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INTERSPECIFIC ISOZYME VARIABILITY IN PORCELLANID CRABS (CRUSTACEA: DECAPODA: ANOMURA) FROM THE COASTAL WATERS OF PAKISTAN

Farah Naz, Sahir Odhano, Noor Us Saher and Mustafa Kamal
Institute of Marine Science, University, Karachi-75270, Pakistan (FN); Centre of Excellence in Marine Biology, University of Karachi, Karachi-75270, Pakistan (SO, NUS); Department of Biotechnology, University of Karachi, Karachi-75270, Pakistan (MK).
email: noorusaher@yahoo.com

ABSTRACT: Polyacrylamide gel electrophoresis was used to investigate the interspecific isozyme variability among three species of Porcellanid crabs: *Petrolisthes ornatus*, *P. rufescens* and *P. boscii* from the coastal waters of Karachi, Pakistan. Seven enzyme systems and the general protein (non-specific) were examined for genetic variations. Among the three species; Nei's heterozygosity for overall loci was observed (0.067), whereas Shannon's information Index (I) for genetic variations based on the heterozygosity was (0.055) in three species of Genus *Petrolisthes*. Species were differentiated from each other as Nei's genetic distance ranged in between 0.0093-0.0314. The genetic relationship among the three species of genus *Petrolisthes* similar from those inferred from morphological features.

KEYWORDS: Isozymes, population structure, Porcellanid crabs, *Petrolisthes*, Anomura, Pakistan

INTRODUCTION

The anomuran crustacean family Porcellanidae commonly known as Porcellanid crabs or false crabs and can easily distinguish from Brachyura (true crabs). Approximately 280 species are being represents the 30 genera from tropical to temperate waters of the world (Osawa and McLaughlin 2010, Osawa and Uyeno 2013, Dolorosa and Werding 2014) along with some fossil (*Pachycheles*, *Pisidia*, *Polyonyx*, *Porcellana)* records. Porcellain crabs are mainly inhabitants of intertidal and shallow subtidal zones characterized by uninterruptedly fluctuating water flows that dominate the wave-induced oscillating flows found beneath humid rocks, dead corals and in muddy bottoms (Werding and Hiller 2007) while, in sub-tidal regions, these crabs occasionally live in association with other invertebrates such as sea urchin, soft corals, worm tubes, sponges and hydrozoans (Werding 1983, Hiller *et al.* 2004, Hiller *et al.* 2006, Osawa and Chan 2010).

Porcellanid crabs usually have small carapace width, less than 15 mm; body plan resembled with squat lobster, nevertheless their bodies additionally compressed or flattened adapted for living and hiding under rocks. These are fragile animals and well known for their escape tactics and by the voluntary shedding of limbs to escape predators (Wasson *et al.* 2002).

Significant works have been done on the diversity and distribution of Porcellanids from Northern Arabian Sea Mustaquim (1972), Ahmed and Mustaquim (1974) Siddiqui and Kazmi (2003), Kazmi and Siddiqui (2006). Beleem *et al*., (2016) described the five species of porcelain crabs from Gujarat western coast of India, among them, *Enosteoides ornatus* (Stimpson 1858) and *Pachycheles tomentosus* (Henderson 1893) were identified as the first records from this region.

