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SIGNIFICANCE OF ELECTROPHORETIC STUDIES IN RELATION TO THE SYSTEMATIC STATUS OF SOME SPECIES OF THE GENUS *ARIUS* (ARIIDAE : SILURIFORMES)

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INTRODUCTION

While the classical systematist studies organisms by counting and measuring various parts, the biochemical systematist uses techniques developed by the protein chemist. Many accounts of the theory and and practice of these techniques are available (Ferguson, 1980).

The maximum amount of biochemical systematic information is obtained when the complete amino acid sequence of a protein is known. To date 20 amino acids are commonly found in the protein of all living organism, sporadically one or two more occur but this twenty is an impressive demonstration of the common kinship of organisms (Needham, 1965).

It is an established fact that the electrophoretic mobility is inversely proportional to the size and weight of the molecule of a particular amino acid, through a supporting media such as starch gel or acrylamide gel. Thus it is evident that the differences in electrophoretic mobilities of different proteins are dependent on the arrangement of various amino acids in a particular protein, besides the pH of the buffer solution (Yureva and Melkova, 1979).

Until recently, the techniques for determining the primary structure of even quite small proteins were too slow and laborious to be of any value in fish systematic work. Recently, however, the process has been automated and sequences can now be determined relatively quickly (Davis, 1964) by 'Disk Polyacrylamide Gel Electrophoresis'.

If a taxanomic problem is not readily resolved by morphological and other comparisions, an experimental approach is needed to clarify the systematic confusion (O' Rourke, 1974). A number of workers such as Thompson (1960), Natarajan, *et al.* (1975) etc., have shown that muscle myogen patterns reveal a high degree of species specificity.

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With the advent of muscle myogen as one of the modern tools of ichthyotaxonomy, soluble eye lens proteins have received much attention. Antisera prepared against lenses of any of the vertebrate species give precipitive reactions with the lenses of all other vertebrates. The possibility, therefore, exists of using these proteins to obtain data on the inter-relationships (Manski and Halbert, 1964). For comparative studies, the eye lens proteins may provide valuable informations because of its inert nature and thus it becomes more suitable for these purposes (Reader and Bell, 1965, Fullhorst and Young, 1966), These nuclear lens proteins are very stable, tolerating temperatures as high as 79°C before precipitating with standing relatively harsh procedures (Smith, 1966). Further, unclear lens proteins do not fluctuate physiologically, rhythmically or seasonally throughout the post embryonic life of the animal. Moreover, these proteins are not present in very high concentration and are readily soluble in many of the commonly available media. Further, they are uncontaminated by proteins of other tissues such electrophoresis. Electrophoretic study of eye lens protein in fishes has thus proved to be an efficient and modern tool to clarify the taxanomic identity. Thus both the tissue i.e. muscle and eye lens were selected for the present investigation.

The aim of the present study is to find out whether protein specificity can serve as an additional aid in determining the taxonomic relationships in closely allied species of the genus *Arius*. This method is applied especially to reinforce the conclusion already drawn by means of conventional method.

Material and methods :

