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Comparative Studies of the Japanese Platycephalid Fishes by Electropherograms of Muscle Proteins, LDH and MDH

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Abstract The electrophoretic patterns of muscle proteins, LDH and MDH of 11 species of platycephalid fishes collected from Japan were described and discussed in connection with the phylogenetic relationships among these species. The specific and generic differences were recognized in the electropherograms of this group. The phylogenetic relationships derived from the comparative morphology by Matsubara and Ochiai (1955) were fundamentally supported by the facts of the electrophoretic analyses made here. A remarkable discrepancy was found in the phylogenetic position of genus *Rogadius*. This genus well accorded with genus *Suggrundus* in the electrophoretic patterns of muscle proteins, LDH and MDH, though rather nearer to genus *Onigocia* than to *Suggrundus* in morphological characters. Two sibling species of *Platycephalus*, *P. indicus* and *P.* sp. usually called "Yoshinogochi" are distinguished from each other by the electropherogram of muscle proteins.

Introduction

The application of the electrophoretic method for the taxonomic study of various fish groups revealed that the electropherogram of proteins such as muscle proteins and hemoglobins represents species specific characteristics (Tsuyuki et al., 1965 a, b; Nyman, 1965; Yamanaka et al., 1965) and is valuable for the analysis of phylogenetic relationships of different groups belonging to one family: e. g. Salmonidae (Tsuyuki et al., 1963, 1966; Yamanaka et al., 1967), Catostomidae (Tsuyuki et al., 1967) and Scorpaenidae (Tsuyuki et al., 1968).

On the other hand, the isozyme having strong specificity in activity for a substrate possesses an advantage that some genetic information can be easily given on the electropherogram of isozymes such as lactate dehydrogenase (LDH) and malate dehydrogenase (MDH). It has already been clarified that the LDH isozyme of fish is coded by two genes on different loci, which is common among the various groups of vertebrates, and its electrophoretic patterns show marked differences among the fish species (Markert et al, 1965; Numachi, 1970). This isozyme pattern is also used in the evidence of hypothesis that the evolution of vertebrate from fish to mammals is realized mainly by gene duplication (Ohno et al., 1968). A remarkable variation among the fish species is also recognized in both of the number and mobility of the LDH isozymes (Numachi, 1970) which are dimers formed by two subunits under the control of separate gene loci (Bailey et al., 1970, Numachi, 1970). These LDH and MDH isozymes as well as soluble proteins and hemoglobins are considered to be useful for the phylogenetic and taxonomic studies of a group of fishes intimately related.

The flathead family Platycephalidae, inhabiting the waters around Japan are suitable group for the study in biochemical taxonomy, since their revisional studies have been well worked out on anatomical and meristic characters by Jordan and Richardson (1908), Jordan and Hubbs (1925), and Matsubara and Ochiai (1955). The purpose of this paper is to determine the specific and generic differences of 11 species of the Japanese flatheads on the basis of the electrophoretic patterns of soluble proteins, lactate dehydrogenase and malate dehydrogenase in the skeletal muscle,

and to campare the results obtained in this study with those derived from morphological studies by Matsubara and Ochiai (1955).

Materials and methods

The samples of 11 species of the flatheads were collected from the waters around Shikoku Province: Kochi, Kochi Pref.: Uwajima, Ehime Pref.: and Takamatsu, Kagawa Pref.

