

## Molecular Systematics of the Thornfishes Genera *Terapon* and *Pelates* (Perciformes: Teraponidae) with Reference to the New Genus *Pseudoterapon*

Sin-Che Lee\* and Mung-Pei Tsai

Institute of Zoology, Academia Sinica, Taipei, Taiwan 115, R.O.C.

(Accepted March 24, 1999)

**Sin-Che Lee and Mung-Pei Tsai (1999)** Molecular systematics of the thornfishes genera *Terapon* and *Pelates* (Perciformes: Teraponidae) with reference to the new genus *Pseudoterapon*. *Zoological Studies* 38(3): 279-286. The aim of this study is to clarify whether the systematic status of 3 externally similar thornfishes can be resolved to posit them at specific or generic levels by using allozyme electrophoresis and partial 12S rRNA sequences of mitochondrial DNA. Three externally similar thornfishes, *Terapon jarbua*, *T. theraps*, and *Pelates quadrilineatus*, can be distinguished by several fixed isozyme loci: *IDHP-2\**, *LDH-A\**, *LDH-B\**, *MDH-1\**, *MDH-2\**, *SDH-1\**, *SOD-1\**, *SOD-2\**, and *SOD-3\**. Nei's unbiased genetic identity of isozymes ranged 0.321-0.454 (or distance 0.790-1.137), and the nucleotide base difference of mtDNA ranged 7.3%-10.9% among the 3 species, enabling them to posit as 3 separate genera. Nei's genetic similarity between congeneric *T. theraps* and *T. jarbua* (0.441) is very low. The position of *T. theraps* with *T. jarbua* on the trees constructed from both isozyme and mtDNA data show similar profiles of grouping on separate nodes. This may support *T. theraps* being removed from the genus *Terapon* as an independent new genus, *Pseudoterapon*. The main diagnostic morphological characters distinguishing *Pseudoterapon* from *Terapon* are: larger scales, absence of teeth on vomer and palatines, and horizontal dark stripes on body sides.

**Key words:** Thornfishes, *Pseudoterapon* n. gen., Allozyme, mtDNA.

Fishes of the family Teraponidae (thornfishes, grunters, or tiger perches) are Indo-West Pacific fishes, living in marine coastal, brackish, and freshwater habitats, with 16 genera and about 45 species (Vari 1978, Nelson 1994). Some species are restricted to freshwaters of Australia, New Guinea, Indonesia and the Philippines (Heemstra 1986). In Taiwan, there are 4 species included in 2 genera, *Pelates* (*P. quadrilineatus*) and *Terapon* (*T. cancellatus*, *T. jarbua*, and *T. theraps*) (Shen et al. 1993). For years, these 2 genera were recognized as valid by several authors because their external features resembled each other, except for the provision of a serrated posterior margin of the post-temporal bone (or suprascapula) in the genus *Terapon* (Weber and de Beaufort 1931, Lindberg and Krasnyukova 1969, Heemstra 1986), while species of the genus *Pelates* have an entire posterior margin of

the post-temporal bone. A third genus, *Pseudoterapon* represented by *Terapon theraps* described in this paper is extracted from *Terapon*. Though both share a serrated post-temporal bone, they differ in scale size, tooth forms, and body stripe feature, as well as possessing unusually low isozyme and mtDNA genetic similarities.

*Pelates* was erected as a genus by Cuvier and Valenciennes (1829), but authors like Bleeker (1873-1876) and Klunzinger (1884) treated them as a subgenus under *Terapon*, recognizing their close resemblance in appearance. However, its systematic status will be clarified from molecular data.

During our investigation of molecular systematics of several thornfishes, we found that discrepancies existed between 2 *Terapon* species as to morphological and molecular criteria. This prompted us to reevaluate the phylogenetic relationships among

\*To whom correspondence and reprint requests should be addressed. Tel: 886-2-27899520. Fax: 886-2-27858059. E-mail: scllee@gate.sinica.edu.tw

the members of thornfish group.

Molecular techniques have proven useful in addressing a variety of systematic questions (Avice 1994). For phylogenetic studies, the sequencing of mtDNA has been thought to be excellent while the protein method is considered only fair (Park and Moran 1995). However, these 2 approaches can be used satisfactorily for estimating the interspecific variation of these 3 externally very similar thornfishes, *Pelates quadrilineatus*, *Terapon jarbua*, and *T. theraps*. Their systematic status at the generic or inter-specific level will also be clarified. *T. cancellatus*, previously recorded in Taiwan, is very rare, and is not considered in this study

## MATERIALS AND METHODS

Thirty-three specimens of *Pelates quadrilineatus* (112-161 mm SL), 44 *Terapon jarbua* (96-172 mm SL), and 34 *T. theraps* (69-117.5 mm SL) collected from Kaohsiung by trawler during 1996-1997 were used for allozyme electrophoresis. Six specimens of each species were randomly chosen from among the above specimens, with an additional 1 *Pomadasys kaakan*, belonging to the Haemulidae as an outgroup, were used for sequencing.

