme data resulting from the electrophoretic analyses are shown in Table 3. The data were genetically interpreted as number of loci with allele frequencies screened in each species. From the total of 38 enzyme loci screened, 28 variable loci were found to be allelically different between the four species, while the other 10 loci were found to be monomorphic, fixed at the same allele (**100*), in every species. In a variable locus of any species, the degree of allele frequencies informs the allelic status of variable alleles as being common (high degree, ≤0.99) or variant (low degree, ≥0.01) in that species or alternatively fixed (1 or 0) between species. In a species, any variable loci that were found with the ≤0.99 commonest allele and other ≥0.01 combined alleles were considered to be polymorphic for that species (Hartl, 1988; Weaver and Hedrick, 1989). Thus, of the 28 variable loci in the four species (Table 3), 11 loci (*ADA*, ADH*, FH-2*, G3PDH-1*, G3PDH-2*, IDHP*, IDDH*, LDH-2*, LDH-3*, MPI** and *SOD-2**) were considered as being polymorphic in *N. notopterus*, while only two loci (*IDHP** and *SOD-2**) in *C.*

Table 2 Screened enzymes, enzyme number, suitable buffer solution, subunit structure, number of loci found, tissue sources and the electrophoretic mobility of each locus found in each enzyme. (Kanasuta and Sodsuk, 1998).

Enzyme	Enzyme no.	Buffer	Subunit structure	No. of loci found	Locus	Tissue source	Electrophoretic mobility
Aspartate aminotransferase (AAT)	2.6.1.1	TCB, TBE	Dimeric	4	*AAT-1**	H, L, E, K	cathodal
					AAT-2*	L	anodal
					AAT-3*	H, K	anodal
					*AAT-4**	H, E, K	anodal
Acid phosphatase (ACP)	3.1.3.2	TBE, TCE	Dimeric	2	*ACP-1**	L, H, K	anodal
					*ACP-2**	L, H, K	anodal, origin
Adenosine deaminase (ADA)	3.5.4.4	TBE	Monomeric	1	*ADA**	L, F, E	anodal
Alcohol dehydrogenase (ADH)	1.1.1.1	TBE	Dimeric	1	*ADH**	L	cathodal
Aconitate hydratase (AH)	4.2.1.3	TBE	Monomeric	1	*AH**	H, L	anodal
Adenylate kinase (AK)	2.7.4.3	TCB	Monomeric	1	*AK**	M, E, L, K	anodal
Alanine aminotransferase (ALAT)	2.6.1.2	TBE	Dimeric	1	*ALAT**	L, K	anodal
Creatine kinase (CK)	2.7.3.2	TBE	Dimeric	1	*CK**	L, M, F, E	anodal
Dihydrolipoamide dehydrogenase (DDH)	1.8.1.4	TCB	Monomeric	1	*DDH**	M, L, K, H, F, E	anodal
Esterase (EST)	3.1.1.-	TBE	Monomeric	1	*EST**	L, F, E	anodal
Esterase-D (ESTD)	3.1.-.-	TCB	Dimeric	1	*ESTD**	L	anodal
Fructose-biphosphate aldolase (FBALD)	4.1.2.13	TCB	Tetrameric	2	*FBALD-1**	L	cathodal
					*FBALD-2**	M, L, E, F, K	anodal
Fumarate hydratase (FH)	4.2.1.2	TBE	Tetrameric	2	*FH-1**	L, H, K	anodal
					*FH-2**	L, H, K	anodal
Glycerol-3-phosphate dehydrogenase (G3PDH)	1.1.1.8	TC, TBE, TCE, TCB	Dimeric	2	*G3PDH-1**	M, L, K	anodal
					*G3PDH-2**	M	anodal
Glucose-6-phosphate isomerase (GPI)	5.3.1.9	TCE, TC	Dimeric	2	*GPI-1**	M	anodal
					*GPI-2**	M, L	anodal
Isocitrate dehydrogenase ($NADP^+$) (IDHP)	1.1.1.42	TC	Dimeric	1	*IDHP**	K, E, L, F	anodal
L- Iditol dehydrogenase (IDDH)	1.1.1.14	TCB	Tetrameric	1	*IDDH**	L	anodal

Table 2 (Contd.).