Samples for cellulose acetate electrophoresis: *Onigocia spinosa*; 10 specimens, 90–100 mm in standard length, collected at Uwajima, in Jan. '70. *Onigocia macrolepis*: 10 specimens, 62–95mm, Kochi, in Sept. '69. *Rogadius asper*: 20 specimens, 138–190 mm, Kochi, in Sept. '69–Jan. '70. *Suggrundus meerdervoorti*; 20 specimens, 158–188mm, Kochi, in Sept. '69–Jan. '70. *Inegocia japonica*; 4 specimens, 114–119 mm, Kochi, in Sept–Nov. '69. *Cociella crocodila*: 7 specimens, 121–125 mm, Kochi, in Nov. '69. *Kumococius detrusus*; 19 specimens, 120–175 mm, Kochi, in Sept.–Dec. '69. *Ratabulus megacephalus*: 17 specimens, 142–191 mm, Kochi, in Sept. '69–Jan. '70. *Platycephalus indicus*; 10 specimens, 185–332 mm, Takamatsu, in Oct. '69. *Platycephalus* sp. (closely related to *Platycephalus indicus,* newly reported by Kamei and Ishiyama, 1968): 6 specimens, 220–290 mm, in Takamatsu, Jan. '70.

Samples for starch-gel electrophoresis of muscle proteins, LDH and MDH: *Onigocia spinosa*; 5 specimens, 66–89 mm in standard length, Kochi, in Jan. '71. *Onigocia macrolepis*: 5 specimens, 90–109 mm, Kochi, in Nov. '70. *Rogadius asper*; 5 specimens, 127–147 mm, Kochi, in Feb. '71. *Suggrundus meerdervoorti*; 5 specimens, 150–202 mm, Kochi, in Feb. '71. *Inegocia japonica*: 5 specimens, 139–175 mm, in Kochi, Jan. '71. *Inegocia guttata*: 1 specimen, 413mm, Kochi, in Oct. '70., 1 specimen, 342mm (in total length), in Kochi, Feb. '71. *Cociella crocodila*; 3 specimens, 163–272 mm, Kochi, in Feb. '71. *Kumococius detrusus*: 5 specimens, 174–188 mm, Kochi, in Nov. '70. *Ratabulus megacephalus*; 3 specimens, 196–240 mm, Kochi, in Nov. '70. *Platycephalus indicus*: 5 specimens, 210–349mm, Takamatsu, Apr. in '71. *Platycephalus* sp.; 5 specimens, 284–327 mm, Takamatsu, in Apr. '71.

Methods for preparation and electrophoresis in cellulose acetate and starch-gel of muscle extracts were the same described in the previous papers (Taniguchi, 1969, 1971). For the localization of LDH and MDH isozymes on starch-gel, the gels were incubated in reaction mixtures consisting of: 5 ml of 0.1 M lactic acid or malic acid, 5 ml of nitroblue-tetrazolium (1.6 mg/ml), 10 ml of phenazine methosulfate (0.4 mg/ml), 10 ml of 1 M TRIS-HCl (pH 8.6) and 1 ml of NAD (1.05 mg/ml).

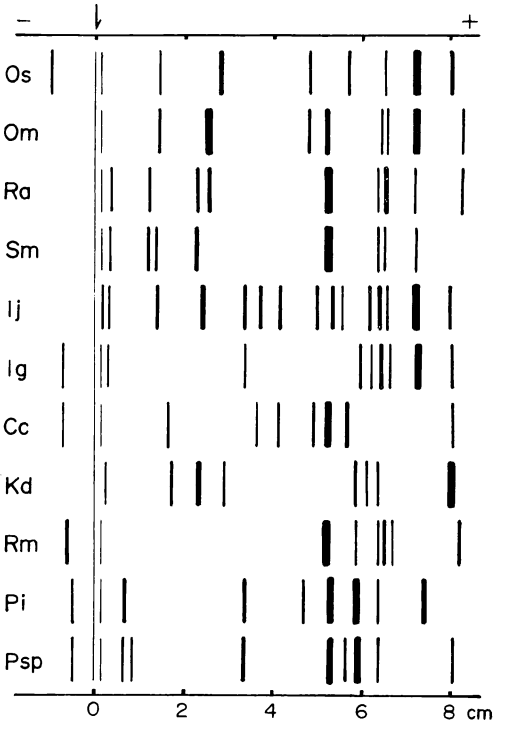

Fig. 1. Muscle protein types by starch-gel method for 11 species of flatheads. Os, *Onigocia spinosa*; Om, *Onigocia macrolepis*; Ra, *Rogadius asper*; Sm, *Suggrundus meerdervoorti*; Ij, *Inegocia japonica*; Ig, *Inegocia guttata*; Cc, *Cociella crocodila*; Kd, *Kumococius detrusus*; Rm, *Ratabulus megacephalus*; Pi, *Platycephalus indicus*; Psp, *Platycephalus* sp. (Yoshinogochi). Arrow shows the origin.

