Genetics of Aquatic Organisms 3(2), 67-77
http://doi.org/10.4194/2459-1831-v3_2_04

RESEARCH PAPER

GENAQUA
GENETICS OF AQUATIC ORGANISMS

Mitochondrial DNA Variation and Population Genetic Structure of Mud Crab, *Scylla serrata* from Pakistan/Northern Arabian Sea

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Article History
Received 26 July 2019
Accepted 23 December 2019
First Online 25 December 2019

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Keywords
16S rRNA
COI
Genetic structure
IWP
Mud crab

Abstract

Amongst the 50 largest Marine Ecosystems (LMEs) that yield 95% of the annual marine fishery revenues throughout the world, the Arabian Sea ranked 32 on a global measure. *Scylla serrata* is an important resource of the aquaculture and commercial fishery in the Northern Arabian Sea (NAS). The mt-DNA variations in *S. serrata* (n16) were estimated from the two populations: Sandspit back waters (n8) and Korangi creek mangrove areas (n8) Pakistan. The study based on 16S rRNA and Cytochrome Oxidase (COI) genes, high haplotype and low nucleotide diversity was observed in the populations of *S. serrata*. The neutrality tests (Tajima's and Fu's F's) were non-significant, whereas mismatch analysis revealed the potential population expansion event occurred in the (NAS). Furthermore, we conducted a phylogeography analysis of *S. serrata* based on the COI obtained from GenBank (n47) determined from specimens of the IWP. Out of all sequences n63 (16 from Pakistan and 47 from Genbank), 46 different COI haplotypes were identified. The AMOVA indicated the phylogeographic regional partition and genetic structure in IWP. In the present study, the partial sequences of the genes provide orientation with the valuation of the genetic structure, phylogeography and genetic affiliation of *S. serrata* in the IWP region.

Introduction

Marine species are proficient for extensive dispersal potential due to planktonic phase or various stages of their life cycle that predicted to strong genetic connectivity throughout their extension range and therefore; expected for an island model of migration (Palumbi, 1994). According to Hellberg (1996); positive correlation between the duration of a planktonic larval phase and levels of gene flow have been shown for a wide range of marine species and estimates that planktonic dispersal endorses genetic and demographic connectivity among populations (Scheltema, 1971, Crisp, 1978, Nanninga and Manica, 2018). Accessibility of marine populations due to numerous nonrandom factors and genetic adaptation in the marine environment may, occur through ecological and geographic limitations such as dispersal capability, niche portioning and/or local adaptation (Hedgecock, 1986; Féral, 2002). Population genetics offer a useful advancement in management of marine systems as a relationship among the dispersive ability of organisms and the genetic differentiation of populations as provide a fundamental link between ecology and evolution (Ayre and Hughes, 2000) and genetic relatedness represent a proxy to the extent of recruitment that is occurring between two areas.

DNA analysis has become the most recent and reliable solution for systematic, population genetics and phylogenetic studies. In principal, the mitochondrial DNA (mt-DNA) has been one of the most widely used molecular markers of taxonomic and phylogenetic

studies in animals (Avise *et al*., 2004; Shekhar *et al*., 2011). *S. serrata* has distinct distribution throughout the Indo West Pacific region as ranged from Tahiti, Australia including the Philippines, Indonesia, Japan, East and South Africa and to the Red Sea (Sakai, 1976; Dai and Yang, 1991). It assumed that high levels of gene flow occur in populations of *S. serrata* and it depends on life history and dispersion pattern (Gopurenko, 2002). Female *S. serrata* releases eggs in offshore waters, thereby facilitating the high levels of oceanic dispersal and mixing of propagules before re-entry into estuarine adult habitats (Hill, 1994).