**Allozyme electrophoresis:** Tissues, including eye, heart, kidney, and liver, were homogenized with 2-3 volumes of extraction buffer (Shaklee and Keenan 1986), and centrifuged at 17 000 g for extraction of enzymes. The extract was electrophoresed on horizontal starch gel consisted of 12% (W/V) starch. Three buffer systems were used: LiOH for resolution of CK, GPI, PGM, LDH, SDH, and XDH; TC8.0 for IDH; and TC7.0 for AAT, MDH, ME, PGDH, and SOD. The staining procedure for enzyme activity followed that of Shaw and Prasad (1970) and Jean et al. (1995).

**DNA sequencing:** Crude DNA was extracted from muscle following the procedure of Kocher et al. (1989). Double-strand PCR amplification was performed using mitochondrial 12 S rRNA primer pair ES1 (5'-AACTGGGATTAGATACCCCACTATG-3') and 12SR (5'-TTTCATGTTTCCTTGCGGTAC-3'). Conditions of PCR were initial denaturation at 94 °C for 2 min, 35 cycles at 94 °C for 1 min, 55 °C for 1 min, 72 °C for 1.5 min, and then maintaining at 72 °C for an additional 10 min. The PCR product was electrophoresed using 1.2% agarose gel by 1X TBE buffer. The single band appearing on the gel was removed, with DNA extraction from the gel being performed by GeneClean III (Bio101). The amount of DNA template for the sequence reaction was 100 ng,

with the quantity of primer at 3.2 pmole; 8 µl of DNA sequencing Kit (Amersham Life Science) was added, and the volume was increased to 20 µl by adding sterilized water before the reaction in a Perkin Elmer PCR 2400. The DNA sequence data was detected by an ABI 377 (Applied Biosystems).

**Data analysis:** The alleles for each locus resolved on the gel were identified and analyzed by the computer program, BIOSYS, to calculate allele frequencies, Nei's unbiased genetic identity (Nei 1978), and F-statistics (Nei 1977). Nei's unbiased genetic identity was used to build the phylogenetic tree by UPGMA and neighbor-joining (Saitou and Nei 1987) methods.

Sequences were aligned using the PILEUP program of GCG sequence analysis software package and were adjusted by visual inspection. Aligned sequences of the 3 thornfish species named above and the outgroup, *Pomadasys kaakan*, were analyzed using the MEGA software package (Molecular Evolutionary Genetics Analysis, version 1.02; Kumar et al. 1993) for calculating the sequence distance using the Kimura 2-parameter model (Kimura 1980), and nucleotide substitution between sequences for constructing a phylogenetic tree by UPGMA and neighbor-joining methods; 100 bootstrap replicates were analyzed.

## RESULTS

**Allozyme electrophoresis:** Among 19 loci and 69 alleles scored from 11 enzymes, only LDH-C and XDH-1 were monomorphic; the remaining 17 were polyallelic (Table 1). The following fixed loci differed among the 3 taxa: *mAAT\**, *CK-B\**, *LDH-A\**, *LDH-B\**, *MDH-1\**, *PGM-1*, *SDH-1\**, *SOD-1\**, and *SOD-2\** between *Terapon jarbua* and *T. theraps*; *mAAT\**, *IDHP-2\**, *CK-A\**, *CK-B\**, *MDH-1\**, *MDH-2\**, *PGM-1\**, *6PGD-1\**, *SDH-1\**, and *SOD-3\** between *T. jarbua* and *Pelates quadrilineatus*; and *mAAT\**, *IDHP-2\**, *CK-A\**, *LDH-A\**, *LDH-B\**, *MDH-1\**, *MDH-2\**, *SDH-1\**, *SOD-1\**, *SOD-2\**, and *SOD-3\** between *T. theraps* and *P. quadrilineatus*, suggesting they belong to separate valid species. Nei's (1978) unbiased genetic distance data in table 2 reveal that the similarity among *T. jarbua*, *T. theraps*, and *P. quadrilineatus* ranged 0.321-0.454 (or distance 0.790-1.137) which fits in well within the generic status (0.45, Shaklee and Tamaru 1981). The subsequent tree (Fig. 1) constructed from the above data indicates that *Terapon jarbua* (TJ) and *Terapon theraps* (TT) could not be grouped as a whole in spite of data treatment with UPGMA (Fig. 1A), neighbor-joining (Fig. 1B)