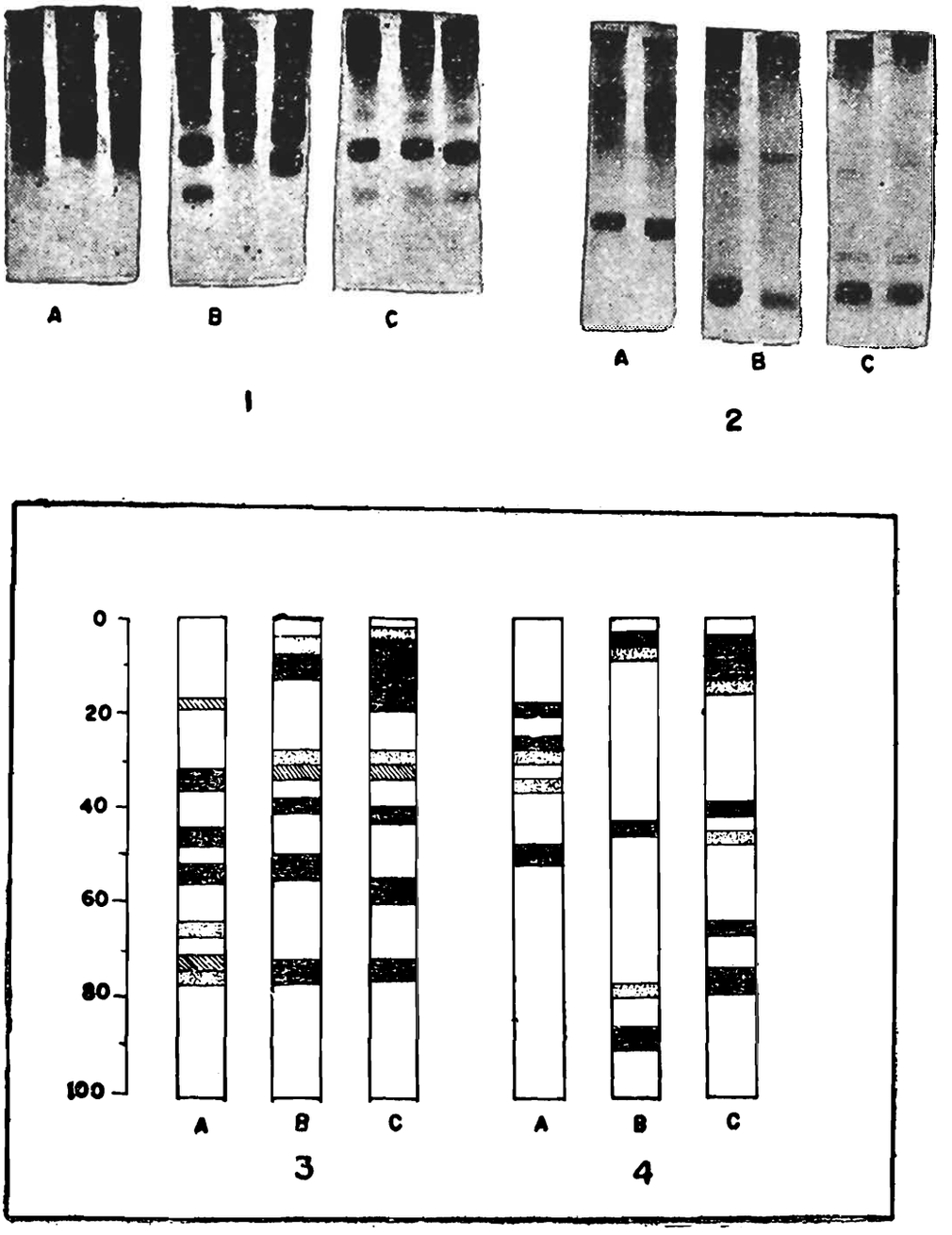
Live specimens of *A. arius* (Hamilton), 300—400 mm SL., were collected with the help of hook and line from Vellar estuary, while *A. caelatus* Val. and *A. thalassinus* (Ruppell) 250—400 mm SL., where procured from the 'commercial trawl catch' at Portnovo fish landing centre (Tamil Nadu). The specimens were transferred to an ice-box immediately after their collection. Electrophoretic experiments were carried out simultaneously on the muscle myogen and eye lens of the three species belonging to same sex and age group.

The muscle tissue was removed just below the first dorsal fin, washed thoroughly in distilled water and 75 mg of this was homogenized with 1 ml distilled water in a hand homogenizer. The extract was centrifuged at 3000 rpm for 15 minutes and the supernatant, containing water soluble proteins was subjected to electrophoretic analysis. The same procedure was repeated for the extraction of eye lens protein.

Three layer polyacrylamide gel electrophoresis (Davis, 1964) was employed.

OBSERVATIONS

In *Arius arius*, there are five major (easily discernible through naked eye) and two minor bands in the electropherogram of muscle myogens. Of the five major bands



Figs : 1. A. Electropherogrrm of muscle myogens of *A. arius*. B. Electropherogram of muscle myogens of *A. caelatus*. C. Electropherogram of muscle myogens of *A. thalassinus*.
2. A. Electropherogram of eye lens protein of *A. arius*. B. Electropherogram of eye lens protein of *A. caelatus*. C. Electropherogram of eye lens protein of *A. thalassinus*.
3. A. Diagrammatic representation of fig. 1A. B. Diagrammatic representation of fig. 1B. C. Diagrammatic representation of fig. 1C.
4. A. Diagrammatic representation of fig. 2A. B. Diagrammatic representation of fig. 2B. C. Diagrammatic representation of fig. 2C.

three are quite dark where as two faint (pl. 1, figs, A, 3A). In case of eye lens protein, there are only three widely separated major bands and two minor bands (pl. 1, figs. 2A, 4A).

In *A. caelatus*: although the total number of bands are same as in the preceding species in respect of both muscle myogens and eye lens proteins, but the intensity and Rm values of different bands are distinguishable. Of the five major bands four are dark and one faint in muscle myogen (pl. 1, figs. 1B, 3B) and the three major bands of eye lens electropherogram are quite apart from each other as compared to *A. arius* (pl. 1, figs. 2B, 3B).