Genus *Scylla* having high commercial and economic significance in catch contribution of tropical and sub-tropical region and also a source of the aquaculture and marketing fishery enterprises (Cristensen *et al*., 2004). Previously single species recognized in the genus *Scylla,* the review of detailed previous work illustrates that genus *Scyll*a includes more than one species (Estampador, 1949a, b; Serene, 1952; Stephenson and Campbell, 1960; Ong, 1964; Joel and Raj, 1980; Radhakrishnan and Samuel, 1982; Fushimi, 1983; Oshiro, 1988; Kathirval and Srinivasagam, 1992; Fuseya and Watanabe, 1995; Fuseya and Watanabe, 1996; Watanabe and Fuseya, 1997; Fuseya, 1998). Keenan *et al*., (1998) identified genus *Scylla* as a four distinct species *S. serrata*, *S. tranquebarica, S. olivacea* and *S. paramamosain* based on the morphological and molecular approaches. Currently, Ma *et al*., (2012) confirmed the work of Keenan *et al*., (1998) through DNA bar-coding technique and confirmed all the four species: *S. tranquebarica, S. olivacea, S. paramamosain* and *S. serrata* from China. After the revision of Keenan *et al*., (1998), Kazmi *et al*., (2000) revised the taxonomy of the species within the genus *Scylla* and confirmed the presence of two species of Genus *Scylla*: *S. serrata, S. tranquebarica* from the coastal waters of Pakistan. According to Avise, (1989) and Brophy, (2004) the accurate taxonomic identification is important to the growth of management strategies and breeding program for sustainable fisheries resource. Sometimes, the external morphology remain insufficient for taxonomic and homogeneous chronological structures and leads to difficulty in the establishment of phylogenetic relationships (Stiassny, 1993; Thomson *et al*., 1997). Taxonomic and molecular exploration in description of sister species has revealed entire species complexes, including economically important species (Matsuoka and Hatanaka, 1991 and Knowlton, 1993).

The current study not only explains the mitochondrial DNA variation of *S. serrata* from the Pakistan Northern Arabian Sea, also determine the phylogeographic structure and levels of population connectivity in the marine environment. The molecular data based on Cytochrome Oxidase COI and 16S rRNA was used to surmise the population genetic structure, genetic relatedness and evolutionary history of *S. serrata* population in the Indo-West Pacific region by using genetic methods (nucleotide diversity tests, network analysis, mismatch analysis and AMOVA). Although features concerning the life history of *S. serrata* and its distribution in the IWP that makes it model for examining hypotheses of genetic structure specific to other marine species.

Materials and Methods

Samples Collection and Morphological Examination

The crabs (n=16) were collected from the field by direct hand pick, purchase fresh catch by local fisherman from the two populations: Sandspit back waters (n=8) and Korangi creek mangrove areas (n=8) from the coastal waters of Pakistan (Figure 1). Capture or purchased live crabs, immediately stored in the icebox, killed by freezing and transferred to the laboratory. The specimen identified up to the possible species level based on morphological characteristics (Fuseya and Wetanabe, 1996; Keenan *et al*., 1998; Kazmi *et al*., 2000; Jirapunpipat *et al*., 2008).

DNA extraction and PCR amplification

Total genomic DNA was isolated from muscle tissue (approximately >25) in the chelipeds of fresh crabs using Qiagens DNeasy Blood and Tissue Kit**,** following the manufacturer's instruction with some modifications of the original protocol to improve the yield and quality of the DNA extraction.

PCR technique was used to amplify the 16S rRNA and COI mt-DNA genes. Selective amplification of a 550-600 base pair product from the 16S rRNA (ribosomal regions of mt-DNA) and approximately 700 base pair product from the COI was carried out through selected primers. Primers used to amplify the 16S fragment and COI were:

16Sar (Forward) (5′-CGCCTGTTTATCAAA AACAT-3′);

16Sbr (Reverse) (5′-CCGGTCTGAACTCAGATCACG T-3′) (Palumbi *et al*., 1991; Schubert *et al.*, 2000b).

COI a (Forward) (5′-AGTATAAGCGTCTGGGTAGTC -3′);

COIf-L (Revers) (5′-CCTGCAGGAGGAGGAGGAGAYCC -3′) (Palumbi *et al*., 1991; Lai *et al*., 2010).

Polymerase chain reactions (PCR) condition was used according to Mantelatto *et al*., (2009), Schubert *et al*., (2006), Fratini *et al*., (2005) and Lai *et al*., (2010). In detail each PCR reaction was performed in 50 µL volumes containing 15 µL of DNA template (150 ng), 25 µL Go Taq Green Master Mix 2X (Promega, Madison, WI, USA), and 5 µL each of forward and reverse primers (10 pmol/ µL). PCR amplification performed in an Applied Biosystems 2720 thermal cycler. The thermal cycling program was different for both gene amplification as for the 16S rRNA amplification the following steps performed; initial denaturation cycle for 10 min. at 95°C, 40 cycles of 1 min. at 95°C, 1 min. at 46°C and 2 min. at

72°C and 72°C for 10 min for final extension. Whereas, for amplification of COI the following steps performed during in thermal cycling profile, initial denaturation cycle for 2 min at 94°C, followed by 40 cycles of 1 min at 94°C, 1 min at 50°C and 1.5 min at 72°C, with the final extension of 72°C for 10 min.