Enzyme	Enzyme no.	Buffer	Subunit structure	No. of loci found	Locus	Tissue source	Electrophoretic mobility
L- Lactate dehydrogenase (LDH)	1.1.1.27	TBE	Tetrameric	3	*LDH-1**	L, F, E, H, K	anodal
					*LDH-2**	M	anodal
					*LDH-3**	L, F, E, K	anodal
Malate dehydrogenase (MDH)	1.1.1.37	TCB, TBE	Dimeric	2	*MDH-1**	L	anodal
					*MDH-2**	M, H, E, L	anodal
Malic enzyme ($NADP^+$) (MEP)	1.1.1.40	TCB, TBE	Tetrameric	2	*MEP-1**	M, L, H, K, F	anodal
					*MEP-2**	M, L, K, F, S	anodal
Manose-6-phosphate isomerase (MPI)	5.3.1.8	TCE	Monomeric	1	*MPI**	M, L	anodal
Phosphogluconate dehydrogenase (PGDH)	1.1.1.44	TCE, TBE	Dimeric	1	*PGDH**	L, M, F, S	anodal
Phosphoglucomutase (PGM)	5.4.2.2	TBE, TCE	Monomeric	2	*PGM-1**	M, L, H, F, E	anodal
					*PGM-2**	M, L, H, F, E	anodal
Superoxide dismutase (SOD)	1.15.1.1	TBE, TCE	Dimeric	2	*SOD-1**	L	anodal
					*SOD-2**	L	anodal

Total = 38 loci

Abbreviation: TBE = Tris-Borate-EDTA (pH 8.5), TC = Tris-Citrate (pH 8), TCB = Tris-Citrate/Lithium Borate (pH 8.5), TCE = Tris-Citrate-EDTA (pH 7) H = Heart, L = Liver, E = Eye, K = Kidney, F = Fin, M = Muscle, S = Spleen

Table 3 Thrity-eight enzyme loci screened with allele frequencies at each locus in each species, and the genetic variabilities measured as loci polymorphism ($P_{0.99}$) and heterozygosities ($H_{observed}$, $H_{expected}$). Results from the χ^2-goodness-of-fit tests for Hardy-Weinberg equilibrium at all polymorphic loci of each species are shown with the *p*-values. (p>0.05 = not significant).

Locus	Allele	*N. notopterus* (χ^2)	*C. ornata* (χ^2)	*C. blanci* (χ^2)	*C. lopis*
*AAT-1**	*-60	1.000	-	-	-
	*-100	-	1.000	1.000	1.000
*AAT-2**	*160	1.000	-	-	-
	*100	-	1.000	1.000	1.000
*AAT-3**	*130	1.000	-	-	-
	*100	-	1.000	1.000	1.000
*AAT-4**	*114	1.000	-	-	-
	*100	-	1.000	1.000	1.000
*ACP-1**	*100	1.000	1.000	1.000	1.000
*ACP-2**	*100	-	1.000	1.000	1.000
	*88	1.000	-	-	-
*ADA**	*160	0.493	-	-	-
	*130	0.507 (p=0.058)	-	-	-
	*100	-	1.000	1.000	-
	*80	-	-	-	1.000
*ADH**	*0	-	-	-	1.000
	*-85	0.090	-	-	-
	*-100	0.910 (p=0.131)	1.000	1.000	-
*AH**	*100	1.000	1.000	1.000	1.000
*AK**	*100	1.000	1.000	1.000	1.000
*ALAT**	*100	1.000	1.000	1.000	1.000
*CK**	*100	1.000	1.000	1.000	1.000
*DDH**	*100	1.000	1.000	1.000	1.000
*EST**	*100	1.000	1.000	1.000	1.000
*ESTD**	*160	1.000	-	-	-
	*100	-	1.000	1.000	1.000
*FBALD-1**	*-100	1.000	1.000	1.000	1.000
*FBALD-2**	*100	1.000	1.000	1.000	1.000
*FH-1**	*100	-	1.000	1.000	1.000
	*74	1.000	-	-	-

Table 3 (Contd.).

