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STUDY ON ISOZYME ELECTROPHORETIC PATTERNS VARIABILITY IN THE THREE SPECIES OF SUPER FAMILY *GRAPSOIDEA* IN KARACHI MANGROVE FOREST

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ABSTRACT

Grapsid crabs inhibit sub tidal and tidal flats of various substrates, such as mud, rock, dead coral reef and seagrass, in the tropical and subtropical Indo-West Pacific region. Grapsids are also important within intertidal food webs. During present study some commonly found grapsid crab species *Parasesarma plicatum, Metaplax indica* and *Metopograpsus thukuhar* were collected from the mangrove areas of Karachi. Genetic and biochemical variations of four enzymes (Carbonate Dehydratase, Creatine kinase, Amylase, Peroxidase, and General protein (non-specific) pattern were determined by the use of native polyacrylamide gel electrophoresis. Crabs muscle samples were homogenized, centrifuged and run on a 10% vertical PAGE gel in the discontinuous buffer system and stained for isozyme variability. The obtained band patterns revealed the biochemical and genetic variability of isozyme and general protein pattern in three species of grapsid crab. Four enzyme system displayed polymorphic loci (38.5%), (27.3%) and (0.21%) in *P. plicatum, M. indica* and *M. thukuhar* respectively.

KEY WORDS: Grapsid, Native PAGE, Electrophoresis, Isozymes, Mangrove, Pakistan.

INTRODUCTION

Grapsid crabs are one of the most dominant macrofauna group within tropical and sub-tropical intertidal mangrove forests and also inhibit the sub tidal and tidal flats of various habitat, such as muddy, rocky, dead coral reef and seagrasses, throughout the world including Indo-west Pacific (Golley *et al*., 1962; Jones, 1984; Smith III *et al*., 1991; Lee, 1998). Grapsid crabs are important faunal components of intertidal food webs. Grapsid crabs including other mangrove fauna have been identified as consumers of mangrove propagules (Dahdouth-Guebas *et al.*, 2002; Smith, 1987a, 1987b; McGuinness, 1997). Dahdouth-Guebas, (2002) have shown that grapsid crabs feed on mangrove propagules and can possibly be a threat to early development of mangrove seedlings by feeding on litterfall, and burrowing habit they influence nutrient cycling and alter the sediment geochemistry (Robertson 1986; Smith III *et al*., 1991; Kristensen, 2008; Nerot *et al*., 2009). Mangroves are major nursery grounds for a variety of juvenile fish and provide important habitat for adult fishes (Robertson *et al*., 1988). Robertson and Duke (1987) showed that many of these fishes are dependent on crab larvae for food. Juvenile and adult crabs are consumed by larger fishes that utilize the forest during high tides (Sasekumara *et al*., 1984; Robertson *et al*., 1988). There is also evidence of competition between grapsid crabs and other invertebrate species in the forest (Fratini *et al*., 2001). During the last decades greatest interest on mangrove ecosystem has been developed in Pakistan, but no Published work is available on the role of this crucial element of the system. Previous studies mostly limited to identification and taxonomic studies (Hashmi, 1963; Tirmizi *et al*., 1983a; Tirmizi and Kazmi, 1986; Tirmizi and Kazmi, 1996a). The six genera belongs to four families were reported from the Pakistan coast (Tirmizi and Kazmi, 1996b; Siddiqui, 2005) studied the few aspects of distribution and abundance of few grapsid species along the coast of Karachi. The present study investigated and described some preliminary biochemical variations by utilizing soluble protein and isozyme analysis, a useful tool used for genetic relationship and also to provide basic data for further studies.

MATERIALS AND METHODS

Study site and field collection: Karachi constitutes a coastal belt of about 100 km long between the Indus delta on the south east coast and the Hub River on the west. The three species (*Parasesarma plicatum*, *Metaplax indica* and *Metapograpsus thukuhar)* of grapsid crabs were selected for the study and the crabs were collected mainly from Sandspit and Korangi creeks mangrove areas of Pakistan. Korangi creek (24º79'N, 67º20'E) is the north most creek of the Indus Delta located in east near the fishing village of Ibrahim Hyedri. It is bounded on its sides by extensive mangrove vegetation of *Avicennia marina*. The Sandspit backwater mangrove area (24º50'N, 66º56'E) comprises of mud flats and dense mangrove vegetation of *Avicennia marina* located north of the sandy coast and is connected to the Arabian Sea through Manora Channel.

