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A COMPARISON OF LDH FROM THE EYES OF SOME NEMIPTERID FISHES OF TAIWAN[1]

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Sin-Che Lee and Jung-Ti Chang (1987) A comparison of LDH from the eyes of some nemipterid fishes of Taiwan. *Bull. Inst. Zool., Academia Sinica* **26**(1): 61-67. The electrophenograms of Lactate dehydrogenase (LDH) from the eyes of nine nemipterid fishes (*Nemipterus hexodon, N. tolu, N. metopias, N. japonicus, N. bathybius, N. delagoae, Scolopsis eriomma, S. inermis* and *Pentapodus nagasakiensis*) are described and the results are discussed in relation to their morphological data in order to demonstrate their possible interrelationships. The interspecific variation of the LDH pattern among the species within one particular genus is very slight, neverthless, the differences at generic level is rather obvious. While treating genetic identity data with UPGMA clustering analysis, the fishes can be subdivided into two main stems: *Nemipterus* and its counterpart of *Scolopsis* and *Pentapodus.* The latter two genera are closer than to the other. This may be supported by their similarity in some internal morphological characters, such as shape and number of pyloric caeca, shape of second to fifth suborbital bones and the shape of epihyal bone. Again, this is an example of the congruence between electrophoretic data and morphological data.

Twenty species in three genera of the nemipterids of Taiwan have been noted previously (Lee, 1986). Though counts of dorsal and anal fin rays of each species are almost identical, they can be recognized from one another by the use of other morphological characters appeared in the artificial key in the paper. It is true that the differences of some external features among these morphologically similar species within one particular genus is difficult to differentiate immediately when certain distinctive color patterns are faded. Fortunately, biochemical method has been proved by many workers as a useful means to solve this problem (Taniguchi *et al.*, 1972; Avise, 1974), and the evolutionary tree derived from electrophoretic data may agree well with the morphological data (Mickevich and Johnson, 1976). The electrophoretic data of soluble muscle protein of other group of fishes, for example, Priacanthidae, has been interpreted well for species identification (Lee, 1986). Lactate dehydrogenase (LDH) of other group of fishes such as *Notropis* (Buth and Mayden, 1981) and *Triboldon* (Sakai and Hamada, 1985) of Cyprinidae are also adopted for clarifying the taxonomic status of the fishes. Among existing three gene loci in the fish, LDH-A, -B and -C, the advanced group of fish like Perciformes usually has highly isozymatic

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activity of subunit C which is primarily restricted in the neural tissues such as eye and brain (Whitt, 1970; Frankel, 1980; 1983). Consequently, eye tissue of this family was chosen for a good reason to demonstrate the possible specific variation of enzyme activity by different mobility. The purpose of this paper is attempted to verify specific and generic differences of 9 Taiwanese nemipterids on the LDH from eye and to compare the biochemical data with morphological differences and to interprete the possible interrelationships among these fishes. Owing to the insufficient fresh materials available during this work, only nine out of 20 Taiwanese species are used for this experiment. Though this report can not cover all the existing Taiwanese nemipterids, however, the selected nine species may fully represent the three genera (*Nemipterus*, *Scolopsis*, and *Pentapodus*) in the family.

MATERIALS AND METHODS

A total of 83 frozen specimens from 9 species were collected near the coastal waters of Taiwan. As soon as the fishes were landed, a pair of eyes were removed immediately and maintained at frozen condition until the samples were stored at −20°C in the laboratory. Methods of gel preparation and the procedure of the vertical slab electrophoresis on polyacrylmide gel followed that of Lee and Chang (1983) and Lee (1984). The electrophoresis was carried out at 4°C under 130 V and 60 mA for about three

TABLE 1

Frequencies of protein bands on the gels of *Nemipterus hexodon* (NH), *N. tolu* (NT), *N. metopias* (NM), *N. japonicus* (NJ), *N. bathybius* (NB), *N. delagoae* (ND), *Scolopsis eriomma* (SE), *S. inermis* (SI) and *Pentapodus nagasakiensis* (PN) appeared in Fig. 1A–B

