

## ELECTROPHORETIC AND IMMUNOTAXONOMIC STUDIES OF THREE SPECIES OF MARINE GASTROPODS FROM PORTONOVO COAST WITH REFERENCE TO POPULATION MANAGEMENT

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### ABSTRACT

Studies on the electrophoretic and serological patterns of foot muscle protein of three species of Cerithiacea viz., *Telescopium telescopium* Linn, *Cerithidea fluviatilis* (Poteiz & Michans) and *Cerithidea obtusum* Lam. were carried out in order to understand their relationships. The living specimens for the study were collected from mud flats and mangrove swamps off Portonovo. Polyacrylamide electrophoresis of proteins from foot muscle extract of *T. telescopium*, *C. fluviatilis* and *C. obtusum* showed that the former had a different densitometric profile as well as more number of protein bands; but the later two species showed a closer related pattern as well as lesser number of protein bands. At the same time the later two species are distinguished from each other in their total number of bands and Rf values. Immunological studies using micro-Ouchterlony double diffusion tests which absorbed antiserum indicated that the *C. fluviatilis* and *C. obtusum* were more closely related as revealed by an identity reactions than the species *T. telescopium* as shown by non-identity reactions. The results are discussed in relation to ecological and morphological adaptations.

### INTRODUCTION

In recent years large number of studies have been made on the electrophoretic and serological studies of molluscs (Davis & Lindsay, 1964, Pace and Lindsay, 1965; Patterson 1967; Burch, 1969; Burch and Lindsay, 1970, 1971 and Davis 1971). Informations on the electrophoretic and serological studies of molluscs in India are meagre (Kasinathan, 1973; and Govindan, 1974).

The present paper is mainly concerned with the electrophoretic and serological pattern of the foot muscle protein of three species of Cerithiacea (*T. telescopium*, *C. fluviatilis*, and *C. obtusum*, from Vellar estuary of Portonovo.

### METHODS

*Electrophoresis* : The methods used in this study were discussed fully by Davis & Lindsay (1964). The standard 7.5% acrylamide gel was used. The buffer was tris-

glycine mixture with a pH of 8.2-8.4. Bromophenol blue served as a tracing dye to indicate the position of the front in the gel.

Rf values for the fractions were determined from the densitometric tracings and were calculated from direct measurements of the gels.

The densitometric tracings were 4 times the actual length. The denser bands were very clear but the diffuse bands were not. In such case, the position was determined by multiplying the Rf value by the length of the chart.

Results were analysed by studying the differences between Rf values and densitometric patterns of the taxa.

*Serology*: Freshly prepared foot muscle extracts were used in all experiments to produce antisera. The immunological technique used here was the same as adopted by Burch and Lindsay (1970).

The micro-Ouchterlony double diffusion tests were conducted by using antigen and antisera. The semisolid medium used was 1% agar with 0.4% NaCl and 0.0001% merthiolate. The gels were washed with 0.005% cadmium sulfate for 5 mts, subsequently washed in barbitol buffer (pH 8.6) for 2 to 3 days (buffer changed thrice daily) stained in amidochiwartz for 1 hour, destained in 8% acetic acid and subsequently washed in water. The stained gels were placed under photographic enlarger and were used as a negatives to make enlarger positives. The precipitation, thus printed on photographic paper, are reproduced here.

#### RESULTS AND DISCUSSION

The results of the electrophoretic study are shown in Fig. 1 & 2. The average Rf values for the separated fractions are listed in Table 1.

Table 1 : Average Rf value for the separated protein components illustrated in Figs 1, & 2

Band No.	<i>T. telescopium</i>	<i>C. fluviatilis</i>	<i>C. Obtusm</i>
1	0.117	0.117	0.117
2	0.147	0.147	0.147
3	0.205	—	—
4	0.264	—	—
5	—	0.370	0.370
6	0.420	—	—
7	0.500	—	—
8	0.505	—	—
9	—	0.529	—
10	—	—	—
11	0.647	—	—
12	—	—	—
13	—	0.823	—
14	—	0.941	0.941
15	1.000	1.000	1.000

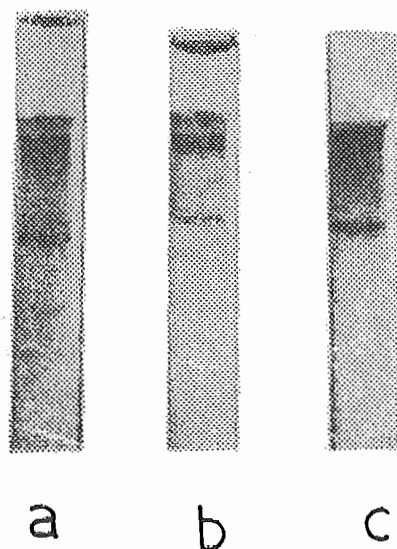


Fig. 1 : Disc electrophoretic pattern of the foot muscle proteins of Cerithiids:  
 (a) *T. telescopium*; (b) *C. fluviatilis* and (c) *C. obtusum*.

