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GEL ELECTROPHORETIC STUDIES OF SOME BLOOD PROTEINS IN THE INDIAN SPINY-EELS (MASTACEMBELIDAE : PISCES)

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Electrophoretic analysis was made on three blood protein systems-plasma, haemoglobin and transferrin-in the three species of spiny-eels occurring in India, namely, *Mastacembelus armatus*, *M. pancalus* and *Macrognathus aculeatum*. The data demonstrated species-specific differences in all the protein systems. All the three species exhibited multiple haemoglobins which could be used as markers in species differentiation as well as for characterization of the family.

INTRODUCTION

The spiny-eels belonging to the family Mastacembelidae are represented by three species in India, *viz. Mastacembelus armatus*, *M. pancalus* and *Macrognathus aculeatum*. Of these, the first two species inhabit both fresh and saline waters while *Macrognathus aculeatum* occurs only in the brackish water within the tidal influence. So far as the authors are aware, electrophoretic studies on the blood proteins of these three species had not been carried out earlier.

In the present investigation, an attempt has been made to analyze the electrophoretic patterns of plasma, haemoglobin and transferrin in the three species of spiny-eels available in India primarily with a view to examining if species-specific differences in their blood protein components could be demarcated and used as diagnositc biochemical markers.

MATERIAL AND METHODS

15 adult specimens each of *Mastacembelus armatus* (Lacepede), *M. pan-*

calus (Hamilton) and *Macrognathus aculeatum* (Bloch) collected from local fish market in living condition constituted the materials for the present study.

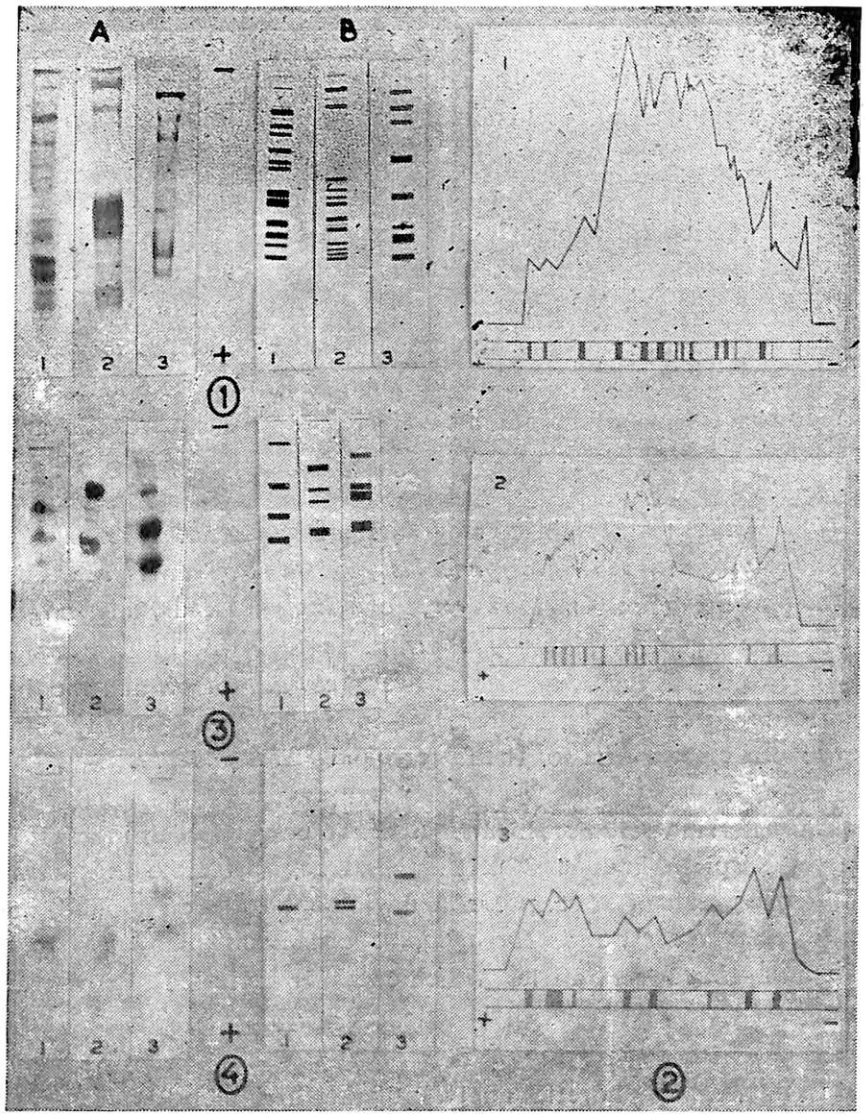
Blood was collected from each specimen separately by caudal puncture into centrifuge tubes rinsed with 3.8% sodium citrate solution (anticoagulant). Blood samples were separated into plasma and red cell fractions by centrifugation. The red blood cells were thoroughly washed in three changes of physiological saline, haemolyzed with an equal volume of water (Beck *et al 1983*; Peck & Biggers, 1975) and centrifuged to remove the cell debris from haemoglobin. The transferrin fractions were separated from the plasma by treating 6.5% rivanol solution (Jamieson & Turner, 1978). The plasma, red cell haemolysate and transferrin were immediately electrophoresed in vertical slab polyacrylamide gel (Ornstein, 1964; Davis, 1964) using Tris-glycine as running buffer (pH 8.6). The gels were stained with 0.1% Amido Black B (E. Merck, Germany) dissolved in acetic acid.