and Wagner trees (Swofford 1981) (Fig. 1C). The shorter branch lengths of *T. jarbua* than *T. theraps* shown in B and C, further confirm the independence of *T. theraps* from *T. jarbua*. Thus a new genus, *Pseudoterapon*, was nominated for *T. theraps* upon consideration of removing it from the original *Terapon* genus. *T. jarbua* remains under the genus *Terapon* based on the type species, *Holocentrus servus* Bloch. The latter is regarded as a synonym of

*T. jarbua* named in 1755 (Forsk. 1755).

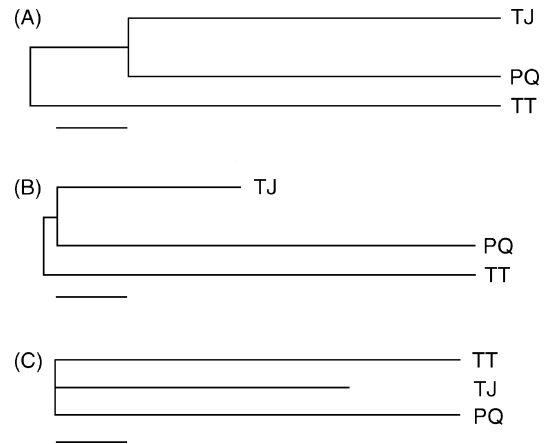
*mtDNA*: A total of 660 base pairs of aligned sequences were studied for genetic analysis. The ratio between transitions (ts) and transversions (tv) obtained from the nucleotide composition data exceeded 1 (1.178-2.335). Such an unsaturated situation inferred that DNA sequences are variable enough for determination of phylogenetic relationships. The pairwise comparisons of observed sub-

**Table 1.** Genotypic data for 19 loci in *Terapon theraps*, *T. jarbua*, and *Pelates quadrilineatus*

Locus	<i>Terapon theraps</i> (N = 33)	<i>Terapon jarbua</i> (N = 44)	<i>Pelates quadrilineatus</i> (N = 34)
<i>mAAT</i> *	116/116 (32) 116/97 (01)	100/100 (41) 100/137 (03)	153/153 (32) 153/123 (02)
<i>IDHP-1</i> *	100/100 (30) 100/80 (02) 100/111 (01)	100/100 (43) 100/84 (01)	100/100 (34)
<i>IDHP-2</i> *	100/100 (33)	100/100 (43) 100/120 (01)	111/111 (27) 111/142 (06) 142/142 (01)
<i>LDH-A</i> *	140/140 (31) 140/40 (02)	100/100 (44)	100/100 (34)
<i>LDH-B</i> *	124/124 (33)	100/100 (44)	100/100 (34)
<i>LDH-C</i> *	100/100 (33)	100/100 (44)	100/100 (34)
<i>CK-A</i> *	200/200 (10) 200/95 (01) 95/95 (22)	100/100 (36) 100/95 (08)	110/110 (34)
<i>CK-B</i> *	103/103 (33)	100/100 (42) 100/97 (02)	103/103 (34)
<i>GPI-A</i> *	100/100 (25) 100/88 (01) 100/92 (04) 100/109 (03)	100/100 (42) 100/64 (02)	109/109 (31) 100/109 (02) 109/116 (01)
<i>GPI-B</i> *	31/31 (30) 100/31 (03)	100/100 (18) 200/200 (13) 100/200 (09) 100/31 (03) 100/300 (01)	100/100 (34)
<i>MDH-1</i> *	128/128 (32) 170/128 (01)	100/100 (44)	114/114 (34)
<i>MDH-2</i> *	100/100 (33)	100/100 (44)	84/84 (34)
<i>PGM-1</i> *	140/140 (31) 140/122 (01) 140/26 (01)	100/100 (34) 100/68 (07)	140/140 (33) 140/138 (01)
<i>6PGD-1</i> *	93/93 (27) 93/84 (06)	93/93 (05) 100/100 (16) 93/100 (12) 107/100 (05) 93/107 (05) 93/103 (01)	83/83 (34)
<i>SDH-1</i> *	74/74 (30) 74/18 (03)	100/100 (34)	113/113 (29) 113/80 (01) 113/20 (03) 20/20 (01)
<i>SOD-1</i> *	109/109 (33)	100/100 (34)	100/100 (34)
<i>SOD-2</i> *	115/115 (33)	100/100 (34)	100/100 (34)
<i>SOD-3</i> *	100/100 (33)	100/100 (34)	60/60 (34)
<i>XDH</i> *	100/100 (33)	100/100 (34)	100/100 (34)