Locus	Allele	*N. notopterus* (χ^2)	*C. ornata* (χ^2)	*C. blanci* (χ^2)	*C. lopis*
*FH-2**	*100	-	1.000	1.000	1.000
	*78	0.918	-	-	-
	*55	0.082 (p=0.077)	-	-	-
*G3PDH-1**	*120	0.948	-	-	-
	*100	0.052 (p=1.000)	1.000	1.000	1.000
*G3PDH-2**	*195	0.075	-	-	-
	*170	0.784	-	-	-
	*136	0.141 (p=0.263)	-	-	-
	*100	-	1.000	1.000	1.000
*GPI-1**	*100	1.000	-	-	1.000
	*70	-	1.000	-	-
	*55	-	-	1.000	-
*GPI-2**	*100	-	1.000	1.000	1.000
	*88	1.000	-	-	-
*IDHP**	*115	0.030	0.417	0.300	1.000
	*100	0.970 (p=0.053)	0.583 (p=0.287)	0.700 (p=0.673)	-
*IDDH**	*100	0.082	-	1.000	1.000
	*80	0.918 (p=0.077)	1.000	-	-
*LDH-1**	*135	-	-	1.000	-
	*100	1.000	1.000	-	1.000
*LDH-2**	*100	0.955	1.000	-	1.000
	*70	0.045 (p=1.000)	-	1.000	-
*LDH-3**	*107	0.067	-	-	-
	*100	0.933 (p=0.971)	1.000	1.000	1.000
*MDH-1**	*120	-	-	-	1.000
	100	1.000	1.000	1.000	-
*MDH-2**	*100	1.000	1.000	1.000	1.000
*MEP-1**	*110	1.000	-	-	-
	*100	-	1.000	1.000	1.000
*MEP-2**	*110	-	-	1.000	-
	*100	1.000	1.000	-	-
	*80	-	-	-	1.000

Table 3 (Contd.).

Locus	Allele	*N. notopterus* (χ^2)	*C. ornata* (χ^2)	*C. blanci* (χ^2)	*C. lopis*
*MPI**	**108*	0.030	-	-	-
	**100*	0.970 (p=0.053)	1.000	-	1.000
	**95*	-	-	0.800	-
	**85*	-	-	0.200 (p=1.000)	-
*PGDH**	**110*	-	-	1.000	1.000
	**100*	1.000	1.000	-	-
*PGM-1**	**100*	1.000	-	1.000	1.000
	**90*	-	1.000	-	-
*PGM-2**	**110*	-	-	1.000	1.000
	**100*	1.000	1.000	-	-
*SOD-1**	**110*	1.000	-	-	-
	**100*	-	1.000	1.000	-
	**90*	-	-	-	1.000
*SOD-2**	**120*	0.022	-	-	-
	**100*	0.948	0.967	1.000	
	**80*	-	-	-	1.000
	**70*	0.030 (p=1.000)	0.033 (p=1.000)	-	-
$P_{0.99}$		0.289	0.053	0.053	0.000
$H_{observed}$		0.046(±0.019)	0.011(±0.010)	0.016(±0.012)	0.000
$H_{expected}$		0.049(±0.017)	0.015(±0.013)	0.022(±0.015)	0.000

ornata and another two (*IDHP** and *MPI**) in *C. blanci*. No polymorphic loci were found in *C. lopis*.

Deviations from Hardy-Weinberg equilibrium as significant differences between the number of the observed and expected genotypes of any polymorphic loci were not detected (Table 3, $\chi^2_{p>0.05}$) in any species. Thus, there are no significant differences between the samples within a species, which means the species were sampled from single panmictic populations (Richardson *et al*., 1986).