Sequencing of COI mt-DNA

All PCR products were checked for confirmation and estimation of the base pair of the amplified products amplification through the agarose gel electrophoresis. The each 5µLof PCR products were checked through 1% of agarose gel by horizontal gel electrophoresis in 80 volts of electric current and also compare with a Gene Ruler 100 bp Plus DNA ladder (Promega, Madison, USA # SM0321). PCR products purified and sequenced from Macrogen Company, (Korea).

Data analysis

The DNA sequence data analyze through Applied Biosystems sequence Scanner v1.0 software. 2.2.4. Species identification and confirmation based on 97-100% homology, assigned to the similar sequence of the same species associated through the best hit (up to high bit score) NCBI, BLASTn 2.2.26 (Zhang *et al*., 2000). Furthermore, the procured coding sequences submitted to the NCBI nucleotide-sequence databases through Barcode submission tools.

Before submission, the procured Cytochrome oxidase (COI) mitochondrial DNA sequences cleaned through CDS annotation than translated into protein. Open Reading Frame finder (ORF finder) used (NCBI link) to translate DNA sequences into protein and as well as for the trimming of a nucleotide sequence. The most suitable open reading frames (ORFs) were selected with its protein translation and nucleotide sequences and also verify the predicted protein by using the Smart Blast and regular Blast P.

Intraspecific genetic variability estimated through 16S and COI gene sequences in *S. serrata*. Sequences (16S rRNA and COI) were analyzed to evaluate the evolutionary model (nucleotide and amino acid substitutions). The later evolutionary divergence analyses were estimated based on the selected evolutionary model. For the overall population, software DNA SP version 5.10 (Librado and Rozas, 2009) was employed to calculate haplotype diversity (hd) and nucleotide diversity (piπ) according to Nei (1987). Neutrality tests Ewens-Watterson (Ewens, 1972;