stitutions are similar, though slightly different among the 3 species. Sixty-five substitutions are shown between *Pelates quadrilineatus* and *Terapon jarbua*, 47 between *P. quadrilineatus* and *T. theraps*, and 53 between *T. theraps* and *T. jarbua* (Table 3). The pairwise between *P. quadrilineatus* and *T. theraps* showed less difference than that between the 2 *Terapon* species alone. Trees in figure 2 including UPGMA (A) and NJ (B), both leave the outgroup *Pomadasys kaakan* isolated, and cluster the rest as a separate group. Three species in the latter are further subdivided into an independent *T. jarbua* and the other with *T. theraps* and *P. quadrilineatus*. Both trees show similar trends, except the branch length can be indicated on the NJ tree. A 100% BCL (bootstrap confidence level) at the branching point between *Pelates quadrilineatus* and *Terapon theraps* on the NJ tree and 71% on the UPGMA tree show a satisfactory grouping of the 2 species. Values are inadequate to associate *T. theraps* with *T. jarbua* in a unique genus. *Terapon theraps* had slightly higher similarity (0.9298) to *P. quadrilineatus* than to *T. jarbua* (0.9220) (Table 4), though the distance was nearly equal between each comparison, with a wide separation of 15.34%-20.14% between the families

Teraponidae represented by the above 3 species and Haemulidae represented by *Pomadasys kaakan*.



**Fig. 1.** UPGMA tree (A) and NJ tree (B) constructed from isozyme data based on Nei's unbiased genetic distance; and Wagner tree (C) constructed from isozyme data based on Roger's genetic distance. PK, *Pomadasys kaakan*; PQ, *Pelates quadrilineatus*; TJ, *Terapon jarbua*; TT, *Terapon theraps*. Scale bar = 0.07.

**Table 2.** Nei's (1978) unbiased genetic distance (below diagonal) and Nei's (1978) unbiased genetic identity (above diagonal)

	<i>Terapon theraps</i>	<i>Terapon jarbua</i>	<i>Pelates quadrilineatus</i>
<i>Terapon theraps</i>	—	0.441	0.321
<i>Terapon jarbua</i>	0.818	—	0.454
<i>Pelates quadrilineatus</i>	1.137	0.790	—

**Table 3.** One hundred forty variable sites among of 661 analyzed mtDNA sequences of *Pomadasys kaakan* (PK), *Pelates quadrilineatus* (PQ), *Terapon jarbua* (TJ), and *Terapon theraps* (TT). “-” indicates a match with sequence of *Pomadasys kaakan*. “.” denotes a gap

	1111111111	1111111111	1112222222	2222222222	3333333333	3333333333
	1113388	0011234455	5566667788	8990122444	4566667888	0012223356
	3673782968	6747241523	7902354513	4054907267	9623587023	6981341213
PK	CGATGCGTGT	CTTACATCTG	AGCCATAGCC	TACGAGCACA	TCCATATTAT	CATTGCGCTC
PQ	--TGATAC-C	TCCCAGCTCT	GAA-CC-ATT	AGTCGAACAC	CTAG-GCGCA	-GCC--TTC-
TJ	T--G--A-AC	--C-GC--A	--TACCGA--	AGTC--AC-T	CTAG--GCA	-G-CT---CT
TT	-A-GATA-AC	T-CT-GC---	--A-C--A--	AGTC-AACAC	CTAGC--ACA	TGCCTATTCT
	3333333333	4444444444	4444444444	5555555555	5555555555	5555666666
	8888889999	0000000001	1455677888	0112233444	5566677888	8899000011
	4567890126	0124567890	1045714234	6585823167	7801629012	5645023701
PK	TCAAATGCTG	CTGGAACAAA	GCCGGGAACC	CGCGGTA AAC	CCCCACTCCT	TCACAAAGCA
PQ	AATTTAA--A	-CAA.CTTGC	ATTACAGGTT	TATTAC-GTT	-AA-CACAAC	AT---GC-C
TJ	-ACTT-AACA	ACA-T--TGC	TT-AT--GG-	TA-C--GGTT	....ACTAC	AT-A--TTAC
TT	-ATTAA--A	A-AA.-TTGC	TT-ATAGGAT	TA-T-C-GTT	AAAT-ACAAC	ATG-CC-T-C