*Telescopium telescopium* :— This species is characterised by fractions 3, 4 and 5 which are slow moving. Further in the densitometric tracings the peaks are 83% and 61% that are characteristic of this species (Fig. 2a).

*Cerithidea fluviatilis* :— Six protein fractions characterise this species, of which 4 dense and other two thinner bands. Fractions 3, 4, 5, (13, 14, 15 Rf value) are closer and slow moving bands. According to the densitometric tracings the maximum peak observed was near the origin (88%) and another (35%) in the mid region (Fig. 2b).

*Cerithidea obtusum* :— This species is characterise by 7 fractions. There are only 3 denser bands and others are thin. Fractions 10 and 12 are characteristic of this species. There is one 99% peak and 40% peak in the densitometric tracings (Fig. 2c).

Of the three species studied in the super family Cerithiacea the species *C. fluviatilis* and *C. obtusum* are closer to each other compared to *T. telescopium*. *C. fluviatilis* and *T. telescopium* occur together in the ecological niche but they are different electrophoretically. *C. fluviatilis* and *C. obtusum* are closer in respect to the densitometric peaks of 35% & 40% occurring in both the species indicating of their close taxonomic relationship. Further fraction 13 and others after it are slow moving in *C. fluviatilis* and *C. obtusum* but in *T. telescopium* fractions 2-7 fractions are slow moving.

The results of specific absorption tests shown in Fig. 3 were derived from antisera obtained 5 days after last injection.

Homologus (identity) results were observed between the two species of *C. fluviatilis* and *C. obtusum* (Fig. 3a).