The fresh samples of adult crabs were randomly collected through handpicked method and on capture, alive crabs were transferred to the laboratory and killed by freezing prior to tissue extraction. Initially preserved in 4ºC for isozyme

variations and taxonomic identification of the species. After taken the tissue sample from the crab, the crab specimen were identified to species level based on their external morphology according to available identification keys (Tirmizi *et al.,* 1983b, Tirmizi and Kazmi, 1996b). For estimation of mean size the morphometric measurements were taken, including Carapace length (CL), along the median line, Carapace width (CW), at its widest point, Carapace height (CH) and total weight of the crab for both sexes. Vernier callipers with an accuracy of 0.5 mm were used for length measurements, and the total weight of the crab was determined to the nearest gram using a digital balance (1 g).

Isozyme variations: Approximately 250-300 mg of muscle tissues was removed from each specimen and placed in hand Homogenizer to homogenize in extraction buffer Tris-Citrate. The homogenate was centrifuged at 13500 rpm for 5 min to remove solid tissue debris. The supernatant (enzyme extract) was filtered. This extract was either immediately used for electrophoresis or stored at -20°C. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed for the general protein as described by (Laemmli, 1970) under reducing conditions in the discontinuous electrode buffer system at room temperature and the gels were stained with Coomassie Brilliant Blue R-250. For the isozyme variation the electrophoresis were performed in vertical native polyacrylamide gels according to (Laemmli1970, Ayala *et al*., 1972, Harris and Hopkinson, 1976) in the discontinuous buffer system. Individual samples of Brachyuran species were stacked in 5% slab gels, built up in 3.0 M Tris-HCl buffer (pH 8.8), followed by separation in resolving gels at concentration 10%, prepared in Tris-HCl. Gels were stained for Isozyme activity, according to (Shaw and Parsad,1970, Hebert and Beaton, 1993).

Migrations for bands were measured in each gel. Relative mobility (Rm) of isozymes was obtained dividing protein migration by dye front (bromophenol blue) migration. The bands produced by each sample of related Isozyme were counted individually and the relative mobility (Rm) was estimated according to Petrokas and Stanys (2008). Alleles at each locus were designated by letters in alphabetical order, starting with the alleles encoding the most undally migrating isozyme. Arabic numerals were given for enzymes coded by more than one locus in order of decreasing anodal mobility. Allele's sizes are given in relation to their anodal mobility relative to the most common allele, the size of which was set to 100. The generated data were statistically analyzed. Individual's genotypes, allele frequencies were determined for all species. Genetic variability was calculated as percentage of polyallelic loci, and the mean heterozygosity per locus. Observed and expected proportions of heterozygous genotypes at each locus were averaged over loci to obtain means.

RESULTS

Mean size distribution: *Parasesarma plicatum*, *Metaplax indica* and *Metapograpsus thukuhar*, are the most abundant grapsid species during high tide at intertidal zone in mangrove areas of Pakistan (Fig. 1). Mean carapace length was observed in *M. indica* in male (1.05± 0.37 cm) and in female (1.18±0.29 cm) ranged in between 0.5-1.7 in male and 0.7-1.6 in female, whereas mean carapace width and carapace height was 1.46±0.536 and 0.78±0.260 in male and 1.61±0.395 and 0.8±0.2252 in female respectively. Mean wet weight of *M.indica* was 1.128±1.1298 in male crab and 1.537±1.193 in female crab. Mean carapace length was observed *P.plicatum* in male (1.469± 0.56) and in female (1.57±0.38). Mean wet weight of *P. plicatum* was 3.9±4.4 in male crab and 3.6±2.7 in female crab. Mean carapace length was observed *M. thukuhar* in male (1.55± 0.6) and in female (1.6±0.4). The mean wet weight of *P. plicatum* was4.35±3.18 in male crab and 4.109±2.625 in the female crab (Table 1).