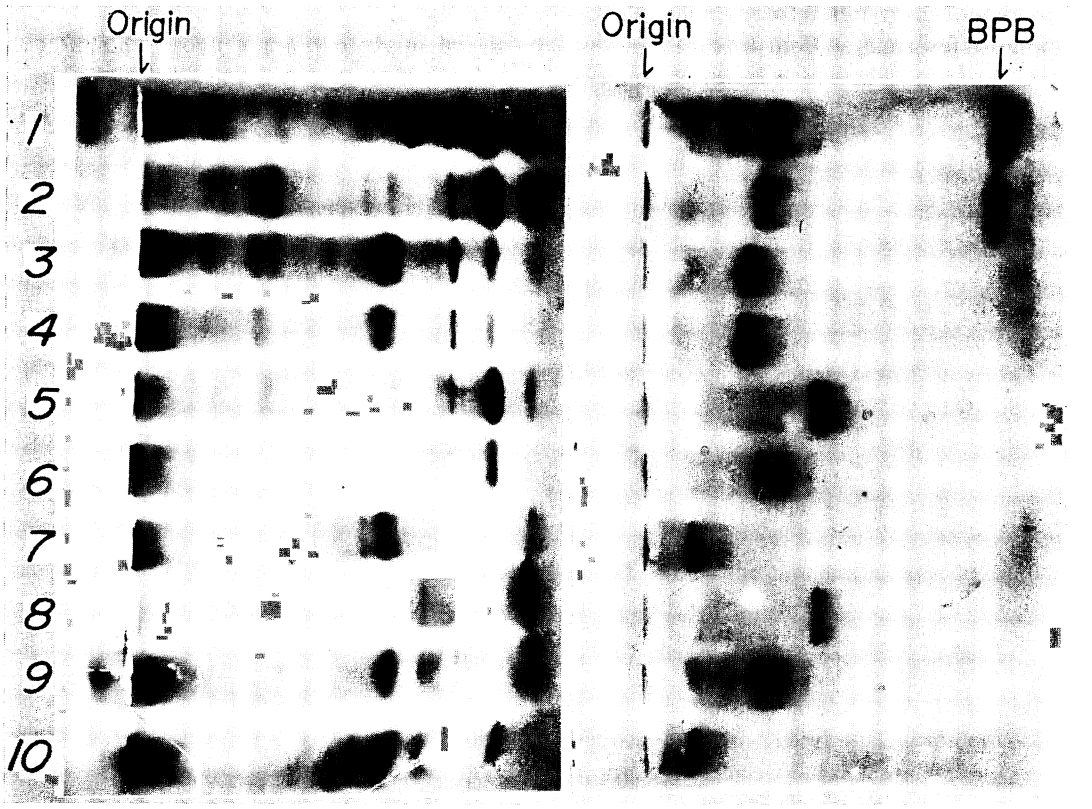

Fig. 2. Starch-gel electrophoretic patterns of muscle proteins (left) and lactate dehydrogenase (right) in a crude extract of skeletal muscle of 10 species of flatheads. 1, *Onigocia spinosa*; 2, *Onigocia macrolepis*; 3, *Rogadius asper*; 4, *Suggrundus meerdervoorti*; 5, *Inegocia japonica*; 6, *Inegocia guttata*; 7, *Cociella crocodila*; 8, *Kumococius detrusus*; 9, *Ratabulus megacephalus*; 10, *Platycephalus indicus*. Arrow shows the origin.