The banding pattern in *A. thalassinus* shows some similarity with that of *A. caelatus* in case of muscle myogen but for the intensity and Rm of first and fourth major bands (pl. 1, figs. 1C, 3C). In case of eye lens, this species is quite distinct from those of preceding two species since there are four dark bands instead of three with different Rm values (pl. 1, figs. 2C, 4C). (Table 1).

TABLE—1

Electrophoretic banding pattern and relative mobility of proteins (Muscle myogen and eye lens) in the three species of the genus *Arius*.

Species	Number of bands		Period of separation	Rm value of different bands as percentage of the total distance travelled by electrolite.								
	Muscle myogen	Eye lens	in minutes	Muscle myogen 1	2	3	4	5	Eye lens 1	2	3	4
Arius arius	5+2	3+2	45	18	34	46	54	72	21	27	50	–
Arius caelatus	5+2	3+2	45	10.5	33	40	53	75	7.5	45	90	–
Arius thalassinus	5+2	3+2	45	15	33	42	57	75	9	40	65	77

The relative mobility of different fractions of the muscle myogens does not depict much significant differences that can readily distinguish the three species from each other. The Rm value of first major band in *A. arius* is 18, *A. caelatus* 10.5 ; and in *A. thalassinus*, 1.5 ; otherwise rest of the bands have more or less identical relative mobility (Table 1). On the other hand the electropherograms developed from the eye

lens proteins show significant differences in respect of the relative mobility of various bands which may prove the species specificity of these proteins in fishes of the genus *Arius* (Table 1).

DISCUSSION

The electrophoretic patterns for proteins from the muscle myogens of *A. arius*, *A. caelatus*, and *A. thalassinus*, although varying interspecifically, appear to have gross contours which are evident from the uniform number of seven bands (5 major and 2 minor). However, the density and the relative mobility of each corresponding band differ in each one of these species. In *A. arius*, the first and the last (5th) major bands having the Rm, 18 and 72 are faint, while in *A. caelatus* and *A. thalassinus* the corresponding bands are dark having the Rm, 10.5, 75 and 15, 75 respectively. The species differ in respect of minor bands association with the major bands. Two minor bands, in *A. arius* seem to be the subfractions of fourth and the fifth major bands whereas in the other two species they are the subfractions of the first and second major bands. Thus it would seem that the species specific nature of the muscle myogens lies in the density and to some extent in the relative mobility of various bands but not in the total number of fractions which are constant at least in the present case.

From the electrophoretic analysis of eye lens proteins, it is seen that the magnitude of differences at specific level is much greater than that of muscle myogens. The three specis (*A. arius*, *A. caelatus* and *A. thalassinus*) differ significantly in respect of the total number of bands, density and concentration of each protein fraction and more particularly in the degree of their relative mobility of each fraction (pl. 1, figs. 1-4, A, B, C) (Table 1). *A. thalassinus* can be easily distinguished from the other two species based on the total number of major bands i.e. four, instead of three in *A. arius*, and *A. caelatus*. The electropherograms developed from the eye lens protein of *A. arius* and *A. cealatus*, although represent three major fractions in each but the species are easily identifiable in respect of the relative mobility of each fraction, i.e. 21. *vs.* 7.5, first band ; 27 *vs.* 45, second band ; 50 *vs.* 90, third band ; in *A. arius vs. A. caelatus*. Further, unlike as the case of muscle myogen, no sexual dimorphism in respect of electrophoretic patterns was noted in the eye lens protein. These findings, perhaps may corroborate the earlier hypothesis of Rabaey (1964) that the lens proteins are species specific. The distribution of the soluble protein components in the crystalline lenses of fishes was demonstrated to be of species specific nature particularly in the family Sciaenidae (Cobb *et al.*, 1968).

It may be concluded that the eye lens proteins have more potentiality as compared to the muscle myogens from Chemo-taxonomic view point as demonstrated in the electropherograms of *A. arius*, *A. caelatus* and *A. thalassinus*. It is worth mentioning

that this method of analysis has a potential for clarifying the taxonomic interspecific affinities of the genera of the family Ariidae.

Summary

A comparative electrophoretic analysis of muscle myogens and eye lens proteins of three species of the genus *Arius* (*Arius arius*, *A. caelatus* and *A. thalassinus*) was carried out by three layer polyacrylamide gel disc-electrophoretic method.

It is seen that the relative electrophoretic mobility of various protein bands are species specific in respect of the muscle myogens, and the total number of protein fractions of the eye lens also differ specifically among the three species, beside the relative mobility. These findings corroborate the conclusion derived from the biometric comparision of the various taxonomic characters.

It may be concluded that the electrophoretic studies are of significant value in clarifying the systematic status of closely allied species of the genus *Arius*.

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