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COMPARATIVE ELECTROPHEROGRAMS OF MUSCLE PROTEIN OF THE FISHES OF FAMILY PRIACANTHIDAE

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Sin-Che Lee (1984) Comparative electropherograms of muscle protein of the fishes of family Priacanthidae. *Bull. Inst. Zool., Academia Sinica* **23**(2): 151-158. The electropherograms of soluble muscle proteins (myogens) of six priacanthid fishes (*Pristigenys niphonia, Cookeolus boops, Priacanthus cruentatus, P. macracanthus, P. hamrur* and *P. blochii*) collected from Taiwan are described and the results discussed in relation to the morphological data for the same specimens to demonstrate possible phylogenetic relationships among them. All the protein patterns show a good specificity and they are suitable for use in the identification when morphological characters or other means are difficult to use. Using protein characters, 6 species examined are divided into two major groups on the basis of genetic distance: *Pristigenys* group with a single species *Pg. niphonia*, and the *Priacanthus* group with 5 species. The latter are further divided into two clusters: the first with *Cookeolus boops* alone and its dichotomous component comprising *Priacanthus cruentatus* and *P. blochii;* and the second being the most closely related pair, *P. hamrur* and *P. macracanthus.* The systematic position shown in the dendrogram, is in agreement with the result of the conventional systematic study of the fishes of this family.

To date, eight species of the family Priacanthidae (Teleostei) have been found in the surrounding waters of Taiwan, namely *Cookeolus boops* (Snyder), *Priacanthus cruentatus* (Lacépède), *P. hamrur* (Forskal), *P. macracanthus* Cuvier and Valenciennes, *P. blochii* Bleeker, *P. tayenus* Richardson, *Pristigenys niphonia* (Cuvier and Valenciennes) and *Pg. multifasciatus* Yoshino and Iwai (Lee, 1980). Because of similar appearance, they are easily confused with one another. In any case, morphological features may not be readily distinguishable between closely related species owing to possible interspecific overlap. Since electrophoretic data can provide an extremely valuable tool for systematists (Avise, 1974), and the electropherogram of muscle proteins mostly represent species specific characteristics, the application of electrophoretic technique is useful for the identification of unknown samples of frozen flesh (Taniguchi *et al.*, 1972) when morphological characters or other means are difficult to differentiate species from one another. The reproducibility of protein is not affected by the storage of the fish in the frozen state (−14°C) for as long as 1 year (Jones and Mackie, 1970), but this is not the case for isozymes which are easily denatured. This suggested to the author to apply the same technique for precise determination of taxonomic position of the species within Priacanthidae. The purpose of this paper is to determine the specific and generic differences of six Taiwanese priacanthids (*P. tayenus* and *Pg. multifasciatus* were not available) on white

skeletal muscle and to compare the result with the external features including meristic data and to interprete relationships among the species. From the electrophoretic separation of the soluble muscle proteins (myogens) examined so far, polymorphic intra-specific variation was not found during the investigation. Apparently, the muscle protein patterns would be regarded as reliable characters in the systematics of this family.

MATERIALS AND METHODS

Samples of six priacanthid species were examined, with a total of 71 specimens caught from the waters around Taiwan, and landed at the following fish markets in frozen condition (Table 1). A piece of white skeletal muscle was removed from the shoulder immediately after purchase, and was maintained at a steady frozen condition on the way back to the laboratory, and later stored at −20°C for test.

Because of the higher resolution power of polyacrylamide electrophoresis (Gaal *et al.*, 1980), vertical polyacrylamide slab gel electrophoresis was employed. Methods for preparation and the procedure of electrophoresis were the same described in the previous paper (Lee and Chang, 1983) adopted from Peck and Biggers (1975). The gel consists of 100 ml 3% upper stacking gel and 25 ml 7.5% lower separating gel. pH for Triglycine buffer (electrode buffer) was 8.3. To ensure cooling, electrophoresis was run in the refrigerator at 4°C. Since temperature is a critical factor in electrophoresis (Gaal *et al.*, 1980), the temperature condition should be kept as constant as possible during the electrophoresis.