Protein Variants \ Species	NH (n=7)	NT (n=7)	NM (n=12)	NJ (n=9)	NB (n=8)	ND (n=4)	SE (n=15)	SI (n=6)	PN (n=8)
1	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
nil	0	0	0	0	0	0	0	0	0
2	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
nil	0	0	0	0	0	0	0	0	0
3	1.0000	0.4286	1.0000	0.5556	1.0000	1.0000	1.0000	1.0000	0.3750
nil	0	0.5714	0	0.4444	0	0	0	0	0.6250
4′	0	0	0	0	0.1250	0	0	0	0
nil	1.0000	1.0000	1.0000	1.0000	0.8750	1.0000	1.0000	1.0000	1.0000
4	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
nil	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	1.0000	0
nil	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0	1.0000
5′	0	0	0	0	0	0	0	0	0.3750
nil	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.6250
6	0.2857	0.5714	0.8333	0.2222	0	0	0	0	0.8750
nil	0.7143	0.4286	0.1667	0.7778	1.0000	1.0000	1.0000	1.0000	0.1250
7	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0	0	1.0000
nil	0	0	0	0	0	0	1.0000	1.0000	0
8	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0	0	0
nil	0	0	0	0	0	0	1.0000	1.0000	1.0000
9	0	0	1.0000	1.0000	0	0	0.0670	0.1667	0
nil	1.0000	1.0000	0	0	1.0000	1.0000	0.9333	0.8333	1.0000
10	0	0	0	0	0	0	1.0000	1.0000	1.0000
nil	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0	0	0
11	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0
nil	0	0	0	0	0	0	0	0	1.0000

hours. Gel was stained with the mixture of 0.1 M Tris–HCl (pH 8.5), DL-Lactic acid (Sodium salt), NAD (Sol.), NBT (Sol.) and PMS (Sol.) and incubated at 37°C. Bands on the gel were counted and the subsequent procedure for the calculation of Genetic distance ($D = -\ln I,\ I = \sum X_iY_i/\sqrt{\sum X_i^2 \sum Y_i^2}$) (Nei, 1972) was obtained from the data in Table 1. The phenograms shown in Fig. 2A–C were constructed respectively by the UPGMA, complete linkage and single linkage methods of clustering analysis (Sneath and Sokal, 1973) in terms of the CLUSTAR program package (Romesburg and Marshall, 1984) on the basis of genetic identity data in Table 2.

RESULTS

Electrophoretic patterns

The LDH from eyes of nine nemipterid species were resolved by vertical slab polyacrylamide electrophoresis into thirteen component bands. The first four bands were present in all nine species studied. The remaining bands differed slightly in staining intensity and electrophoretic mobility among species. *Nemipterus hexodon* has dark-staining bands of 1, 2, 3, 4, 8, 11 and light-staining bands of 6, 7. *N. tolu* has dark-staining bands of 1, 2, 3, 4, 8, 11 and light-staining bands of 6, 7. *N. metopias* has dark-staining bands of 1, 2, 3, 4, 8, 11 and light-staining bands of 6, 7, 9. *N. japonicus* has dark-staining bands of 1, 2, 3, 4, 11 and light-staining bands of 6, 7, 8, 9. *N. bathybius* has dark-staining bands of 1, 2, 3, 4, 7, 8, 11 and light-staining band 4′ *N. delagoae* has dark-staining bands of 1, 2, 3, 4, 7, 8, 11. *Scolopsis eriomma* has dark-staining bands of 1, 2, 3, 4, 10, 11 and light-staining band 9. *S. inermis* has dark-staining bands of 1, 2, 3, 4, 5, 10, 11 and light-staining band 9. *Pentapodus nagasakiensis* has dark-staining bands of 1, 2, 3, 4, 6, 7, 10 and light-staining band 5′.

Among the above 13 bands, the different mobility of bands 10 and 11 is the critical point to separate the three genera, *Pentapodus*, *Scolopsis* and *Nemipterus*. Band 10 of *Pentapodus* and band 11 of the remaining species