A molecular technique allozyme electrophoresis is a powerful technique to study genetic variation (Ward and Grewe 1995), intra-specific population studies (Sodsuk 1996, Sodsuk and Sodsuk 1998a 1998b, Sodsuk *et al.* 2001) and also used in invertebrate systematics of swimming crab species includes; *Callinectus sapidus* (Weber *et al.* 2003), species of *Scylla* (Keenan *et al.* 1998); *Chionoecetes bairdi* and *C. opilio* (Merkouris and Seeb 1997), marine invertebrates (Thrope *et al.* 2000) and some walking crabs like; *Sesarma* and *Uca* species complex (Felder and Staton 1994). Recently; some studies was conducted on isozyme variation in different species of crabs from the coastal waters of Pakistan. Odhano *et al.* (2018) describe the isozyme variations in three populations of *Austruca sindensis* (Alcock 1900), Naz *et al.* (2017) studied the isozyme variation in genus *Thalamita* of family Portunidae whereas, Saher *et al.* (2016) describe the variation in Carbonic Anhydrase (Ca) isozyme in muscles of five species of Portunid crabs. However, there is no information available on the genetic isozyme variations for the porcellain crabs. The current study aimed and initiated to estimate the genetic variations of morphologically identical species of Porcellanids through isozyme study in order to reveal the isozyme variations and interspecific genetic diversity among the three species of Porcellanid crabs with providence of basic genetic data for further studies.

Fig. 1. Some Porcelain crabs (Crustacea: Decapoda: Anomura): a, *Petrolisthes rufescens*; b, *P. ornatus*; c, *P. boscii* (collected from the coastal waters of Pakistan).

MATERIALS AND METHODS

The fresh specimens of Porcellain crabs were randomly collected by handpicked from the coastal waters of Mubarak village, Buleji and Sandspit areas of Pakistan. Capture live crabs transferred to the laboratory killed by freezing, before tissue extraction. Specimens initially preserved in 4° C for isozyme variations. Morphological identification based on taxonomic keys of Mustaquim (1972); Ahmed and Mustaquim (1974), Siddiqui and Kazmi (2003). Three species were identified belonging to genus *Petrolisthes; Petrolisthes ornatus, Petrolisthes rufescens, Petrolisthes boscii* (Fig.1).

Forisozyme variations approximately 250-300 mg crab muscle was removed and homogenized in extraction; Tris-Citrate buffer according to Naz *et al.* (2017). For the isozyme variations, Carbonate dehydratase (*CD*) EC 4.2.1.1, Peroxidase (*PER*) EC 1.11.1.7, Creatine Kinase (*CK*) EC2-7.3.2, Amylase (*AMY*) EC 3.2.1.1, Catalase (*CAT*) EC 1.11.1.6, Octanol Dehydrogenase (*ODH*) EC 1.1.1.73, Glucose -6- Phosphate Dehydrogenase (*G6PDH*) EC 1.1.1.49 and General protein (*GP*) EC (non-specific) were selected for observation. Electrophoresis was performed in vertical native polyacrylamide gels (Native-PAGE) as described by Laemmli (1970) under the reducing conditions in the discontinuous buffer system at room temperature. Gels stained for isozyme activity according to Hebert and Beaton (1993), whereas the standardized genetic nomenclature used to the designated the loci and alleles (Shaklee *et al.* 1990). Shannon and Weaver (1949) described the index for information for the diversity of species and population and also, a relatively good measure for the effective allele number (a reciprocal of homozygosity) as estimated in the study. The genetic distance was calculated by using Nei's Unbiased Measures of Genetic Identity and Genetic distance (Nei 1978). Dendrogram was constructed based on genetic distance through the UPGMA method.

RESULTS AND DISCUSSION

The isozyme variations observed in three species of Porcellanid crabs from the coastal waters of Pakistan; the results indicated that a total of five numbers of loci was observed as polymorphic (CD-1*, CK-3*, GP-3* and AMY-1*, OCT-1*) in *P. boscii* whereas in *P. rufescens* a single locus was observed as polymorphic, in case of *P. ornatus* all loci expressed as monomorphic loci. In three porcelain crabs, the overall observed heterozygosity was 0.0741±0.0889 and expected heterozygosity was 0.0687±0.0793,

Table 1. Summary of homozygosity and heterozygosity statistics for all loci (Leven 1949 and Nei 1973) and genic variation Nei (1987) of genus *Petrolisthes* Porcelain crabs.