Basically, the band counting method (Ferguson, 1980) was employed for those visualized on the gels using amido black 10B general protein stain. Genetic distance (D) was calculated using Nei's formula (1972): $D = -\ln I$ ($I = \sum X_i Y_i / \sqrt{\sum X_i^2 \sum Y_i^2}$), on the basis of data appeared in Table 3. The mean genetic distance ($\bar{D}$) and genetic indentity ($\bar{I}$) are the mean overall loci studied. The phenogram in Fig. 3 was arranged on the basis of the UPGMA (unweighted pair group method with arithmetic means) method of cluster analysis (Sneath and Sokal, 1973) based on the data in Table 4.

The morphological parameters chosen for interspecific comparison were: standard length/body depth proportion; number of gill-rakers on lower limb of first left gill arch, and number of soft dorsal and anal fin rays.

RESULTS

Morphological comparison

Six species examined were in three genera: genus *Pristigenys* with a single *Pg. niphonia*, genus *Cookeolus* with *C. boops* and genus *Priacanthus* with *P. curentatus*, *P. macracanthus*, *P. hamrur* and *P. blochii*. Presence of vertical bands on body sides of *Priacanthus cruentatus* is the only species with dark patches while the others are uniformly red on the body sides. *Cookeolus boops* is the only one with black pelvic fins, and *Priacanthus hamrur* and *P. blochii* commonly have a black spot at the base of the pelvic fins. Vertical fins of *P. cruentatus*, *P. blochii* and *P. macracanthus* are spotted with reddish or yellowish. Fourth and 5th dorsal spines are highest in *Pristigenys*, while the height of the spinuous profile raising progressively toward the rear end, in any fishes of *Cookeolus* and *Priacanthus*. Although *Cookeolus* has such a common feature with that of *Priacanthus*, *Cookeolus* is recognizable by its much enlarged pelvic fins. Other morphological features and measurements are given in Fig. 1 and Table 1. The body of *Pristigenys* (SL/BD, 2.02) is the deepest, which is followed by that of *Cookeolus* (2.45) and *Priacanthus* (2.55–3.05). The range of body depth within *Priacanthus* species is followed by the order of declining body depth of *P. blochii*, *P. cruentatus*, *P. hamrur* and *P. macracanthus*. Number of gill-rakers in *Pg. niphonia* (19.5), *P. macracanthus* (19.6) and *P. hamrur* (19.61) are more numerous than those of *C. boops* (17.88), *P. cruentatus* (17.38) and *P. blochii* (17.60). The modal dorsal fin ray number of 11 in *Pg. niphonia* is the lowest (Table 1), and followed by the order of increasing number of *C. boops*

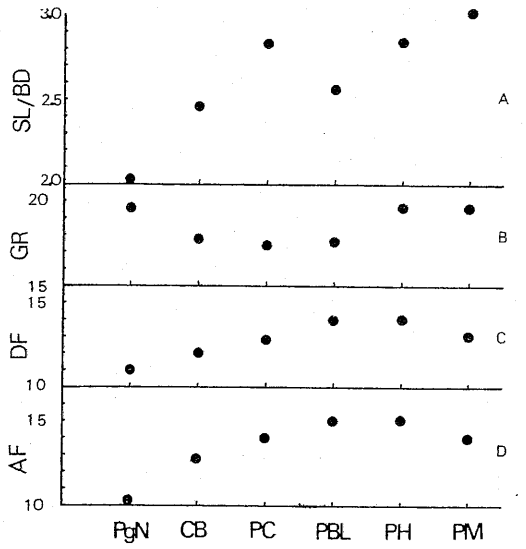

Fig. 1. Body proportional measurements and meristic counts of *Pristigenys niphonia* (PgN), *Cookeolus boops* (CB), *Priacanthus cruentatus* (PC), *P. blochii* (PBl), *P. hamrur* (PH) and *P. macracanthus* (PM). A, the ratio of standard length to body depth; B, number of gill-rakers on lower limb of first left gill arch; C, number of soft dorsal fin rays; D, number of soft anal fin rays.