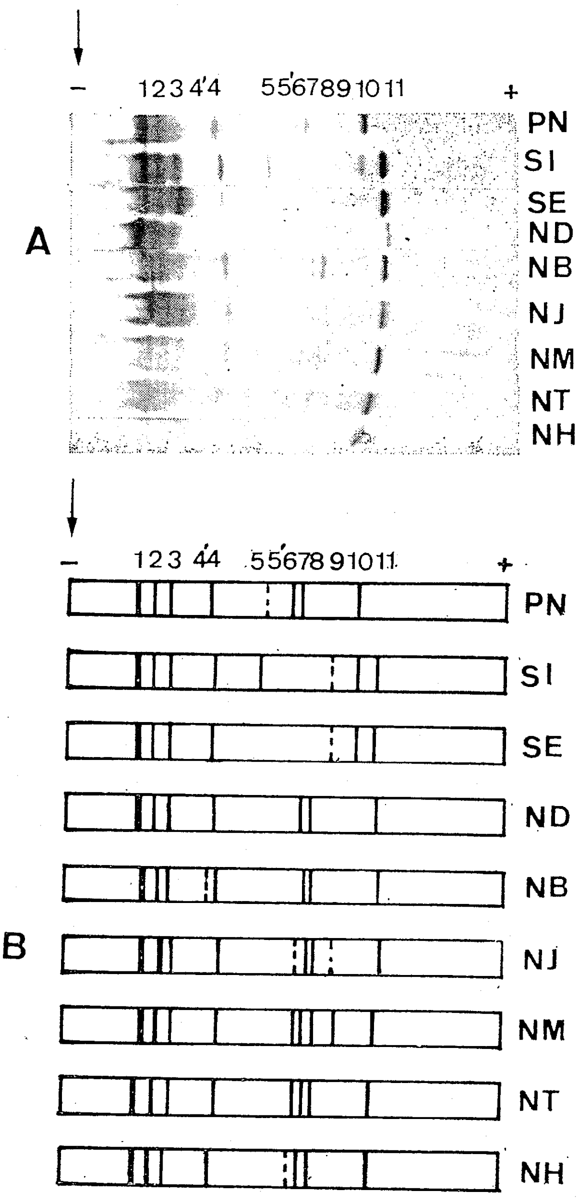

Fig. 1. Electrophoretic patterns of lactate dehydrogenase (LDH) from the eyes of *Nemipterus hexodon* (NH), *N. tolu* (NT), *N. metopias* (NM), *N. japonicus* (NJ), *N. bathybius* (NB), *N. delagoae* (ND), *Scolopsis eriomma* (SE), *S. inermis* (SI) and *Pentapodus nagasakiensis* (PN). A, upper figure, photograph; B lower figure schematic diagram of the same scale traced from the above photograph.