The loci polymorphism ($P_{0.99}$ = 0 - 0.289) and heterozygosities (both the observed, H_o = 0 - 0.046±0.019, and Hardy-Wienberg expected, H_e = 0 - 0.049±0.017) resulting for each species are shown at the end of Table 3. The results of P = 0 and H = 0 in *C. lopis* do not mean that there is no genetic variation in this species. Instead, it should be because of that this species status in nature is so uncommon that only few number of the fish could be collected for sample analyses, and therefore has affected the detection of its genetic polymorphism. However, regardless of *C. lopis*, the heterozygosity values of these fish could generally compare well with a range of the levels observed in other fishes, H = 0.013 - 0.049, that have significance for population fitness (Purdom, 1993).

Based on the allozymes resulting, *N.*

notopterus would generally seem to be of much higher genetic polymorphism than the other three species would do, although there was difference in the number of samples used between species. Small numbers of the samples of two species, *C. blanci* and *C. lopis*, were unavoidably employed in this study, due to their specific status of being very rare in nature. Distributions of the fish in this group have been surveyed and reported recently by Roberts (1992). It is apparent that the commonest species of these fish in Thailand should be *N. notopterus* which can be found widely in many natural freshwater-bodies over the country. The next relatively common one is *C. ornata*, while the other two, *C. blanci* and *C. lopis*, are locally endemic species. It has been known that *C. blanci* is restricted to the Mekhong River and its tributaries in the Northeast, while *C. lopis* is restricted to the Tapi River and its tributaries in the South and very few to the Meklong River in the West. The species status as being threatened and endangered have been remarked respectively for *C. blanci* and *C. lopis* (Bain and Humphrey, 1980), which means rareness of these two species has been realised since many years ago.

Genetic differentiation

Table 4 shows the genetic differentiations performed over all loci using the F_{ST} index of Wright (1978) and the heterogeneity contingency chi-square analyses of Workman and Niswander (1970). All results of the differentiations hierarchically performed to all four species, the three *Chitala* species and each pair of every species are shown.

As described by Hartl (1988), F_{ST} has a theoretical minimum of 0 (indicating no genetic divergence) and a theoretical maximum of 1 (indicating fixation for alternative alleles in the subpopulations, or species). All values of the F_{ST} resulting from this study are close to 1, which, therefore, are related to a number of alternatively fixed alleles detected in the species (Table 3). One of the qualitative guidelines for the interpretation of F_{ST} suggested by Wright (1978) is that the values of F_{ST} above 0.25 indicate *very great* genetic differentiation among subpopulations/species. Consistently, all of the *p*-values ($p<0.001$, Table 4)

Table 4 Hierarchical genetic differentiations detected over all loci using F_{ST} values (Wright, 1978) and contingency chi-square analysis (Workman and Niswander, 1970).

Species	*FST*	Contingency χ^2 analysis		
		χ^2	df	*p*
NN & CO	0.865	3144.580***	28	0.000
NN & CB	0.890	2982.120***	33	0.000
NN & CL	0.919	2980.501***	32	0.000
CO & CB	0.873	630.829***	12	0.000
CO & CL	0.954	781.667***	13	0.000
CB & CL	0.933	210.769***	12	0.000
Among three species of *Chitala*	0.936	1293.492***	38	0.000
Among all species	0.931	6328.634***	123	0.000

Abbreviation: NN = *N. notopterus*, CO = *C. ornata*, CB = *C. blanci*, CL = *C. lopis*
$p < 0.001$ = very highly significant (***)

from the heterogeneity contingency chi-square analyses of all the categorized hierarchies indicate highly significant differences between these species. Therefore the resulting F_{ST} values and the heterogeneity analyses consistently imply that there is rather large genetic divergence between the species studied, although they all appear to have several similar phenotypes (Sodsuk *et al*., 1983; Donsakul and Magtoon, 1990; Roberts, 1992). The consistent implication can be more strengthened by a number of different diagnostic loci found for each species pair (Table 5), which can readily be used to discriminate between one and the other of each pair of the species. With these diagnostic or discriminating loci, Kanasuta and Sodsuk (1998) have successfully established a reliable allozyme identification key for the group of these fish in Thailand.