(12), *P. cruentatus* (13), *P. macracanthus* (13), *P. hamrur* (14) and *P. blochii* (14). The number of anal fin rays also follows the similar trend, being 10, 13, 14, 14, 15 and 15 respectively. Caudal fin shape is rounded in *Pg. niphonia*; truncate or slightly rounded in *P. blochii* and *C. boops*; truncate in *P. cruentatus* and *P. macracanthus*, and lunate in *P. hamrur*, the latter being reported becoming protruded with age (Masuda *et al.*, 1975). Although, the above morphological features and measurements show somewhat interspecific differences, there are some overlapping (Table 1).

Electrophoretic patterns

Fig. 2 shows the characteristic protein pattern of six priacanthid species. All the patterns are consistent within a particular group. The patterns of muscle protein from a total of 71 specimens were classified into six types, based on the mobility of electrophoretic bands. Each type is specific to a valid species. There are 11 component bands designated from 1 to 11 by their position from cathodal to anodal side. *Pristigenys niphonia*

TABLE 1

Some meristic counts and proportional measurements of the priacanthid fishes

Species	Collecting localities	No. Specimens examined (Standard length mm)	Dorsal rays Mean (range)	Anal rays Mean (range)	Lower limb gill-rakers Mean (range)	Standard length / Body depth Mean±SD (range)
C. boops	Keelung	14	12.14	12.93	17.88	2.45±0.10
	Hengchun	(210.2-336)	(12-13, mode 12)	(12-13, mode 13)	(17-20, mode 17)	(2.21-2.61)
P. cruentatus	Hengchun	8	12.88	14	17.38	2.82±0.04
		(161-205)	(12-13, mode 13)	(14)	(16-18, mode 18)	(2.77-2.89)
P. macracanthus	Keelung	20	13.05	13.95	19.60	3.05±0.14
		(211-260)	(13-14, mode 13)	(13-14, mode 14)	(18-22, mode 19)	(2.70-3.38)
P. hamrur	Keelung	18	14	15	19.61	2.82±0.09
	Hengchun	(181-358)	(14)	(15)	(18-21, mode 20)	(2.60-2.94)
P. blochii	Hengchun	5	14	15	17.60	2.55±0.05
		(234-276)	(14)	(15)	(17-18, mode 18)	(2.47-2.60)
Pg. niphonia	Kaohsiung	6	11	10	19.50	2.02±0.07
		(168-216)	(11)	(10)	(18-21, mode 20)	(1.93-2.08)

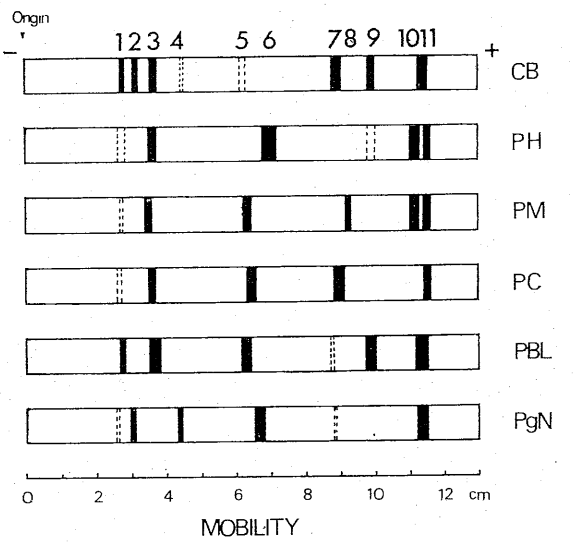

Fig. 2. Electropherograms of soluble muscle proteins of Priacanthidae, traced after the gel. CB, *Cookeolus boops*; PH, *Priacanthus hamrur*; PM, *P. macracanthus*; PC, *P. cruentatus*; PBl, *P. blochii*; PgN, *Pristigenys niphonia*. Arrow shows the origin. Black and white bands indicate deep and light-staining bands respectively.