Densitometric tracings of plasma bands were recorded from transparencies fitted into built-in-recorder (Texacon, India) and the relative concentration of the protein components as revealed from their optical density presented in a curve.

RESULTS AND DISCUSSION

Plasma proteins : The plasma profile in both *M armatus* and *M pancalus* (Fig 1) exhibited 15 components as against 8 exhibited by *Macrognathus aculeatum*. However, the two profiles with 15-band phenotype in *M. armatus* and *M. pancalus* could be easily distinguished from each other for the differential net negative charges of their respective components; while the bands in *M pancalus* were clearly distributed in two zones (3 bands in the cathodal set including one 'linear' band and 12 bands migrating more anodally including two 'linear' bands forming the anodal set), those of *M. armatus* were distributed all along the migrating column without showing any zonal distinction. Further, the major components of plasma protein in *M pancalus* appeared to show lesser concentration as revealed from the densitometric tracing (Fig. 2) and the 'linear' band were missing. Thus, from the zymogram of plasma protein components, the three species could be differentiated from one another.

Haemoglobin : Multiple haemoglobin bands occurred in gels of red cell haemolysate of all the three species (Fig 3). However, while the red cell haemolysate in *M. pancalus* yielded two major haemoglobin fractions and two minor bands the same in *M armatus* revealed three relatively less pronounced major bands and a fourth lighter minor band. On the other hand, *Macrognathus aculeatum* showed four intensely stained bands, 3 major and one minor. Two bands were



Figs. 1–4 **1.** Photographic display (A) and diagrammatic representation (B) of electropherogram of plasma protein system in *Mastacembelus armatus* (1), *M pancalus* (2) and *Macrognathus aculeatum* (3). **2.** Densitometric tracing of plasma profiles in *M. armatus* (1) *M. pancalus* (2) and *Macrognathus aculeatum* (3). **3.** Photographic display (A) and diagrammatic representation (B) of electropherogram of haemoglobins in *M armatus* (1), *M. pancalus* (2) and *Macrognathus aculeatum* (3). **4.** Photographic display (A) and diagrammatic representation (B) of electropherogram of transferrin in *M. armatus* (1), *M. pancalus* (2) and *Macrognathus aculeatum* (3).

so closely spaced that the two appeared to be a common 'broad' band in *Macrognathus aculeatum*. Thus, although all the three species showed multiple haemoglobin band, the nature and staining intensity of the bands differed among them, as also the mobility pattern. Therefore, the haemoglobin data could also be useful as a diagnostic biochemical parameter in distinguishing these three species from one another.

Multiplicity of haemoglobins is relatively less common, but has also been reported to occur in some other teleostean fishes (Beck *et al*, 1983; Chandrasekhar, 1959; Cross & O'Rourkee, 1978; Wilkins, 1970) However, the umbiquitious occurrence of multiple haemoglobins in all the available species of Indian mastacembelids may be of special evolutionary significance and also may form a basis for interpretation of taxonomic relationships, both within and between families.

Transferrin : While transferrin zymograms of *M. armatus* revealed a single-banded phenotype, those of *M. pancalus* and *Macrognathus aculeatum* yielded a two-band profile (Fig. 4). However, of the two bands, one migrated quite faster than the other in *Macrognathus aculeatum* while the net negative charges of the two bands in *M. pancalus* were close, for which the bands were closely spaced Interestingly, the rate of anodal mobility of the single band in *M. armatus* was close to that of the faster band of *M. pancalus*.

Transferrin polymorphism is stated to be very high in most of the fish taxons (see Kirpichnikov, 1981). It is therefore, probable that we might have dealt with individuals of *M. armatus* (showing one transferrin band) homozygous and those of *M. pancalus* and *Macrognathus aculeatum* (showing two transferrin bands) heterozygous for Tf alleles which can only be tested by a thorough population study.

In fine, electrophoretic analysis of the three blood protien systems revealed that the data could be used for demarcating species-specific differences at the biochemical level among the Indian spiny-eels. However, additional data from two-way electrophores is, further analysis of sub-fractions and sequence studies etc are needed to examine more critically the degree of their relatedness at the blood protein level.

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