Genetic structure and relationships

Genetic structure and relationships of the four featherbacks are presented in Figure 1 by the phylogenetic tree (dendrogram) reconstructed using the Cavalli-Sforza and Edwards (1967) chord distances (Table 6, above diagonal) with the tree reconstruction method, the distance Wagner algorithm of Farris (1972). The subsequently resulting patristic distances, the similarities due to common ancestry (Lawrence, 1989), are also shown in the below diagonal of Table 6 and represent branch lengths among species in the tree. The tree presented genetic structure of the four species as two clades: one is the *N. notopterus* clade and the other is the clade of *Chitala* species, *C. ornata, C. blanci* and *C. lopis*. This means that the three *Chitala* species are genetically related more closely to each other than to *N. notopterus*. Considering between the two generic clades, *Notopterus* and *Chitala*, *N. notopterus* seems to have genetic relationship more closely to *C. ornata* than to *C. blanci* and *C. lopis*. In the *Chitala* clade, *C. blanci* and *C. lopis* are closely related as sister species whereas *C. ornata* is distantly related. With morphological characters resulting from the most recent revision study of Roberts (1992), the decision of which one of the *Chitala* species was the closest species to *N. notopterus* could not be made. However, based on the uniquely shared character of a black spot on the pectoral fin base, Roberts (1992) has considered *C. blanci* and *C. lopis* to be close relatives to each other. The genetic structure and relationships presented here are consistent with those previously reported by Kanasuta and Sodsuk (1998), although the form of the data input for phylogenetic tree reconstruction between the two studies is different. Binary coded allozyme data as 1 or 0 for the presence or absence of alleles together with the bootstrap resampling data of Felsenstein (1985) were employed in the Kanasuta and Sodsuk (1998) study. The same results from both studies support the present nomenclature of the fish that has recently been revised by Roberts (1992). Based merely on morphological characters information, Roberts (1992) retained the only one species possessing the most generalized characters as *Notopterus notopterus* and reconsidered other relatives which probably evolved from *Notopterus*-like ancestor, such as the other three species of Thailand (*C. ornata, C. blanci* and *C. lopis*), as the species of *Chitala*.

CONCLUSION

The study on genetic diversity of four economically important fish in the family Notopteridae or 'featherbacks' of Thailand was carried out using a molecular genetic technique called allozyme electrophoresis to result in genetic variation, differentiation and structure informations, that were of great benefit to the sustainable utilization and conservation of their genetic resources both in cultural stocks and wild

Table 5 Diagnostic enzyme loci found for each pair of all species studied.

Species pair	Diagnostic enzyme loci
NN & CO	*AAT-1*, AAT-2*, AAT-3*, AAT-4*, ACP-2*, ADA*, ESTD*, FH-1*, FH-2*, G3PDH-2*, GPI-1*, GPI-2*, MEP-1*, PGM-1*, SOD-1** (15 loci)
NN & CB	*AAT-1*, AAT-2*, AAT-3*, AAT-4*, ACP-2*, ADA*, ESTD*, FH-1*, FH-2*, G3PDH-2*, GPI-1*, GPI-2*, LDH-1*, MEP-1*, MEP-2*, MPI*, PGDH*, PGM-2*, SOD-1** (19 loci)
NN & CL	*AAT-1*, AAT-2*, AAT-3*, AAT-4*, ACP-2*, ADA*, ADH*, ESTD*, FH-1*, FH-2*, G3PDH-2*, GPI-2*, MDH-1*, MEP-1*, MEP-2*, PGDH*, PGM-2*, SOD-1*, SOD-2** (19 loci)
CO & CB	*GPI-1*, IDDH*, LDH-1*, LDH-2*, MEP-2*, MPI*, PGDH*, PGM-1*, PGM-2** (9 loci)
CO & CL	*ADA*, ADH*, GPI-1*, IDDH*, MDH-1*, MEP-2*, PGDH*, PGM-1*, PGM-2*, SOD-1*, SOD-2** (11 loci)
CB & CL	*ADA*, ADH*, GPI-1*, LDH-1*, LDH-2*, MDH-1*, MEP-2*, MPI*, SOD-1*, SOD-2** (10 loci)