Variables	**Abbreviations**	***Values***
Observed heterozygosity	*Obs Het*	0.0741±0.0889
Expected heterozygosity	*Exp Het*	0.0687±0.0793
Observed homozygosity	*Obs Hom*	0.9259 ±0.0889
Expected homozygosity	*Exp Hom*	0.9313 ±0.0739
Observed alleles	*na*	1.5833±0.5149
Effective number of alleles	*ne*	1.0802±0.0993
Nei's heterozygosity	*Nei*	0.0677±0.0782
Polymorphic loci	*P*	58.33%.
Shanon's Information Index	*I*	0.0550±0.0557

Whereas, the observed homozygosity was 0.9259±0.0889 and expected homozygosity was 0.9313 ±0.0739. The mean number of observed alleles was 1.5833±0.5149 with the mean effective number of alleles was 1.0802±0.0993. Genetic variations were also observed as the allele variability was estimated through Nei's heterozygosity (0.0677±0.0782) in *P. ornatus*, *P. rufescens* and *P. boscii*. The total number of polymorphic loci was 7, whereas the percentage of polymorphic loci was 58.33%. Shannon's Information Index (I) was also estimated to observe the genetic variations based on the distribution of different alleles. The estimated average heterozygosity was (0.0550±0.0557) that was very similar to other crustaceans (Table1).

Genetic Diversity and Genetic Identity:

The genetic identity and genetic distance was calculated by using Nei's formula to estimate the degree of genetic divergence between pairs of three species of Porcellanid crabs were also estimated in *P. ornatus*, *P. rufescens* and *P. boscii* (Table 2). The genetic distance between the *P. boscii* and *P. rufescens* was 0.0314 whereas in between *P. boscii* and *P. ornatus* was 0.0189 and it was 0.0093 in between *Petrolisthes rufescens* and *P. ornatus*. Similarly, the genetic identity between *P. boscii* and *P. rufescens* was 0.9691 whereas in between *P. boscii* and *P. ornatus* was 0.9812 and 0.09908 in between *P. rufescens* and *P. ornatus***.**

Table 2. Estimates of (Nei's 1978) unbiased genetic distance (*D*) (below diagonal) and genetic identity (*I*) (above diagonal).

	Petrolisthes boscii	*Petrolisthes rufescens*	*Petrolisthes ornatus*
Petrolisthes boscii		0.9691	0.9812
Petrolisthes rufescens	0.0314		0.9908
Petrolisthes ornatus	0.0189	0.0093	

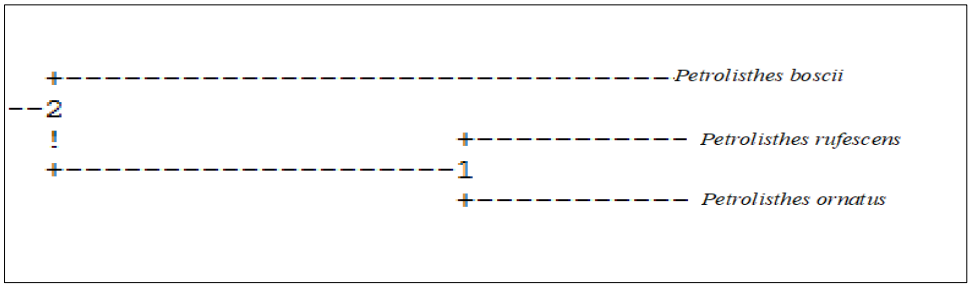

Fig. 2. Dendrogram showing relationship among the of Porcelanid crabs according to UPGMA method of cluster analysis.

Genetic divergence:

Dendrogram based on cluster analysis with unweighted Pair-Group Method with arithmetic mean (UPGMA) showed that *P. rufescens* and *P. ornatus* cluster together whereas *P. boscii* radiated from this cluster and showed the close relationship (Fig. 2).