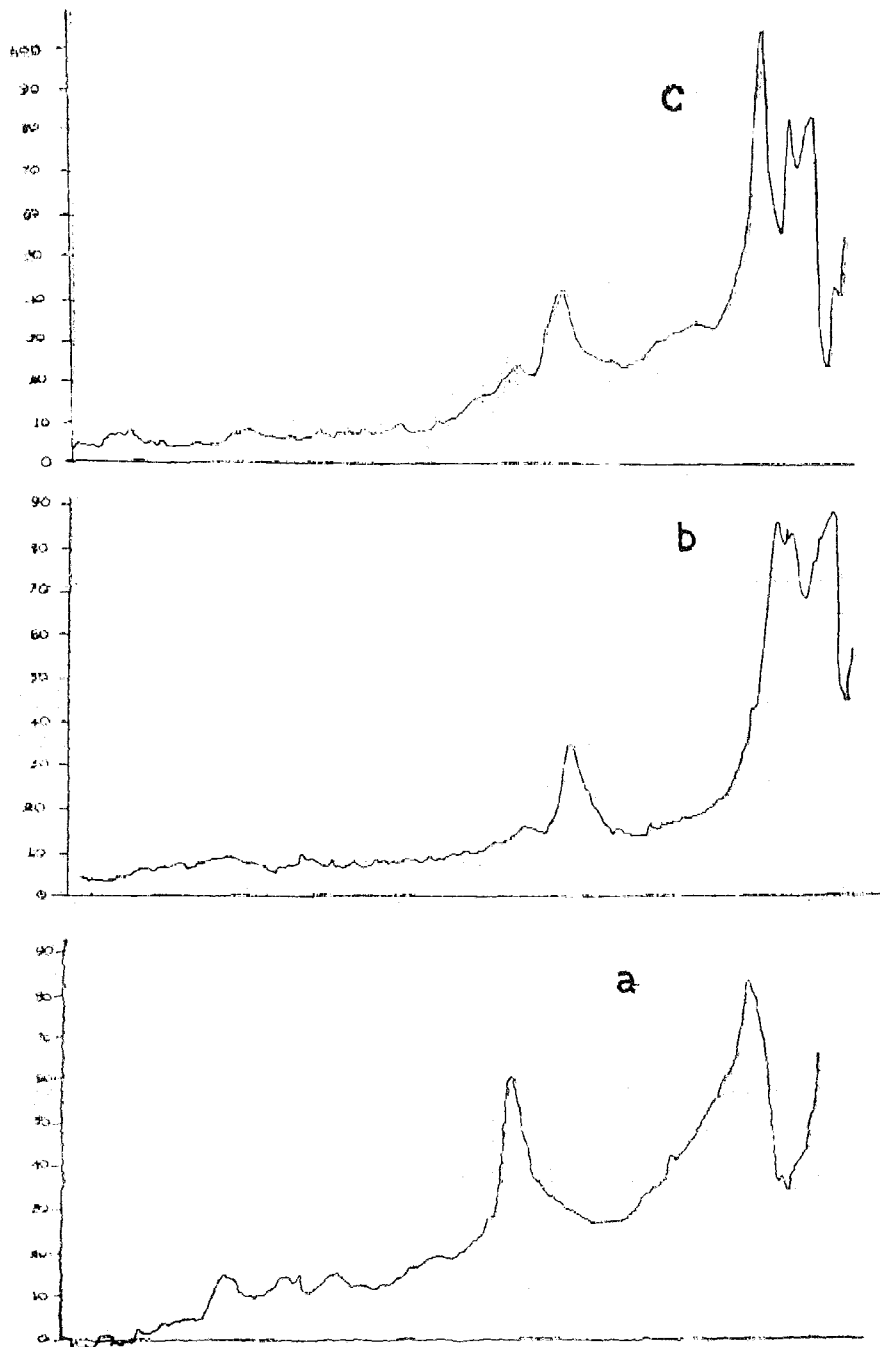


Fig. 2 : Densitometric tracings of the gels : (a) *T. telescopium*; (b) *C. fluviatilis* and (c) *C. obtusum*.

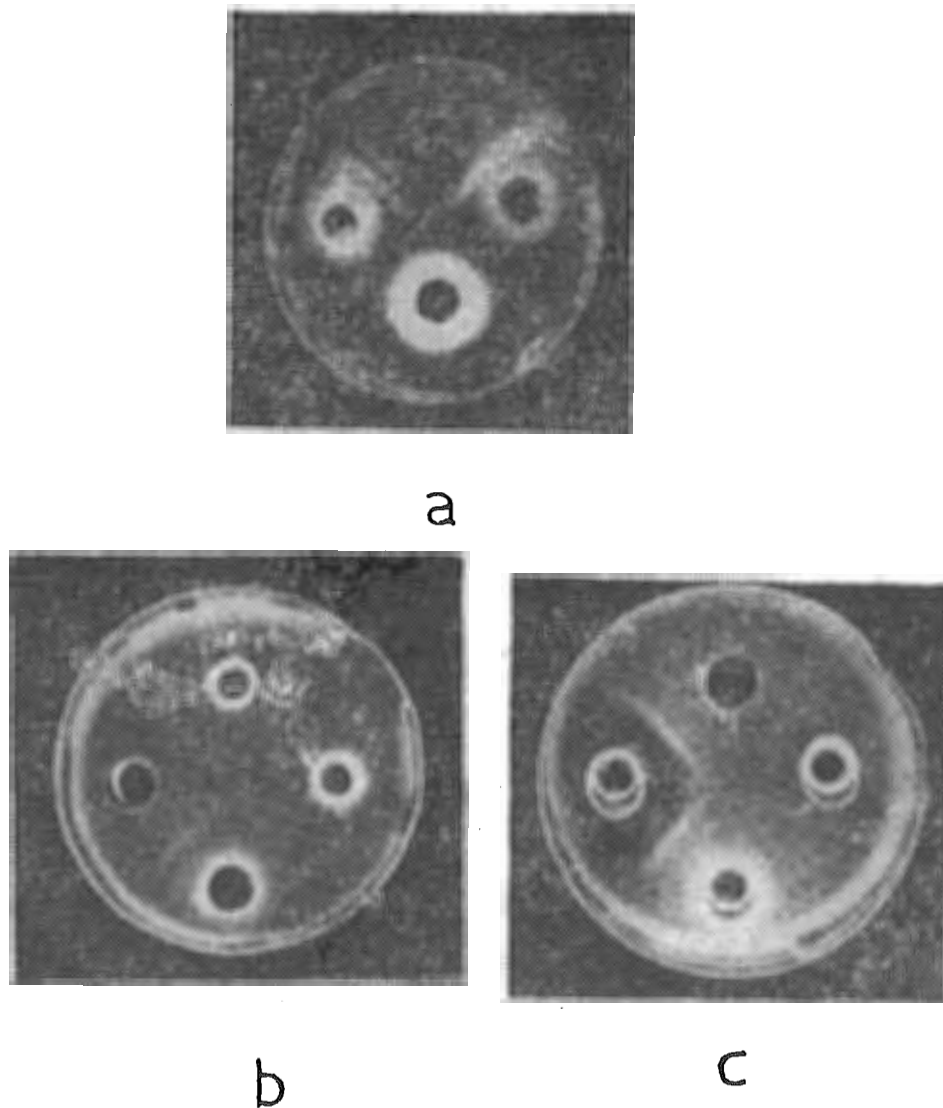


Fig. 3 : Serological assesement :

- (a) Precipitation reactions of *C. fluviatilis* antigen with antiserum of *C. obtusum*
- (b) Precipitation reactions of *C. obtusum* antigen with antiserum of *T. telescopium*.
- (c) Precipitation reactions of *C. fluviatilis* antigen with antiserum of *T. telescopium*.

*C. obtusum* and *T. telescopium* (Fig. 3b) give strong non-identity bands, showing that they are distinct with no indication of any close relationship whatsoever. The antigen-antibody reactions of *C. fluviatilis* and *T. telescopium* (Fig. 3c) indicates a single non-identity reaction and 2-3 identity reactions. The presence of the identity reactions on the later can be taken as an indication that these two may be related to each other more than *C. obtusum* to *T. telescopium*.

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