Results

Muscle protein patterns by starch-gel electrophoresis. The muscle protein patterns of the Japanese flatheads were reproducible, and remarkable variations were recognized in number, mobility and density even among the related species of a genus (Figs. 1, 2). The muscle protein patterns of 11 species may be divided into 7 types on the basis of the arrangement, density and position of zones on electrophoresis. The type number of the pattern is named mainly based on the systematic arrangement given by Matsubara and Ochiai (1955).

First type represented by two species of genus *Onigocia, O. spinosa* and *O. macrolepis*, is characterized by having a thick zone at the position, 7 cm measured from the origin, and a few zones occured from 6 cm to 8 cm from the origin. Both species of this type have 9 zones respectively, but these were distinguishable from each other by 4 zones, positions of which are more or less slided. In 2nd type comprising two species of genus *Inegocia, I. japonica* and *I. guttata,* several zones occured from 6 cm to 8 cm from the origin, including one characteristic thick zone at the position 7 cm. The number of all zones is much more numerous in *I. japonica* than in *I. guttata.* Third type is represented by *Rogadius asper* and *Suggrundus meerdervoorti,* in which there are 3 faint zones from 6 cm to 7.5 cm and one thick zone at 5 cm from the origin. Both species

of this type slightly differ from each other by position of a few zones. Fourth type represented by *Cociella crocodila,* and 5th type by *Ratabulus megacephalus* resemble to 3rd type in at least having a thick zone at the position, 5.3 cm from the origin. In 4th type, no zone is present from 6 cm to 8 cm from the origin. Fourth type is separable from the 5th type by having a few faint zones from 3 to 5 cm from the origin. Sixth type comparing *Kumococius detrusus* is provided with a thick zone at the position, 8 cm from the origin. Seventh type is represented by two species of genus *Platycephalus, P. indicus* and *P.* sp., in which 2 thick zones occurred respectively from 5 cm to 6 cm from the origin. Comparing the patterns of those two species of this type, 7 zones well accord in both position and thickness, but a few rest ones are different in position.

Muscle protein patterns by cellulose acetate electrophoresis. In contrast to the starch-gel electrophoresis, the muscle proteins on the ceparax membrane migrated towards cathode from anode side in the cellulose acetate method. The muscle components separated by this method are fewer in number than those of starch-gel method. The pattern of each species of this group is represented by one specific zone bending towards anode terminal and a few common zones. Species specificity in this method appeared to exist for 10 species of the flatheads as shown in Fig. 3. The patterns of these fishes may be divided into 5 types on the position of the specific zone and general arrangement of some common zones. First type represented by two species of genus *Onigocia* has specific zone at middle part of the sheet and 1 common zones occurred both sides of the specific zone respectively. *Onigocia spinosa* is distinguished from *Onigocia macrolepis* by having 3 common zones instead of 2 ones as seen in the latter. Second type comprising *Inegocia japonica, Rogadius asper, Suggrundus meerdervoorti* and *Cociella crocodila* well resembles to 1st type in this pattern. In this type, common zones occurred on both sides of the specific zones are widely separated from the specific zone. Slight difference is observed in the pattern of two species, *Rogadius asper* and *Suggrundus meerdervoorti.* In *Inegocia japonica,* there is no zone in the cathodal space. Among 4 species of this type, *Cociella crocodila* is peculiar in having 3rd common zone slightly moved towards anode. Third type is represented by *Ratabulus megacephalus,* and 4th by *Kumococius detrusus,* respectively. The specific zone of those types moves more to the anode side than that of 1st and 2nd ones. *Ratabulus megacephalus* is distinctive from *Kumococius detrusus* in the position of the specific zone, and has 3 common zones, the position of which well accord with that of 2nd type. Fifth type comprising two species of *Platycephalus* is distinctive from other types by the specific zone migrating slowly. Both species of *Platycephalus* resemble each other in general arrangement of 3 zones except for the position of one common zone.