Isozyme variation: Four enzyme systems were studied, total 20 activity zones or loci were detected in the present study. Carbonate Dehydrates (*Cd*) EC 4.2.1.1, Creatine kinase (Ck) EC 2.7.3.2, Peroxidase (*Per*) EC 1.11.1.7, Amylase (*Amy*) EC 3.2.1.1 and General protein (*GP*) EC (non-specific), In the four enzyme systems and general protein non-specific studied, the 20 loci were detected in the present study, 5 loci for the Carbonate Dehydrates (*Cd*1, *Cd*2, *Cd3*, *Cd4*, *Cd5*), two for Peroxidase (*Per*1, *Per*2), three for Amylase (*Amy*1, *Amy*2, *Amy*3), five for Creatin Kinase (*Ck*1,*Ck*2, *Ck*3, *Ck*4, *Ck*5) and five for General protein (*Gp1*, *Gp2*, *Gp3*, *Gp4*, *Gp5*) were detected (Table 2). In *P. plicatum,* (*Cd*) activity was expressed on polyacrylamide gels and coded by two loci (*Cd1*) and (*Cd2*) both loci appeared as monomorphic. In *M. indica* (*Cd*) activity was expressed only one common polymorphic locus (*Cd1*) out of three loci, where as in *M. thukuhar (Cd)* resolved by five loci whereas the (*Cd1*) and (*Cd4*) expressed as a polymorphic. Peroxidase (*Per*) was shown two loci (*Per 1*) and (*Per 2*) in *P. plicatum,* and *M. thukuhar* was showed andsingle loci in .*M. indica.* Five loci of Creatine kinase also were expressed in three species of grapsid crabs. General protein (*Gp*) EC (non-specific) was expressed as 5 loci in three species of grapsid crabs. (*Gp3*) appeared as polymorphic loci in *P. plicatum,*(*Gp1*) in *M. indica* and (*Gp4*) in *M. thukuhar*. Amylase was shown three loci only (*Amy*1) express as a polymorphic in *P.* (Fig. 2).

Numerical analyses: Genetic variation was estimated through the proportion of polymorphic loci, observed heterozygosity. The allele frequencies and observed heterozygosity (H) for each locus were determined by direct census of the data. Expected heterozygosity (H) of each population was calculated from alleles which was equal to or less than 0.99. Four enzyme system displayed polymorphic loci (38.5%), (27.3%) and (0.21%) and mean of alleles per locus 1.385, 1.273 and 1.211 *P. plicatum, M.indica* and *M.thukuhar* respectively. The average heterozygosity (expected), in *P. plicatum* (0.0267): *M. indica*(0.023) and *M. thukuhar* (0.01049) for the polymorphic loci (Table 3).

Fig. 1. *P. plicatum*, *M. indica* and *M. thukuhar*, are the most abundant grapsid species at high tide from the intertidal zone in mangrove from coastal waters of Pakistan .A, *P. plicatum.* B, *M. thukuhar*. C, *M. indica. D*, *P. plicatum* on a mangrove tree. E, *P. plicatum* at high tide.

Table 1. Morphometric measurement of grapsid crab found in mangrove area of Pakistan.

S. No.	N	Mean	St. Dev.	Min.	Max.	N	Mean	St. Dev.	Min.	Max
	Male					Female				
M. indica										
CL (mm)	23	1.058	0.3701	0.5	1.7	8	1.188	0.290	0.7	1.6
CW (mm)	23	1.461	0.536	0.8	2.5	8	1.619	0.395	1.0	2.50
CH (mm)	22	0.78644	0.2606	0.4	1.3	8	0.8	0.2252	0.5	1.25
Wet weight (gm)	22	1.128	1.298	0.07	4.71	7	1.537	1.193	0.15	3.148
P. plicatum										
CL (mm)	61	1.4697	0.5684	0.6	2.5	47	1.5745	0.3836	0.8	2.2
CW (mm)	61	1.8607	0.7244	0.8	3.1	47	2.0638	0.5215	1.0	3
CH (mm)	61	1.130	0.966	0.4	8	47	1.1404	0.3591	0.5	2.2
Wet weight (gm)	61	3.954	4.405	0.130	14.370	47	3.676	2.708	0.424	10.233
M. thukuhar										
CL (mm)	6	1.55	0.647	0.9	2.4	14	1.614	0.487	0.6	2.3
CW (mm)	6	1.95	0.812	1.1	3.0	14	2.107	0.609	0.8	2.8
CH(mm)	5	0.920	0.409	0.5	1.4	14	1.0857	0.3278	0.4	1.6
Wet weight (gm)	6	4.35	3.18	0.42	7.29	12	4.109	2.625	0.085	9.077

Table 2. The enzymes assayed, with their code (EC).