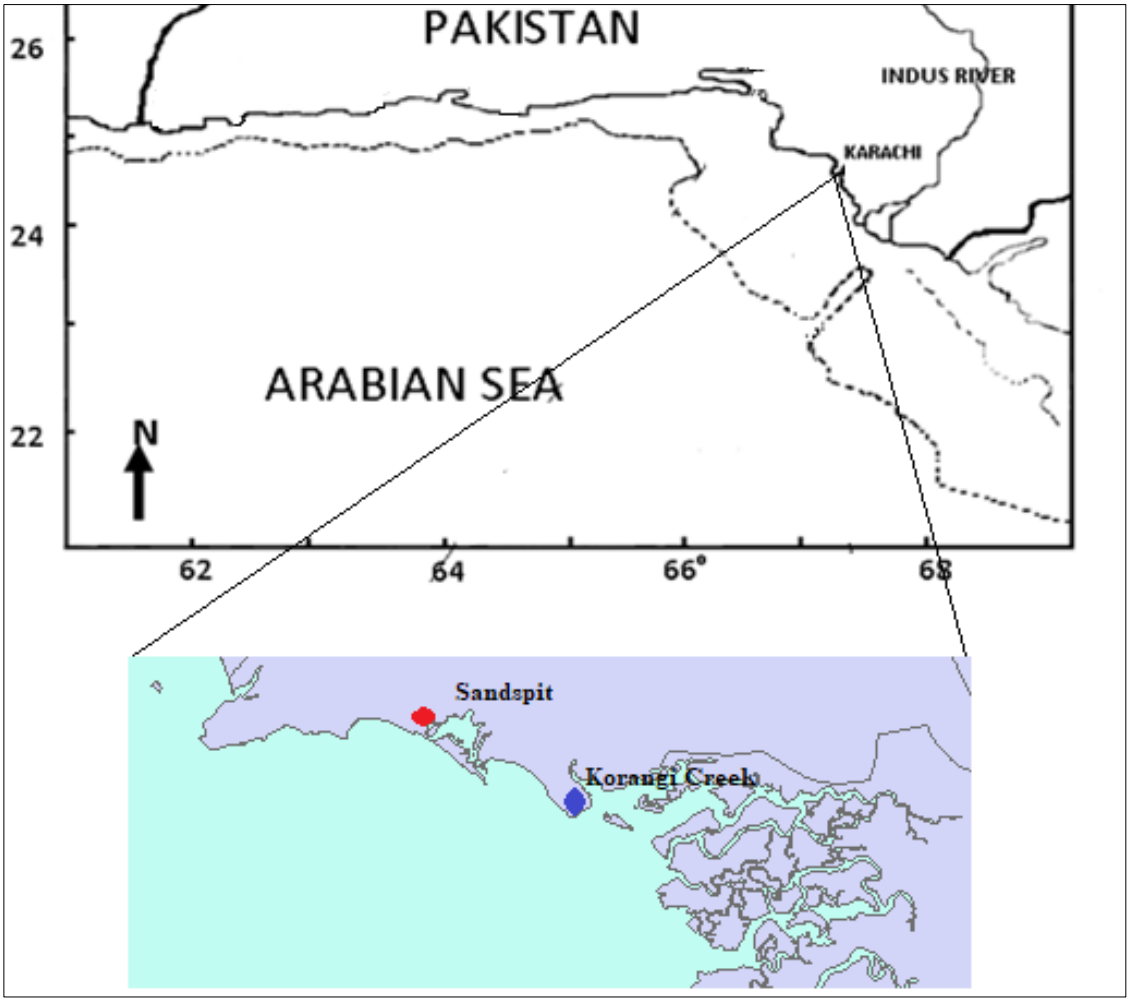

Figure 1. Map of the stud area from the coastal waters of Pakistan northern Arabia Sea

Watterson, 1978; Chakraborty, 1990) including Tajima D (Tajima, 1989), and Fu's Fs (Fu, 1997) performed. Arlequin v. 3.1 (Excoffier *et al*., 2005) used for the AMOVA, mismatch distribution, Tajima's D, and Fu's Fs test of neutrality.

Furthermore, the new procured mitochondrial COI gene data (n=9) along with data (n=54) from 24 locations (Table 1) in the IWP obtained from Genbank as described by He *et al*., (2011) included in the current study for the phylogenetic analysis. All sequences were ATG transformed aligned in CLUSTAL W (Thompson *et al*., 1997). The Maximum Likelihood evolutionary model (T92+G) were determined using MEGA 7. A median-joining (MJ) network (Bandelt *et al*., 1999) of phylogenetic relationships among haplotypes was used to clarify the evolutionary relationship of *S. serrata* constructed by using software Network version 4.51 (version 4.5.1.0; Fluxus engineering, 2008) (Polzin and Daneshmand, 2003).

All currently available molecular data to infer the genetic structure, genetic relatedness and evolutionary history of *S. serrata* in the Indo-West Pacific region by using nucleotide diversity tests, network analysis, mismatch analysis and AMOVA.

Results

General findings

The crabs (n= 16) were collected from two populations Sandspit back water areas (n=8) and Korangi mangrove creek areas (n=8) by direct hand pick or purchase fresh catch from local fisherman. After amplification, 16S and COI gene exhibit a band presence of UV light, whereas 100 base pair plus DNA ladder Gene Ruler used for the comparison. Sequence similarity searched by using the Basic Local Alignment Search Tool (BLAST), species homology based on at least a 99-100% homology. DNA sequences submitted to GenBank and the accession number received for each isolate (Table 2). The BLAST search showed high sequence similarity (97-100%) to *S. serrata* sequences in GenBank, indicated that misidentification of species does not occur in this study.