LDH isozyme pattern. As shown in some cottid fishes by Numachi (1970), the zones of LDH of this group were fewer in comparison with those of the primitive groups of teleostean fishes. The LDH pattern of each species of this group consists of 2 zones closely approached, the one being denser and the other fainter as shown in Figs. 2, 4. In this case, these zones migrated faster in some species but slower in others. The main LDH zone occurred at the position, 1.5 cm (4th type) from the origin in *Cociella crocodila* and two species of *Platycephalus,* 2.5 cm (2nd type) in *Rogadius asper* and *Suggrundus meerdervoorti,* 3 cm (1st type) in two species of *Onigocia, Inegocia guttata* and *Ratabulus megacephalus,* and 4 cm (3rd type) in *Inegocia japonica* and *Kumococius detrusus,* respectively.

MDH isozyme pattern. There are one or two zones occurred from 2 cm to 6 cm from the origin in MDH patterns of each species of the flatheads (Fig. 4). A single zone appeared at the position, 3 cm (3rd type) in *Kumococius detrusus,* 3–4 cm (1st type) in species referable to the genus *Onigocia, Inegocia,* and *Cociella,* 5 cm (2nd type) in *Rogadius asper* and *Sug-*

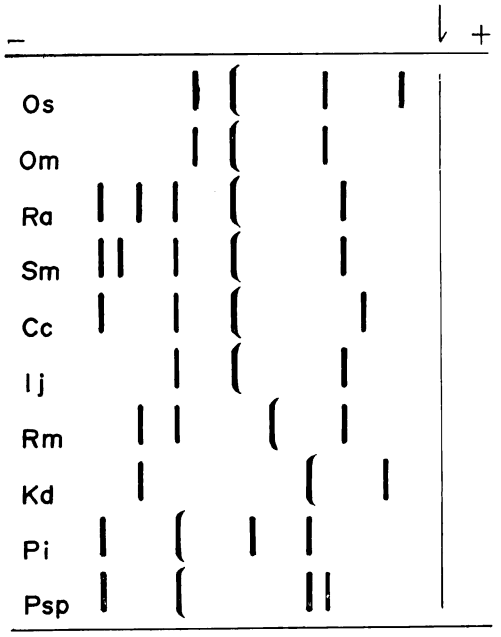

Fig. 3. Muscle protein types by cellulose acetate method for 10 species of flatheads. Os, *Onigocia spinosa*; Om, *Onigocia macrolepis*; Ra; *Rogadius asper*; Sm, *Suggrundus meerdervoorti*; Ij, *Inegocia japonica*; Ig, *Inegocia guttata*; Cc, *Cociella crocodila*; Kd, *Kumococius detrusus*; Rm, *Ratabulus megacephalus*; Pi, *Platycephalus indicus*; Psp, *Platycephalus* sp. (Yoshinogochi). Arrow shows the origin.

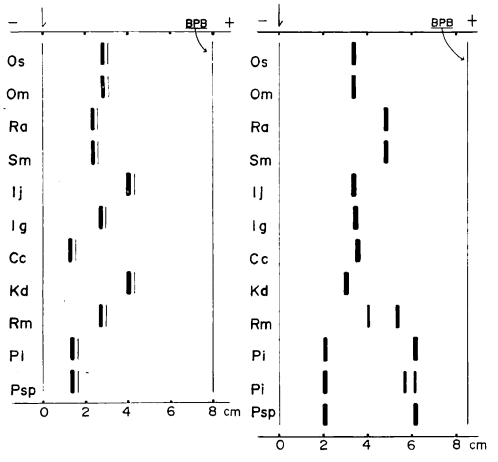

Fig. 4. LDH (left) and MDH (right) types by starch-gel method for 11 species of flatheads. Two types are recognized in the MDH patterns of *P. indicus*. Arrow shows the origin. For the abbreviation of species names see Fig. 3.

grundus meerdervoorti, respectively. *Ratabulus* has 2 zones occured from 4 cm to 6 cm (4th type). Two species of *Platycephalus* including a variant type with 3 zones are characterized in having at least 2 zones at 2 cm and 6 cm (5th type). Three species, *Ratabulus megacephalus*, *Platycephalus indicus* and *Platycephalus* sp. are remarkably different from remaining species in having two MDH zones under the control of two genes of separated loci. The variant pattern of *Platycephalus indicus* shows that the anodal MDH is controlled by heterozygous alleles of the locus which is different from the other locus coding cathodal MDH.