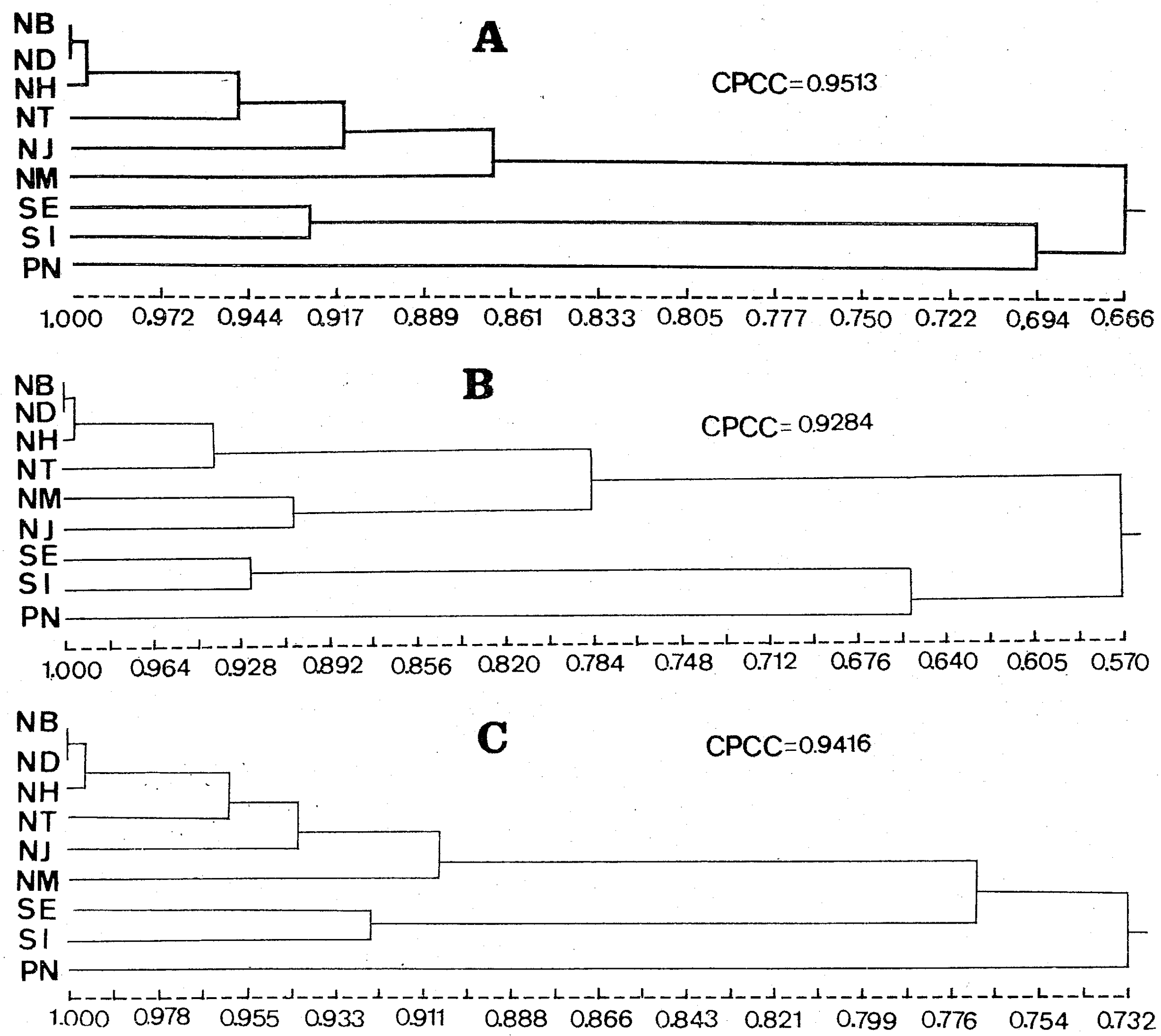

Fig. 2. Dendrogram constructed from UPGMA (A), Complete-linkage (B), and Single-linkage (C) of 9 nemipterid species based on the genetic identity data in Table 2. NB, *Nemipterus bathybius*; ND, *N. delagoae*; NH, *N. hexodon*; NT, *N. tolu*; NJ, *N. japonicus*; NM, *N. metopias*; SE, *Scolopsis eriomma*; SI, *S. inermis* and PN, *Pentapodus nagasakiensis*. Cophenetic correlation value for three above different phenograms are 0.9513, 0.9284, and 0.9416 respectively.

are equivalent to the locus LDH–C. *Pentapodus* is distinct from *Scolopsis* and *Nemipterus* by having slow anodal eye-band (band 10) compared to the others with fast anodal eye-band (band 11).

A further discrimination between *Scolopsis* and *Nemipterus* is clearly indicated by the lack of band 10 in *Nemipterus*. Within the genus *Nemipterus* alone, bands of all six species are almost indentical except few minor variations of frequencies in certain light-staining bands. As for two *Scolopsis* species, *S. eriomma* and *S. inermis* can be distinguishable by having band 5 in the latter species.

Genetic identity and dendrogram

The data of genetic identity were treated separately with three clustering methods of UPGMA, complete-linkage and single-linkage, all with high cophenetic correlation of over

TABLE 2

Mean genetic identity (I) (above diagonal) and genetic distance (D) (below diagonal) calculated from the frequencies of the electrophoretic bands of LDH from eyes of *Nemipterus hexodon* (NH), *N. tolu* (NT), *N. metopias* (NM), *N. japonicus* (NJ), *N. bathybius* (NB), *N. delagoae* (ND), *Scolopsis eriomma* (SE), *S. inermis* (SI) and *Pentapodus nagasakiensis*

Species	NH	NT	NM	NJ	NB	ND	SE	SI	PN
NH		0.9580	0.8882	0.9414	0.9937	0.9945	0.7635	0.6139	0.6824
NT	0.0429		0.8848	0.9050	0.9377	0.9385	0.6306	0.6293	0.6167
NM	0.1186	0.1224		0.9059	0.7835	0.8612	0.7072	0.5686	0.6439
NJ	0.0988	0.0998	0.0604		0.9026	0.9033	0.6780	0.6107	0.6310
NB	0.0063	0.0643	0.2440	0.1025		0.9992	0.7683	0.6900	0.7194
ND	0.0055	0.0635	0.1494	0.1017	0.0008		0.6921	0.6908	0.6549
SE	0.2698	0.4611	0.3464	0.3886	0.2636	0.3680		0.9225	0.7316
SI	0.4879	0.4631	0.5646	0.4931	0.3711	0.3700	0.0807		0.6534
PN	0.3821	0.4834	0.4402	0.4600	0.3293	0.4233	0.3125	0.4256	

0.9 (Fig. 2A–C). Resulting from the first two approaches, the phenograms are rather similar, with subdivision of two main stems including *Nemipterus* and its counterpart of *Scolopsis* and *Pentapodus*. In the third case of single-linkage, the phenogram was divided into a more widely distinct *Pentapodus* and the other with *Scolopsis* and *Nemipterus*.