Table 1. Sample size, Clade, haplotypes and GenBank accession numbers in geographic distribution range of *Scylla serrata*

Sequence No	Clade	Hap	Accession no	Sequence No	Clade	Hap	Accession no	Sequence No	Hap	Clade	Accession no
1	IC	1	AF097003	24		20	AY373349	47	32	II	AF279318
2		2	AF097007	25		19	AY373350	48	33		AF279323
3		3	AY373342	26		21	AF097016	49	34		AF279330
4		4	AY373344	27		21	AF097016	50	26		AF279312
5		3	AF097002	28		21	AF097018	51	35		AF279311
6		3	AF097006	29		21	AY373346	52	36		AF279329
7		5	AF097005	30		21	AF097017	53	37		AF279324
8		3	AF097004	31		21	AF097016	54	38		AF279311
9		6	AY373343	32		22	AF097019	55	39	III	KY290374.1
10		7	AY373345	33		23	AY373348	56	40		KY290376.1
11		8	AF203943	34		17	AF279321	57	41		KY290378.1
12		9	AF097009	35	IA	24	AF279315	58	42		KY290381.1
13		10	AF097010	36		25	AF279322	59	43		KY428865.1
14		10	AF097008	37		26	AF279331	60	40		KY587766.1
15		11	AF203946	38		27	AF279326	61	44		KY587767.1
16		12	AF097012	39		28	AF279327	62	45		KY587779.1
17		13	AF097011	40		26	AF097013	63	46		KY587393.1
18	IB	14	AF203945	41		29	AF279313				
19		15	AF203947	42		27	AF279326				
20		16	AF279321	43		30	AF279332				
21		17	AF279321	44		26	AF279310				
22	II	18	AF093715	45		26	AF279317				
23		19	AY373341	46		31	AF279328				

Table 2. GenBank accession numbers for 16S and COI sequence of *S. serrata* from the coastal waters of Pakistan

Portunid crabs	16S rRNA GenBank accession numbers	No	COI GenBank accession numbers	No
Subfamily: Portununie Rafinesque, 1815 Genus *Sylla* (De Hann, 1833) *Scylla serrata* (Forskal, 1775)	KU296942.1, KU296943.1, KY062994.1, KY062995.1, KY062996.1, KY062997.1, KY062998.1,	7	KY290374.1, KY290376.1, KY290378.1, KY290381.1, KY428865.1, KY587766.1, KY587767.1, KY587779.1, KY587393.1	9

Genetic diversity

16S and COI alignment consisted of 580bp and 697bp with exclusion of hyper variable regions, whereas the remaining 511bp of 16S and 426bp of COI used for the phylogenetic analysis. Likelihood ratio test revealed the selected optimum model (The T92+G Tamura 3-parameter + Gamma distribution) under the Akaike information criterion (AIC). However, the models with the lowest BIC scores were considered to describe the DNA substitution pattern the best as implemented in MEGA 7 (Kumar *et al*., 2016).

The number of haplotypes and their diversity for 16S and COI estimated for the assessment of genetic diversity and differentiation within two different populations' of Sandspit back water areas and Korangi mangrove creek area from the coastal waters of Pakistan. In 16S rRNA four haplotypes (2 from Korangi and 2 from Sandspit) determined out of 7, haplotype diversity (hd) (0.810 P<= 0.0168), whereas the nucleotide diversity (0.01 P<=0.000) (Table 3). In COI five haplotypes (3 from Korangi and 2 from Sandspit) were determined (hd 0.873, P<=0.003) from 9 sequences, whereas the nucleotide diversity was (0.007 P<=0.000). The maximum numbers of haplotype description likely due to the selection of morphological difference individual (morphotype) from each population.

Neutrality and Mismatch Analysis

Tajima's D (Neutrality test) was estimated for COI in *S. serrate* D= -1.14965 (P>0.10) whereas Fu's Fs -1.09284 (P > 0.10). In addition, neutrality test was also performed for the 16S rRNA gene (D= -1.53047) and was non-significant P > 0.10, whereas Fu's Fs 1.7354 (Table 4). The number of base substitutions per site from an average of overall sequence pairs, within *S. serrata* was also estimated. The estimated inter population distance within *S. serrata* was (0.001 ± 0.001) for 16S and (0.006 ± 0.002) for COI. Mismatch analysis showed the bimodal pattern in two selected regions of Northern Indian Ocean bounded on the north by Pakistan and Iran, on the west by the Gulf of Aden, Guardafui Channel and the Arabian Peninsula, on the southeast by the Laccadive Sea, on the southwest by the Somali Sea, on the east by India and relate inadequately with their similar distribution this recommend the population underwent population expansion in Northern Indian Ocean Northern Arabian Sea (Figure 2).