Genetic diversity considered as the overall expression of genotypic features within a species, whereas the genetic variability is the tendency to discriminate species on the basis of their genetic characteristics, which may vary within or among the species (Brown and Hodgkin, 2016; Saher *et al.*, 2016; Saher *et al.*, 2015). The electrophoretic technique has made it possible to study variations at a very basic level that of gene expression (Mustaquim 1998) and enzyme variations observed by electrophoretic technique are genetic in origin and expressed at each gene loci (Mustaquim 1998). Protein structures are not destroyed in electrophoresis accomplished under native conditions, allowing exploiting differences in their sizes, charges and shape (Mateus *et al.* 2009). The electrophoretic separation of protein, including enzymes is a useful tool for differentiating species for taxonomic identification. Isozymes commonly known as isoenzyme or multiple forms of enzymes that differ in amino acid sequence but catalyze the same chemical reaction. The isozymes used as independent markers to evaluate the relationship between the species and populations (Huss *et al.* 1996). The current study is the first isozyme studies through Polyacrylamide gel electrophoresis PAGE to estimate the interspecific variation in porcellanid crabs from the coastal waters of Pakistan. Allele frequency, monomorphic and polymorphic loci, genetic identity and distance was estimated to detect the genetic variation in three Porcellanid species. A total of five numbers of loci was observed as polymorphic in *P. boscii* whereas a single locus in *P. rufescens* was observed as polymorphic, in case of *P. ornatus* all loci expressed as monomorphic loci. In three species of porcellain crabs, the overall observed heterozygosity was 7.4% and expected heterozygosity was 6.85% whereas the observed homozygosity was 93% and expected homozygosity was 93%. Nei's heterozygosity, showed 6.7%, an estimate of genetic variations in *P. boscii*, *P. rufescens* and in *P. ornatus*. The total number of polymorphic loci was 7 whereas the percentage of polymorphic loci was 58.33%. Shannon's Information Index (I) was 5.5% based on the distribution of different alleles that was very similar to other crustaceans. The genetic identity and genetic distance between pairs of three species of Porcellanid crabs were also estimated in *P. ornatus, P. rufescen s*and *P. boscii*. The genetic distance between the *P. boscii* and *P. rufescens* was 3% whereas in between *P. boscii* and *P. ornatus* was 1% in between *P. rufescens* and *P. ornatus*. This value is also similar with the average heterozygosity of other crustaceans as studied by various researchers such as coconut crab 1.8%; Penaeid shrimps 0.6% (Lavery and Fielder 1993), Mulley and Latter (1980), Norway lobsters 18% (Stamatis *et al.*2004); Portunid crabs1.5-2.5%. (Saher *et al.* 2016) and invertebrates 11% (Nevo 1978), *Macropthalamus spp* 0.4%- 0.5% (Horii *et al.* 2001), 15% in invertebrate (Powell 1975), in Ocypodid crabs 3.48-4.5% (Sin and Jones 1983) *Macropthalamus hirtipes* (0-11%) in *Uca* Malaysian fiddler crabs (Suzawa *et al.* 1993) were observed lowest heterozygosity. Such higher level of genetic variation might be the cause of higher gene flow or random matting of individuals within each site such results can be compared with the previous studies (Huang and Shih 1995, Wright 1946, 1978). Whereas, Albrecht and Hagen (1981) did not find any intraspecific variation in the muscle proteins among various species of *Uca* collected from different localities.

Present study helps us to assess the genetic diversity existing among species of genus *Petrolisthes* and also derive diagnostic loci and alleles that are useful for identification of closely related species. Similarly, the genetic identity between *P. boscii* and *P. rufescens* was 96% whereas in between *P. boscii* and *P. ornatus* was 98%. *P. rufescens* and *P. ornatus* cluster together whereas *P. boscii* radiated from this cluster that was showed the close relationship. Present study helps us to assess the genetic diversity existing among species of genus *Petrolisthes* and also derive diagnostic loci and alleles that are useful for identification of closely related species.

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