Discussion

The results of the present investigation showed that the examination of electrophoretic patterns of muscle protein, LDH and MDH is useful not only to compare closely related species in a group but also to estimate the phylogenetic relationships among genera.

Species specificity. The muscle protein patterns by both methods made here are also distinctly differerent in closely related species of a genus and appear to be as a characteristic as fingerprints for identification. It is interesting to note that there are two cases in the interspecific diversity of muscle protein pattern by starch-gel electrophoresis. As seen in two species of *Inegocia*, remarkable differences are found in total number of zones. When total zones are nearly equal in number in sibling species of a genus, such as two species of *Onigocia* and *Platycephalus*, it seems that the specific diversity is observed in position of a few faint zones.

Recently, Kamei and Ishiyama (1968) have reported *Platycephalus* sp. (Yoshinogochi) from Inland Sea of Japan, describing the morphological and ecological characters of this species which are distinguishable from those of *Platycephalus indicus*. In *Platycephalus indicus* the body is blackish brown, with 3 to 10 cross bars on its dorsal and lateral surfaces, the

pectoral fins are not dusky, the pored scales on lateral line being less than 80 in number. The species in question is characterized by following features: color lighter without cross bars, pectoral fins dusky, pored scales numerous more than 80. The muscle protein patterns of two species by both cellulose acetate and starch-gel methods support result of morphological analysis by Kamei and Ishiyama (1969).

Phylogenetic relationships. The proteins showing the same electrophoretic mobility do not always mean that these are the same in structure. But we assumed that zones appearing at the same position are homologous in the case of comparison of closely related species within a family, because these different patterns have developed on the accumulation of gene mutations from the same pattern. Tsuyuki et al. (1963, 1968) marked the position of zones as a criterion for the relationship and recognized that the more intimate species, the more common zones. In this study, we remarked the number, density, position of zones and classified muscle protein patterns by starch-gel method into 7 types, by the cellulose acetate method into 5 types, LDH patterns into 4 types and MDH patterns into 5 types (Fig. 6). The classification of muscle protein by cellulose acetate method was principally parallel to that by starch-gel method. The type of muscle protein is more easily identified on the position of specific zone of the pattern in the former than in the latter.

When discussed the phylogenetic relationships of the Japanese flatheads, Matsubara and Ochiai (1955) divided this group into two main stems, *Onigocia* and *Platycephalus*, on the basis of important morphological characters (Fig. 5). According to them, *Platycephalus* stem comprising genus *Platycephalus* alone is more differentiated than *Onigocia* stem represented by several genera, *Onigocia, Rogadius, Suggrundus, Inegocia, Cociella, Kumococius* and *Ratabulus* which are diversified continuously. These assumptions for differentiation are fundamentally supported by the electrophoretic

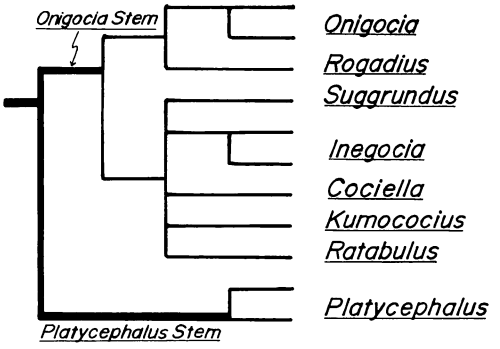

Fig. 5. Suggested differentiation of the Japanese flatheads derived from morphological study by Matsubara and Ochiai (1955).