Abbreviation: NN = *N. notopterus*, CO = *C. ornata*, CB = *C. blanci*, CL = *C. lopis*

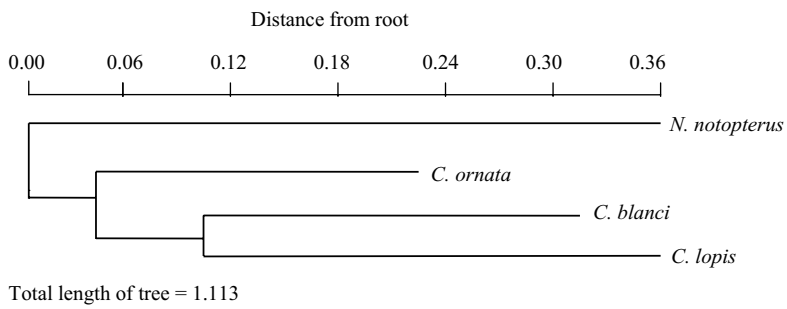

Figure 1 Phylogenetic tree presenting genetic structure and relationships of the four featherback species. Total length of tree is the number of all branch lengths in the tree.

Table 6 Pairwise matrices of the Cavalli-Sforza and Edwards (1967) chord distances (above diagonal) used in the phylogenetic tree building, and the subsequent patristic distances (below diagonal) representing branch lengths between species in the tree.

Species	*N. notopterus*	*C. ornata*	*C. blanci*	*C. lopis*
N. notopterus	-	0.586	0.677	0.675
C. ornata	0.586	-	0.439	0.492
C. blanci	0.677	0.439	-	0.472
C. lopis	0.730	0.492	0.472	-

populations. The obtained results could be summarized as the following :

1) Based on the allozymes resulting (Table 3), *N. notopterus* would seem to be of much higher genetic polymorphism than the other species would do; eleven polymorphic loci were found in *N. notopterus* ($P_{0.99}$ = 0.289) while only two found in *C. ornata* and *C. blanci* ($P_{0.99}$ = 0.053) and none in *C. lopis*. The undetectable genetic variation in *C. lopis* ($P_{0.99}$ & $H_{o,e}$ = 0) should probably be due to the species status in nature as being so uncommon that quite a small number of the fish could be sampled unsuitably for the analyses. However, except *C. lopis*, the range of heterozygosity levels of the three other species, both the observed (H_o = 0.011 - 0.046) and estimated (H_e = 0.015 - 0.049), were comparable with the range of the levels observed in many other fishes, H = 0.013 - 0.049, that had significance for population fitness (Purdom, 1993).

2) The observed genotypes in any species were not significantly deviated from Hardy-Weinberg equilibrium ($\chi^2_{p>0.05}$), suggesting no significant differences among the samples within a species, while the F_{ST} values (above 0.25) and the heterogeneity contingency chi-square analyses (p<0.001) suggested rather large genetic divergence among these species.

3) All the species studied could be discriminated from each other by a number of different diagnostic loci found for every species pair (Table 5), which were acceptable as genetic markers for the identification between one and the other of each pair of the species.

4) The genetic structure and relationships of the four species phylogenetically presented as two separate clades, the *N. notopterus* and the three species of *Chitala* (Figure 1), revealed that the three *Chitala* species were genetically related more closely to each other than to *N. notopterus*. This revelation supports the present nomenclature of the fish that has recently been revised by Roberts (1992).

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