S.No.	Enzyme	Abbreviation	Enzyme code
1.	Carbonate dehydrates	CD	EC 4.2.1.1
2.	Creatine kinase	CK	EC 2.7.3.2
3.	Amylase	α-AMY	EC 3.2.1.1
4.	Peroxides	PER	EC 1.11.1.7
5.	General protein	GP (nonspecific)	

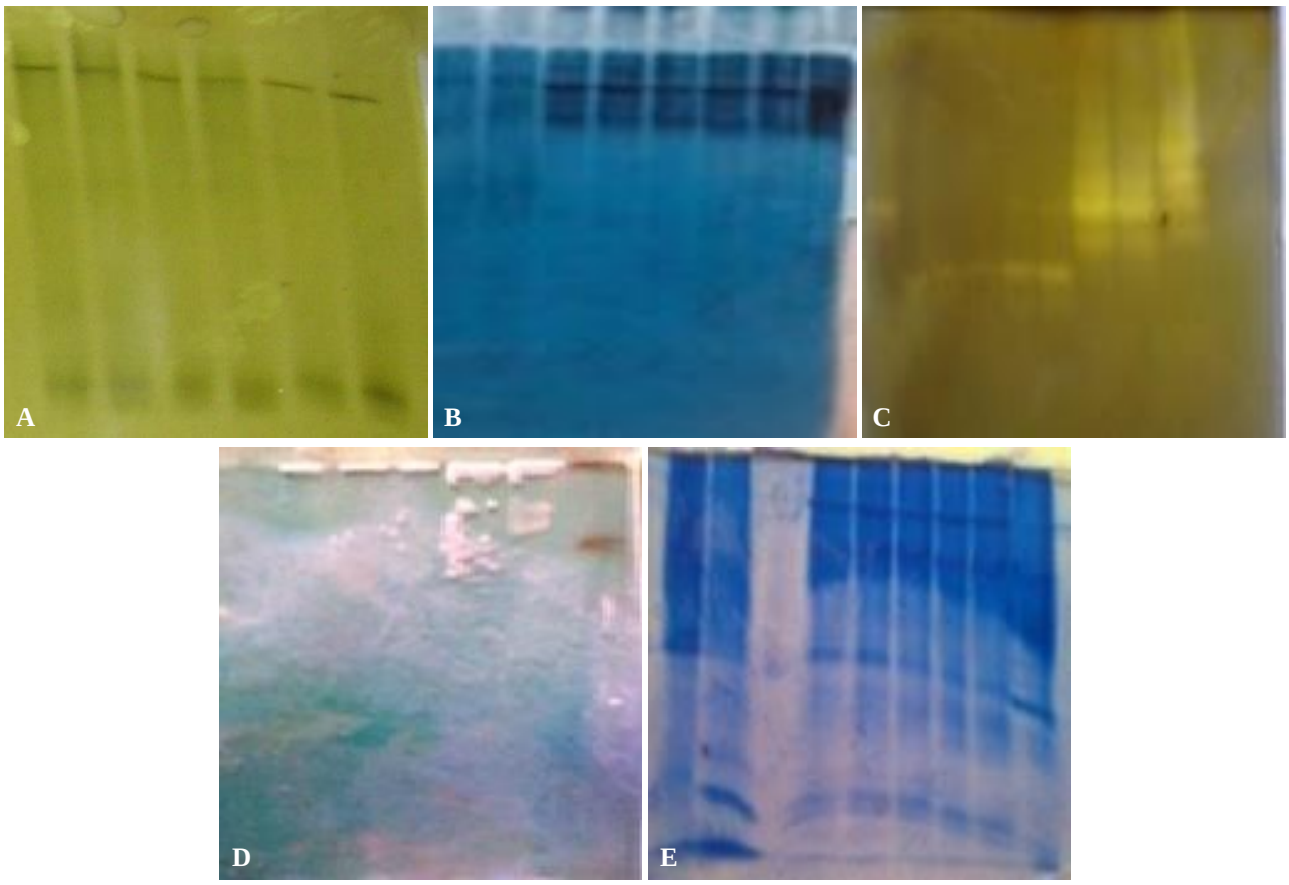

Fig. 2. Isozyme and protein (Carbonate Dehydratase (*Cd*) EC 4.2.1.1 (A), Creatine kinase (*Ck*) EC 2.7.3.2 (B), Amylase (*Amy*) EC 3.2.1.1 (C), Peroxidase (*Per*) EC 1.11.1.7 (D) and General protein (*Gp*) EC (non-specific) (E) pattern of grapsid crab.

Table 3. Parameters of genetic variation in the three species of family Grapsidae collected from coastal waters of Pakistan.

	P. plicatum	***M. indica***	***M. thukuhar***
Total loci	13	11	19
Polymorphic loci	5	3	4
Allele expressed by loci	18	14	23
% of polymorphic loci	0.385	0.273	0.211
% of Allele expressed by polymorphic loci	6	6	8
Mean no of locus	1.385	1.273	1.211
Average Heterozygosisty	0.02674101	0.02310293	0.010497157
χ^2	0.017	0.00	0.0027

DISCUSSION

The present study based on some preliminary information about isozyme variability in three species of the grapsid crabs M. *indica*, *P. plicatum*and *M. thukuhar*. A total of 90 males and 69 females of grapsid crabs were randomly collected by hand pick method in mangrove areas along the coast of Pakistan including Sandspit and Korangi creeks.

Four enzyme systems Carbonate Dehydrates (*Cd*), Peroxidase *(Per)*, Amylase (*Amy*), Creatin Kinase (*Ck*) and General protein were studied and the results provide the evidences of interspecific genetic variations among the three species. A total 20 loci was expressed for three species. In *M. thukuhar* total 19 loci were resolved, out of four loci were polymorphic and 15 monomorphic. The 11 loci expressed by *M. indica,* out of this 3 loci expressed as polymorphic and 8 loci expressed as monomorphic, in *P. plicatum* total 