has deep-staining bands 2, 4, 6, 11 and light-staining bands 1, 7. *Cookeolus boops* has deep-staining bands 1, 2, 3, 7, 9, 11 and light-staining bands 4, 5. *Priacanthus cruentatus* has deep-staining bands 3, 5, 7, 11 and light-staining band 1. *P. macracanthus* has deep-staining bands 3, 5, 8, 10, 11 and light-staining band 1. *P. hamrur* has deep-staining bands 3, 6, 10, 11 and light-staining bands 1 and 9. *P. blochii* has deep-staining bands 1, 3, 5, 9, 11 and light-staining band 7. Band 10 is only found in *P. macracanthus* and *P. hamrur*. Comparing the number of bands commonly shared with those in *Pg. niphonia* (Table 2): *C. boops* has 5 common bands and followed by *P. blochii* (3), *P. cruentatus* (3), *P. hamrur* (3) and finally *P. macracanthus* (2) with genetic identity of 0.67, 0.38, 0.49, 0.53 and 0.21 respectively. When comparing *Cookeolus boops* with the rest of *Priacanthus* group (*Cookeolus* and *Priacanthus*), number of commonly shared bands are 6 (*Priacanthus blochii*), 5 (*P. cruentatus*) and 4 (*P. hamrur* and *P. macracanthus*) with genetic identity of 0.68, 0.69, 0.46 and 0.41 respectively. Within genus *Priacanthus* alone, number of commonly shared bands between *P. cruentatus* and three other species are 5 (*P. blochii*), 4 (*P. macracanthus*) and 3 (*P. hamrur*) with genetic identity of 0.80, 0.65 and 0.58 respectively.

The indices of genetic distance converted from the indices of genetic identity (or similarity) which in turn are calculated on the basis of the frequency occurrence of protein bands (or loci) appeared in Table 3, are present in Table 4. The indices of genetic distance are then arranged into a dendrogram of all the priacanthid species studied by using UPGMA clustering method applied on genetic distance data (Fig. 3). Species on the dendrogram are divided into two main stems. The stem of *Pristigenys niphonia* is well separated from the *Priacanthid* stem. The latter is divided into two clusters, the first represented by *P. hamrur* and *P. macracanthus*, and the remaining three being divided into *C. boops* and the other branch with *P. cruentatus* and *P. blochii*.

TABLE 2

Occurrence of muscle protein bands on the gels of each priacanthid species

Species	Number of muscle protein bands											No. common bands compared with *Pg. niphonia*
	1	2	3	4	5	6	7	8	9	10	11	
Pg. niphonia	+	+	−	+	−	+	+	−	−	−	+	
C. boops	+	+	+	+	+	−	+	−	+	−	+	5
P. cruentatus	+	−	+	−	+	−	+	−	−	−	+	3
P. macracanthus	+	−	+	−	+	−	−	+	−	+	+	2
P. hamrur	+	−	+	−	−	+	−	−	+	+	+	3
P. blochii	+	−	+	−	+	−	+	−	+	−	+	3

TABLE 3

Frequencies of protein bands on the gels of *Cookeolus boops* (CB), *Priacanthus hamrur* (PH), *P. macracanthus* (PM), *P. cruentatus* (PC), *P. blochii* (PBl) and *Pristigenys niphonia* (PgN) appeared in Fig. 2.

Protein variants	CB (n=14)	PH (n=18)	PM (n=20)	PC (n=8)	PBl (n=5)	PgN (n=6)
1	1.0000	0.8889	0.3000	0.2500	0.8000	1.0000
null	0	0.1111	0.7000	0.7500	0.2000	0
2	0.7857	0	0	0	0	1.0000
null	0.2143	1.0000	1.0000	1.0000	1.0000	0
3	1.0000	1.0000	1.0000	1.0000	1.0000	0
null	0	0	0	0	0	1.0000
4	0.9286	0	0	0	0	1.0000
null	0.0714	1.0000	1.0000	1.0000	1.0000	0
5	0.6429	0	1.0000	1.0000	1.0000	0
null	0.3571	1.0000	0	0	0	1.0000
6	0	1.0000	0	0	0	1.0000
null	1.0000	0	1.0000	1.0000	1.0000	0
7	1.0000	0	0	1.0000	0.2000	1.0000
null	0	1.0000	1.0000	0	0.8000	0
8	0	0	1.0000	0	0	0
null	1.0000	1.0000	0	1.0000	1.0000	1.0000
9	1.0000	0.2222	0	0	1.0000	0
null	0	0.7778	1.0000	1.0000	0	1.0000
10	0	1.0000	1.0000	0	0	0
null	1.0000	0	0	1.0000	1.0000	1.0000
11	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
null	0	0	0	0	0	0