## Status of new genus

### *Pseudoterapon* gen. nov.

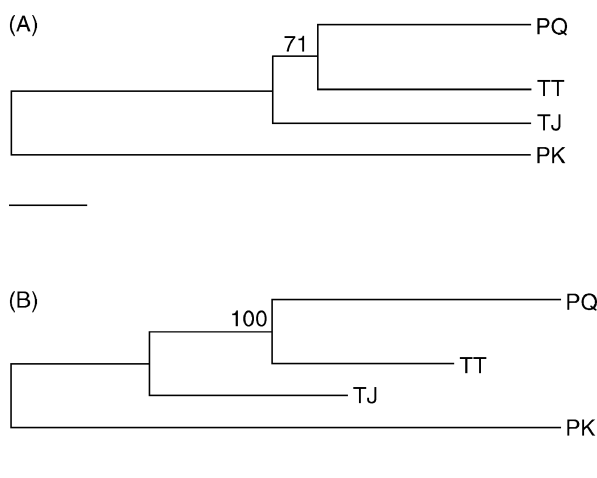
Type: *Terapon theraps* Cuvier and Valenciennes, 1829

**Diagnosis:** *Pseudoterapon* is erected as a new genus, separated from the original *Terapon* genus. Scales moderate in size, the number on lateral line (LLp) 46-56, between middle of spinuous dorsal to lateral line 6-8, anal origin to lateral line 14-16. Suprascapula bone serrated posteriorly. Teeth on vomer and palatines absent. Lower branch of gill rakers 15-18. Dorsal rays XI-XII, 9-11, anal rays III, 7-9. Dark horizontal bands on body sides.

**Remarks:** As summarized in table 4, the significant difference in isozyme patterns and mtDNA sequences between *Pseudoterapon* and *Terapon* enables them to be recognized as separate genera. However, although they closely resemble each other in external appearance, yet *Pseudoterapon* can be distinguished from *Terapon* in the following points: scales larger, with LLp 46-56 in *Pseudoterapon* vs. 75-100 in *Terapon*; teeth on vomer and palatines absent vs. denticulated in *Terapon*; and straight horizontal dark stripes on body sides vs. curved in *Terapon*.

## DISCUSSION

Data represented by neighbor-joining and Wagner trees are more adequate than those by the UPGMA tree, since the former 2 trees can indicate



**Fig. 2.** UPGMA tree (A) and neighbor-joining tree (B) constructed from mtDNA data based on Kimura 2-parameter distance. PK, *Pomadasys kaakan*; PQ, *Pelates quadrilineatus*; TJ, *Terapon jarbua*; TT, *Terapon theraps*. Scale bar = 0.0127.

branch lengths. The tree derived from DNA sequence data with both UPGMA and neighbor-joining methods group *Terapon theraps* and *Pelates quadrilineatus* together, having a bootstrap confidence level of 71% and 100% respectively, for this node, the latter approach being more suitable for phylogenetic implication. On the contrary, both trees produced from isozyme data cluster *Terapon jarbua* and *Pelates quadrilineatus* together. Although the mtDNA data is not congruent with that of isozyme data, their similar tree profiles which do not group *Terapon theraps* with *T. jarbua* are regarded as evidence to show that *T. theraps* is an independent taxon as it is from *Pelates*.

A survey of the literature of allozyme electrophoretic estimates of intergroup genetic differentiation for fishes reveals that genetic distances between genera range from 0.58 to 1.21, while those of congeneric species range from 0.025 to 0.609 (Shaklee et al. 1982). The genetic distances (0.790-1.137) obtained among the 3 species are far beyond the species level and fall well within the generic level. The slight anatomical difference between thornfish taxa can be considered to evaluate their taxonomic status using genetic distance derived from isozyme data or base difference derived from mtDNA data. The respective values below 0.7 and 7%, or even lower, will be justified as species or population level. The exact values for the latter will be confirmed further when the population genetic studies of any thornfish species is completed.

The range of nucleotide diversity of 12S rRNA between congeneric or closely related species of fishes differs among lineages. For example, the percentage of nucleotide difference between genera of Antarctic Notothenioidei ranges 1%-5.6% (Bargelloni et al. 1994) while intergeneric difference is close to 5% as obtained in American electric fishes (*Gymnotiformes*) (Alves-Gomes et al. 1995). The range of genetic diversity may reach 10%-15% in aplocheiloids (Murphy and Collier 1997).