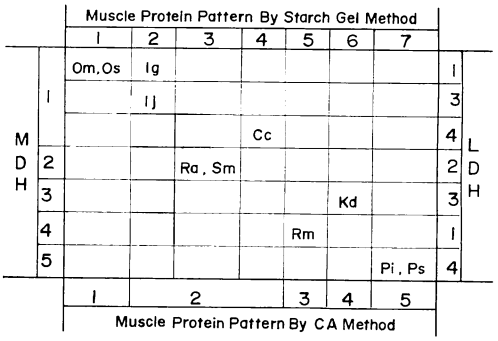

Fig. 6. Relationships of 11 species of flatheads based on the types of electropherograms of muscle proteins, LDH and MDH. For the abbreviation of species names see Fig. 1.

results as shown in Fig. 6.

Among the group of *Onigocia* stem, genus *Onigocia* with many primitive morphological characters observed in the ancestral scorpaenid fish belongs to 1st type in each electrophoretic character. Jordan and Hubbs (1925) erected a new genus *Wakius* for the accomodation of *Platycephalus spinosus*. Matsubara and Ochiai (1955), however, have been unable to retain *Wakius* as a distinct genus from *Onigocia*, since the differences seen between them in armature of the head and the lateral line scales, are all specific in their opinion. Their opinion obtained from morphological examination is also supported by the present study.

According to Matsubara and Ochiai (1955), genus *Rogadius* is much closer to genus *Onigocia* (subfamily Onigocinae) than to other

member of *Onigocia* stem (subfamily Inegociinae) in having some primitive features that the side of head is unicarinate, horny process from posterior margin of the opisthotic bone is rudimentary, and the head is larger. Meanwhile, the genus *Rogadius* is easily separable morphologically from genus *Suggrundus* by the following diagnoses: presence or absence of an anterose spine on the lower face of the preopercle, one or two carinates on each side of the head, and denticulation on upper side of head. Judging from the electrophoretic characters, the genus *Rogadius* is much closer to genus *Suggrundus* than to *Onigocia* (Fig. 6). Since these two genera have nearly identical patterns and the differences in the electrophoretic patterns are minor, the phylogenetic position of genus *Rogadius* ought to be discussed again synthetically.

Matsubara and Ochiai (1955) also stated genus *Inegocia* is more differentiated than genus *Suggrundus* at least in having 2 or 3 preopercular spines, the third being rudimentary, if present. Considering from some intimate accordance in patterns of muscle protein and LDH, it seems reasonable that *Inegocia* is rather nearer to *Onigocia* than to *Suggrundus* and *Rogadius* (Fig. 6). Matsubara and Ochiai's view that 3 genera, *Cociella, Ratabulus* and *Kumococius* seem to be highly specialized offshoots among the groups of *Onigocia* stem, may be supported by the present examination in which these genera belong to the different types respectively.

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筋肉たん白，LDH および MDH の電気泳動像による日本産コチ科魚類の比較研究

谷口 順彦・落合　明・宮崎 嗣生

日本の沿岸で採集されたコチ科魚類 11 種の筋肉たん白，LDH および MDH を電気泳動法により分析してその泳動パターンを比較した．筋肉たん白の泳動パターンは属や種で異なり，近似種間でも明らかな差が認められた．最近瀬戸内海などから報告されたマゴチに近縁なヨシノゴチ (*Platycephalus* sp.) はマゴチとは異なる泳動像を示し，両者が異種であることを立証した．筋肉たん白はでん粉ゲル法で 7 型，セルロース・アセテート法で 5 型，LDH で 4 型に，MDH で 5 型に類型化された．電気泳動の結果から得られた類縁関係は形態にもとづくそれと基本的には一致した．両者間の一つの大きな差異は形態的特徴からオニゴチ属に近縁と考えられていたマツバゴチ属が，形態的差異の著しいメゴチと電気泳動パターンで全く同じ型に属していることであった．

（高知県南国市物部　高知大学農学部栽培漁業学科）