TABLE 4

Mean genetic identity ($\bar{I}$) (above diagonal) and genetic distance ($\bar{D}$) (below diagonal) calculated from the frequencies of the soluble muscle protein electrophoretic bands of each species

	CB	PH	PM	PC	PBl	PgN
CB (*C. boops*)	—	0.4632	0.4096	0.6940	0.6752	0.6718
PH (*P. hamrur*)	0.7695	—	0.6771	0.5771	0.6580	0.5284
PM (*P. macracanthus*)	0.8925	0.3899	—	0.6540	0.5903	0.2132
PC (*P. cruentatus*)	0.3654	0.5497	0.4246	—	0.7981	0.4856
PBl (*P. blochii*)	0.3927	0.4186	0.3729	0.3290	—	0.3830
PgN (*Pg. niphonia*)	0.3977	0.6380	1.5455	0.7223	0.9598	—

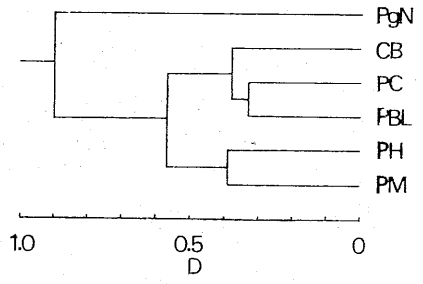

Fig. 3. Dendrogram showing the relationships of three genera and six species of priacanthid fishes by using UPGMA clustering method, based on the genetic distance data in Table 4. PgN, *Pristigenys niphonia*; CB, *Cookeolus boops*; PC, *Priacanthus cruentatus*; PBl, *P. blochii*; PH, *P. hamrur*; PM, *P. macracanthus*.

DISCUSSION

Although the component bands of general protein extracted from white skeletal muscle are few and indistinct, however, the electrophoretic patterns are species specific and do not show variation under different environmental and physiological conditions (Tsuyuki *et al.*, 1965). Although some fishes, for instance, walleye (Atherinidae) (Uthe and Ryder, 1970), frigate mackerel (Taniguchi and Konichi, 1971) and Japanese crucian carp (Taniguchi and Ishiwatari, 1972) showed intraspecific polymorphism in muscle proteins. It is neverthless a rare occurrence and usually muscle myogens show good species specific, the main exception being identity of patterns between related species rather than polymorphism within a species (Uthe and Ryder, 1970). Other fishes like *Merluccius* (Jones and Mackie, 1970), Platycephalidae (Taniguchi *et al.*, 1972), *Girella* (Lee and Chang, 1981), *Kyphosus* (Lee and Chang, 1983) and the present species all exhibit good intraspecific uniformity without polymorphism. Thus the present report of muscle protein may be considered as a good approach for the phylogenetic study of priacanthid species and this may be supported by some morphological evidence. The muscle protein patterns may then be used for the accurate identification of fish to prevent error in this family.