**Table 4.** Percentage of overall base difference (below diagonal) and similarity (above diagonal) among *Pelates quadrilineatus* (PQ), *Terapon jarbua* (TJ), *T. theraps* (TT), and the outgroup *Pomadasys kaakan* (PK), obtained with MEGA software

	PK	PQ	TJ	TT
PK	–	0.8237	0.8627	0.8378
PQ	0.2014	–	0.9048	0.9298
TJ	0.1534	0.1087	–	0.9220
TT	0.1798	0.0729	0.0872	–

The genetic divergence of 7.29%-10.87% obtained among the congeneric species in the present study though barely reaching the upper limit of that of the aplocheiloids (10%-15%) (Murphy and Collier 1997), still is far beyond that obtained in the Antarctic notothenioids (1%-5%) (Bargelloni et al. 1994). Thus generic separation for the 3 species in this study is warranted. Many fish genera were erected long before the rise of genetics and molecular phylogenetics, when old-time ichthyologists had no idea about genetic divergence and a poor idea of divergence time. Thus, it seems obvious that many genera may not homogeneously fit divergence times and genetic divergences. In general, the more closely related species are, the greater the similarity in their isozyme pattern and mtDNA sequences. Any nominal genus should have a higher level of difference than species, and this criterion can be used for designating systematic status. Lower isozyme genetic similarities of 0.321-0.454 and lower sequence of similarity of 0.9048-0.9298 among the 3 species examined suggest that they are posited far beyond species level.

Morphologically, the existing genus *Pelates* is quite distinct from the genus *Terapon* by having more numerous dorsal soft rays (11 vs. 10) and anal soft rays (10 vs. 8) though with slight overlap. The feature of post-temporal bone differs significantly between them: *Pelates* has an entire posterior margin ending with a spine, while that of *Terapon* is serrated,

without a sharp ending. This character used in the key for South African fishes (Heemstra 1986) has been ignored by many authors. The most recent key prepared by Senou (1993) for the family Teraponidae is classified into 4 genera: *Terapon*, *Pelates*, *Rhyncopelates*, and *Mesopristes*. *Pelates* differs from *Rhyncopelates* and *Mesopristes* by the lack of a serrated post-margin on the post-temporal bone, but the provision of a serrated margin on the post-temporal bone and lower gill raker numbers (less than 20) in the latter 2 genera reveal their close resemblance to the genus *Terapon*. There is no reason to separate them as 3 individual genera, and their generic status should be reconsidered. When considering *Terapon* alone, the new genus *Pseudoterapon* represented by *Terapon theraps* is now separated from *Terapon* due to its large-sized scales (pored scales on lateral line 66-75 vs. 75-100; transverse scale rows from dorsal origin to lateral line 9-11 vs. 13-17) and by having greater generic divergence from both allozyme and mtDNA sequences (Table 5). Thus the genera *Rhyncopelates* and *Mesopristes* are prospectively included in *Pseudoterapon* rather than *Terapon*. Nevertheless, this can not be finalized until their molecular data are well established in the future. As to the status of *Pelates* which is morphologically distinguishable from *Terapon* and *Pseudoterapon*, their allozyme and DNA data reveal a greater divergence which is almost at the generic level.

**Table 5.** Morphological characters and molecular data for the newly revised genera *Terapon*, *Pelates*, and *Pseudoterapon*

	<i>Terapon</i> ( <i>T. jarbua</i> )	<i>Pseudoterapon</i> ( <i>T. theraps</i> )	<i>Pelates</i> ( <i>Pelates quadrilineatus</i> )
Scale size	smaller	larger	medium
Pored scales on lateral line	75-100	46-56	66-75
Post-temporal bone	serrated	serrated	not serrated
Vomerine teeth	present in young	absent	absent
Palatine teeth	present in young	absent	absent
Horizontal dark stripes	curved	straight 3-4	straight 5-6
Fixed allele difference with <i>Pelates</i>	<i>mAAT</i> *	<i>mAAT</i> *	
	<i>IDHP-2</i> *	<i>IDHP-2</i> *	
	<i>CK-A</i> *	<i>CK-A</i> *	
	<i>CK-B</i> *	<i>LDH-A</i> *	
	<i>MDH-1</i> *	<i>LDH-B</i> *	
	<i>MDH-2</i> *	<i>MDH-1</i> *	
	<i>PGM-1</i> *	<i>MDH-2</i> *	
	<i>6PGD-1</i> *	<i>SDH-1</i> *	
	<i>SDH-1</i> *	<i>SOD-1</i> *	
	<i>SOD-3</i> *	<i>SOD-2</i> *	
		<i>SOD-3</i> *	
mtDNA sequences	base difference with <i>Pelates</i> 10.87%	base difference with <i>Pelates</i> 7.29%	