13 loci expressed out of which 5 loci polymorphic. The expected heterozygosity of each population was calculated from allele frequencies. The average heterozygosity in the present study for grapsid crab ranged in between 0.0104-0.0267 and these heterozygosity values were low then the other invertebrate values (0.15) given by Powell (1975). The low values of expected heterozygosity in *M. indica, P.plicatum*

and *M.thukuhar* supports the suggestion that a relatively low genetic variation is a phylogenetic character of decapod crustacean (Gooch, 1977). Hetrozygosity estimates obtained in this study were relatively similar to the values obtained in other studies, for Japanese sentinel crabs (Decapoda: Ocypodidae: genus *Macrophthalamus*) ranged in between 0.004-0.051 (Horii *et al*., 2001), and other Ocypode crabs (0.038-0.045) in *Macrophthalamus hirtipes* (Sin and Jones, 1938), 0.012 in *Ocypode quadrata* and 0.046 in *Ocypode occidentalis* (Nelson and Hedgecock, 1980) and 0-0.11 in Malaysian fiddler crabs of *Uca* (Suzawa *et al*., 1993). Low genetic variability in decapod crustaceans has also been reported by Hedgecock *et al*. (1982). Gooch (1977),Turner and Lyerla, (1980) and Hedgecock *et al*. (1982). Some studies have suggested that the low genetic variability in crustacean's species in fact reflects low rates of mutation (Nemeth and Tracey, 1979) or their mobility (Hedgecock *et al*., 1982, Horii *et al*., 2001). The isozyme patterns provide additional information about the biochemical and genetic structure of these crabs and these preliminary results presented here, confirmed the genetic variability and detail study will provide the information for the genetic diversity and phylogeny of grapsid crab species found along the coast of Pakistan. .

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