DISCUSSION

It is shown from previous taxonomic report (Lee, 1986) that three genera (*Nemipterus*, *Scolopsis* and *Pentapodus*) in the family can be distinguished from some external features. *Scolopsis* is characterized by having a suborbital spine, smooth preopercular edge and the absence of canine teeth to separate from *Nemipterus* and *Pentapodus*. A further difference between the latter twos arises from the horizontally directed canine teeth and the scaled interorbital space in *Pentapodus*. When dealing with some internal characters alone (Akazaki, 1962), the possession of 7–8 slender pyloric caeca in *Nemipterus versus* 6–7 stouter ones in *Scolopsis* and *Pentapodus*, the stouter second to fifth suborbital bones in *Nemipterus versus* the slender ones in *Scolopsis* and *Pentapodus*, as well as the trapezoid epihyal in *Nemipterus versus* the triangular one in *Scolopsis* and *Pentapodus*, all of them could evidently support that *Nemipterus* is far apart from the others (Table 3). The preliminary grouping of the above three genera set by

TABLE 3

Some selected morphological characters of *Nemipterus*, *Scolopsis* and *Pentapodus*, summarized from Akazaki, 1962

	Nemipterus	*Scolopsis*	*Pentapodus*
Scales on interorbital	naked	scaled except *S. inermis*	scaly
Scales on posterior ½ of preopercle	naked	scaly	scaly
Pyloric caeca	slender, 7–8 in number	stouter, 6–7 in number	stouter, 6–7 in number
Shape of second to fifth suborbital bones	stouter	slender	slender
Shape of epihyal	trapezoid	triangular	triangular

electrophoretic patterns of LDH on eyes reveals a more similar result to internal characters rather than the superfitial external features. The application of LDH from eyes may not explain the whole story of the phylogenetic relationships among species of Nemipteridae since the interspecific difference within one particular genus, *eg.*, *Nemipterus* are very slight. However, when considering only generic level, the most significant bands of 10 and 11 are supposed to be a critical point to distinguish these genera. *Nemipterus* has band 11 only and *Pentapodus* has band 10 only while *Scolopsis* has both of them. It may be true that *Scolopsis* is intermediated between *Nemipterus* and *Pentapodus*. Although the match of clustering data of LDH with internal characters supports that *Scolopsis* and *Pentapodus* are more closely related than the other, neverthless, in the view of interorbital squamosus patters, all *Nemipterus* species are naked, and *Pentapodus* are scaled entirely. In the case of *Scolopsis*, only *S. inermis* and *S. eriomma* are much alike *Nemipterus* with naked interorbital space while the other *Scolopsis* species resemble interorbitally scaled *Pentapodus*. The possesion of two types of interorbital squamosus patterns in the *Scolopsis* may further support that *Scolopsis* has an intermediate combination between the other two genera. It is concluded that the systematic grouping of these fishes may be sufficient when judging only from the single LDH isozyme from eyes, however the present result shows more or less similar to the morphological data at least at the generic level.

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數種臺灣產金線魚眼部乳酸去氫酶之比較

李 信 徹　　張 羲 悌

本文敍述虹色金線魚、 薔薇金線魚、 姬金線魚、 日本金線魚、 底金線魚、 蝶金線魚、 紅赤尾多、 橫帶赤尾冬及長崎錐齒鯛等九種金線魚科魚類眼部乳酸去氫酶並比較 各魚種電泳帶 與其形態差異之關連性， 進而探討彼此間之可能親緣關係。 雖然任何一屬內各魚種間電泳譜帶之差異性極微，但若僅比較各屬級間之差異性則較明顯 。 根據各魚種之遺傳相似值所求出之 UPGMA 類聚分析結果 可將 這些 魚類分爲二大羣：一爲金線魚屬，另一支則包括赤尾冬及錐齒鯛屬， 後二屬間之親緣關係似較前者接近， 這可從彼此間幽門囊形狀及數目，第二至第五眼下骨以及上舌骨之形狀之相似程度看來 ， 似乎可應證之。由本實驗的結果再度證明電泳資料與某些形態資料是相符的。