One could suggest the transfer of another doubtful species, *Helotes sexlineatus*, which is recorded from Taiwan (Chen and Yu 1984), to the genus *Pelates* since its post-temporal bone is completely hidden by scales and skin (Vari 1978), but the lower gill-raker counts (15-17) resemble those of *Terapon*. Its status is still ambiguous.

We conclude from the morphological comparisons among these fish genera existing in the family Teraponidae that *Rhyncopelates* and *Mesopristes* group with *Pseudoterapon*, *T. jarbua* is retained in *Terapon* while *Helotes* is combined with *Pelates*. The feature of post-temporal bone allows enough difference for these genera to be subdivided into 2 main groups: one with *Pseudoterapon* and *Terapon*; and the other with *Pelates*. The results of molecular examination in this work further support that the 3 taxa examined in this study are equally independent at the generic level.

**Acknowledgments:** This paper was financially supported by the Council of Agriculture, R.O.C. under Grant 84 AST-1.2-FID-07(2). The authors wish to express their sincere thanks to Dr. S.K. Wu of Colorado Univ. at Boulder for reading this manuscript.

## REFERENCES

- Alves-Gomes J, G Orti, M Haygood, W Heiligenberg, A Meyer. 1995. Phylogenetic analysis of the South American electric fishes (Order Gymnotiformes) and the evolution of their electrogenic system: a synthesis based on morphology, and mitochondrial sequence data. *Mol. Biol. Evol.* **12**: 298-318.
- Avise JC. 1994. *Molecular marker, natural history and evolution*. New York: Chapman & Hall.
- Bargelloni L, PA Ritchie, T Patarnello, B Battaglia, DM Lambert, A Meyer. 1994. Molecular evolution at subzero temperatures: mitochondrial and nuclear phylogenies of fishes from Antarctica (Suborder Notothenioidei), and the evolution of antifreeze glycopeptides. *Mol. Biol. Evol.* **11**: 854-863.
- Bleeker P. 1873-1876. *Atlas ichthyologique des Indes Orientales Néerlandaises*. Vol. 7. Amsterdam: Frederic Muller.
- Chen JTF, MJ Yu. 1984. A synopsis of the vertebrates of Taiwan. (revised and enlarged edition). Vol. 2. Taipei: Shanwu. XXXVI + pp. 443-1092.
- Cuvier G, A Valenciennes. 1829. *Histoire naturelle des poissons*. Vol. 4. Paris: Academic Press.
- Forsk. 1755. Descriptions, animalium, avium, amphibiorum, piscium, insectorum, quae, in itinere orientali observavit: post mortem auctoris edidit. Carstem Niebuhr. 164 pp.
- Heemstra PC. 1986. Family Teraponidae. In MM Smith, PC Heemstra, eds. *Smiths' sea fishes*. Berlin: Springer-Verlag, pp. 543-544.
- Jean CT, SC Lee, CF Mui, CT Chen. 1995. Tissue-specific isozymes in fishes of the subfamily Sparinae (Perciformes: Sparidae) from the coastal waters of Taiwan. *Zool. Stud.* **34**: 164-169.
- Kimura M. 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *J. Mol. Evol.* **16**: 111-120.
- Klunzinger CB. 1884. *Fische des Rothen Meeres*. I. Acanthopteri veri Owen. Stuttgart: E. Schweizerbart'sche Verlagshandlung.
- Kocher TD, WK Thomas, A Meyer, SV Edwards, S Paado, FX Villablanca, AC Wilson. 1989. Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. *Proc. Natl. Acad. Sci. USA* **86**: 6196-6200.
- Kumar S, K Tamura, M Nei. 1993. MEGA (Molecular Evolutionary Genetic Analysis) version 1.02. University Park: Pennsylvania State Univ.
- Lindberg GU, ZV Krasnyukova. 1969. *Fishes of the sea of Japan and the adjacent areas of the Sea of Okhotsk and the Yellow Sea*. Part 3. Moskova-Leningrad: Academy of Sciences of the USSR (translated from Russian in 1971, IPST).
- Murphy WJ, GE Collier. 1997. A molecular phylogeny for aplocheiloid fishes (Atherinomorpha, Cyprinodontiformes): the role of vicariance and the origins of annualism. *Mol. Biol. Evol.* **14**: 790-799.
- Nei M. 1977. F-statistics and analysis of gene diversity in subdivided populations. *Ann. Hum. Genet.* **14**: 225-233.
- Nei M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* **89**: 583-590.
- Nelson JS. 1994. *Fishes of the world*. 3rd ed. New York: John Wiley & Sons, Inc.
- Park LK, P Moran. 1995. Developments in molecular genetic techniques in fisheries. In GR Carvalho, TJ Pitcher, eds. *Molecular genetics in fisheries*. London: Chapman & Hall.
- Saitou N, M Nei. 1987. Neighbor-joining method: a new method for reconstructing phylogenetic trees. *Proc. Natl. Acad. Sci. USA* **74**: 5463-5467.
- Senou H. 1993. Family Teraponidae. In T Nakabo, ed. *Fishes of Japan with pictorial keys to the species*. Tokyo: Tokai Univ. Press, pp. 646-648.
- Shaklee JB, CP Keenan. 1986. A practical laboratory guide to the techniques and methodology of electrophoresis and its application to fish fillet identification. CSIRO. *Aust. Mar. Lab. Rep.* **179**: 1-59.
- Shaklee JB, CS Tamaru. 1981. Biochemical and morphological evolution of Hawaiian bonefishes (*Albula*). *Syst. Zool.* **30**: 125-146.
- Shaklee JB, CS Tamaru, RS Waples. 1982. Speciation and marine fishes studied by the electrophoretic analysis of protein. *Pacif. Sci.* **36**: 141-157.
- Shaw R, R Prasad. 1970. Starch gel electrophoresis of enzymes—a compilation of recipes. *Biochem. Genet.* **4**: 297-320.
- Shen SC, SC Lee, KT Shao, HK Mok, CH Chen, CT Chen. 1993. *Fishes of Taiwan*. Taipei: Dept. of Zoology, National Taiwan Univ., XX + 960 pp.
- Swofford DL. 1981. On the utility of the distance Wagner procedure. In VA Funk, DR Brooks, eds. *Advances in cladistics*. Proceedings of the First Meeting of the Willie Hennig Society. New York: New York Botanical Garden. pp. 25-43.
- Vari RP. 1978. The terapon perches (Percoidei, Teraponidae). A cladistic analysis and taxonomic revision. *Bull. Amer. Mus. Nat. Hist.* **159**: 179-340.
- Weber M, LF de Beaufort. 1931. *The fishes of the Indo-Australian Archipelago*. Vol. 6. Leiden: EJ Brill, XII + 448 pp.