Comparing the dendrogram with the morphological characters of the fishes studied, *Pristigenys* alone is more differentiated than other group (*Cookeolus* and *Priacanthus*) at least by having fewer dorsal and anal fin rays, deeper body, together with the extended 4th and 5th spines in the middle of the spinuous dorsal. The above grouping on superficial features corresponds exactly to the division by protein patterns at generic level. Among the remaining 5 species, *Cookeolus boops*, *Priacanthus blochii* and *P. cruentatus* are clustered as a whole in constrast to other group with *P. macracanthus* and *P. hamrur*. For the previous group, all three species commonly have fewer gill-rakers than those in *P. macracanthus* and *P. hamrur*, but the range of body depth as well as the number of soft dorsal and anal rays show somewhat overlapping between the above two groups. When considering the previous group alone, *Cookeolus boops* is more widely separable from the other two member of the group. This is why the species *boops* was previously placed under the subgenus *Cookeolus* within the genus *Priacanthus* (Fowler, 1928), in order to distinguish it from the other member of *Priacanthus* species. However, the status of *Cookeolus* was raised to generic level (Munro, 1955; Masuda *et al.*, 1975; Nelson, 1976; Randall, 1983), and the species *boops* has been naturally included in this valid genus. In the view of Fowler (1928), two subgenera, namely *Cookeolus* and *Priacanthus*, were combined in the genus *Priacanthus: Cookeolus* was closely related to the subgenus *Priacanthus* but distinguishable by the extremely enlarged pelvic fins. Resulting from the close examination of the above five species during the present study, *C. boops* can be easily separated from all *Priacanthus* species by the conspicuously large ventral fins and fewer modal dorsal rays of 12 against 13-14 in *Priacanthus* species and fewer modal anal rays of 13 against 14-15 in *Priacanthus*. In addition, *C. boops* has 5

electrophoretic bands shared commonly with *Pg. niphonia* while the remaining species have only 3 (*P. cruentatus*, *P. blochii* and *P. hamrur*) or 2 (*P. macracanthus*) common bands. Thus, *Pg. niphonia* is more likely closely related to *Cookeolus* rather than to *Priacanthus*. Within *Priacanthus* group, *Cookeolus boops* is more closely related to *Priacanthus blochii* and *P. cruentatus* rather than to *P. hamrur* and *P. macracanthus*, this may be supported by the evidence with higher genetic identity and more commonly shared bands between *C. boops* and *P. blochii* and *P. cruentatus* than to other two species. The more closely related species the more bands of same electrophoretic mobility they share. As for the discussion on the couple *P. macracanthus* and *P. hamrur*, the presence of doubled protein bands (namely 10 and 11) is the characteristic biochemical feature to differentiate them from the other member of *Priacanthus* species. Morphologically, both species can also be readily distinguished from other *Priacanthus* by having higher gill-raker numbers but the numbers of dorsal and anal rays overlap. The application of caudal fin shape to key-out the fishes of this family (Lee, 1980) is controversial. It may change with the growth of fish, and therefore, the grouping by the caudal fin shape may not truly reflect that set by protein types. It is concluded from the present study that the phylogenetic relationships of the priacanthid species by protein patterns are likely associated with some morphological features. Thus, the electrophoretic and conventional approaches may be used in concert (Avise, 1974), and are thus suitable for use in systematic problem involving closely related species.

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大眼鯛科魚類肌肉蛋白之比較

李　信　徽

本文記載二屬六種臺灣產大眼鯛魚類（*Pristigenys niphonia*, *Cookeolus boops*, *Priacanthus cruentatus*, *P. blochii*, *P. macracanthus*, *P. hamrur*）肌肉蛋白之電泳分析結果，同時對照各魚種之形態特徵，以期闡明各魚種間之可能類緣關係。由於電泳譜帶，均顯示其特有之種別性，且均無種內多型現象，若遇本科魚類之外形特徵均無法辨認時（如只有冷凍之魚肉），則本實驗所得之電泳分析資料將有助於解決種別之鑑定。

由遺傳距離資料所推判之系統樹，可將六種大眼鯛分成 *Pristigenys*（只有一種 *Pg. niphonia*）及 *Priacanthus* 二大種羣。後者可進而分歧爲：第一支包括單獨之 *Cookeolus boops* 及其對立之 *Priacanthus cruentatus* 與 *P. blochii*；第二支則包括 *P. macracanthus* 及 *P. hamrur* 等二種。彼此間之關係與本科魚類之傳統分類系統尙稱符合。