## 花身雞魚屬(*Terapon*)及四線雞魚屬(*Pelates*)之分子系統分類，並兼記 擬花身雞魚屬(*Pseudoterapon*)一個新屬(鱸形目：條紋雞魚科)

李信徹<sup>1</sup> 蔡孟蓓<sup>1</sup>

臺灣常見之花身雞魚(*Terapon jarbua*)、條紋雞魚(*T. theraps*)及四線雞魚(*Pelates quadrilineatus*)等三種外形酷似之雞魚科魚類，能由本文表一所示之若干基因座之某些固定基因位點之呈現而輕易區分，用以印證上述雞魚確呈不同獨立種地位。若進一步權衡數值偏低得自同功異構酶之 0.321-0.454 Nei氏不偏遺傳相似度 0.321-0.454 (遺傳距離 0.790-1.137) 以及粒線體去氧核糖核酸 7.3%-10.9%之核苷酸鹼基差異度，則三種間之地位不應有從屬的關係，而宜分隸三個不同的屬別。由 Nei氏不偏遺傳相似數值看來，條紋雞魚(*T. theraps*)與同屬之花身雞魚(*T. jarbua*)間所得到之極低數值(0.441)，以及無論由同功異構酶及mtDNA資料所架構出的系統圖，均顯現不出此同屬之二種雞魚間有聯結的情形，益增條紋雞魚(*T. theraps*)有由原來之*Terapon*自立為一個新屬的可能性(擬花身雞魚屬*Pseudoterapon*)。 *Pseudoterapon*與*Terapon*主要的差別在於具有較大型之鱗片、鋤骨及顎骨均無齒，體部若有水平側帶，則排列平直而不呈弧形，以及若干分子生物學分析出來的差異。

关键词: 雞魚，擬花身雞魚屬，同功異構酶，粒線體去氧核糖核酸。

<sup>1</sup> 中央研究院動物研究所