Phylogeography of *S. serrata*

Cytochrome oxidase (COI) sequence of *S. serrata* from Northern Arabian Sea (present study) along with sequences from four geographic regions according to He

Table 3. Summary 16S rRNA and COI sequences, sites, Haplotypes (P) and Haplotype diversity (Hd) at significance level (P<=0.000); nucleotide diversity (π) at significance level Theta per site (P<=0.000) by using Dna SP V5 of 16S rRNA and COI

	Sequence	Sites	Haplotype	hd	hd (P <=0.000)	π	Pi (P<=0.000)
16S rRNA	7	511	4	0.810	0.01686	0.01044	0.000***
COI	9	426	5	0.873	0.00352	0.00743	0.000***

Table 4. Tajima's *D* and Fu's *F* Test for COI and 16S rRNA mitochondrial DNA from the coastal waters of Pakistan

Neutrality test	COI			16S		
	Tajima's D	Fu's Lis D	Fu's Lis F	Tajima's D	Fu's Lis D	Fu's Lis F
S. serrata	-1.14695	-1.09284	-1.23922	-1.53047	-1.58858	-1.73574

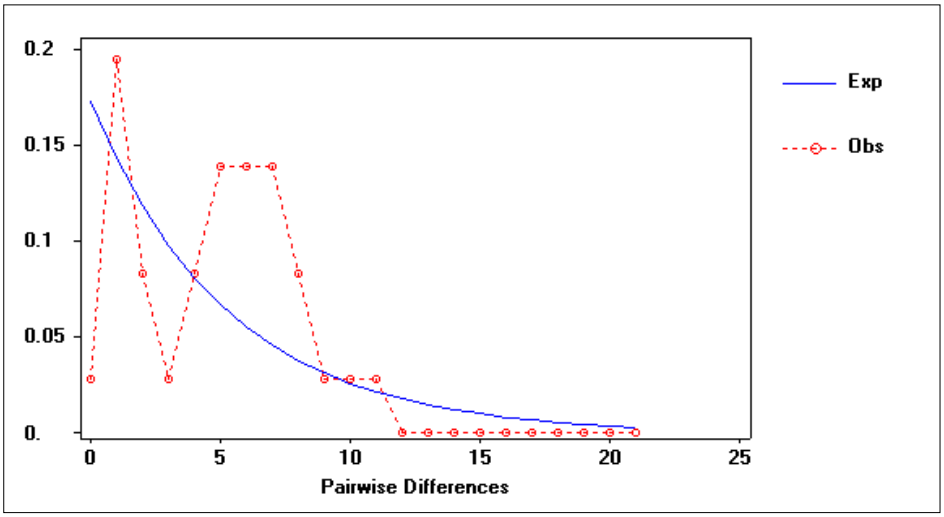

Figure 2. Bimodal pattern of mismatch analysis of *S. serrata* in coastal waters of Pakistan Northern Arabian Sea.

et al., (2011): West Indian Ocean (IA), Red Sea-South China Sea (IB) West Pacific (IC) Northwest Australia (II) archived in molecular databases from the Indo Pacific region used for phylogentic analyses. Total 46 different haplotypes identified from 63 sequenced (Table 1) 99 variable sites, 84 informative sites, no insertions or deletions found. Based on the Akaike informative criterion (AIC), the best evolutionary model, the T92 +G model selected proportion of invariable sites; base frequencies, A = 0.333, C = 0.1665, G = 0.1655, T = 0.333; (AC) = 0.01, (AG) =0.15, (AT) = 0.02, (CG) = 0.01, (CT) = 0.3, (GT) = 0.02. The sequence alignment of major haplotypes for *S. serrata* was shown in (Figure 3). The three clusters had a disjunct distribution corresponding to five geographic groups: West Indian Ocean (IA), Red Sea-South China Sea (IB) and West Pacific (IC), Northwest Australia (II), and the Northern Indian Ocean Northern Arabian Sea (III) (Figure 4). Tajima's D negative and non-significant deviation found in mutation-drift equilibrium except Western Indian Ocean (IA) (D = -2.07591, P>0.05), whereas Fu's F's shows similar observation (F's= -12.41699, P>0.05) exception of Western Indian Ocean (IA), West Pacific (IC) and Northern Arabian Sea (III) (Table 5). The AMOVA (molecular variance analysis) intimated that phylogeographic basis for the regional partitioning of genetic structure (FST = 90.193%, p = 0.000) found in five geographic groups (Tamura and Nei distance method), within group 90.19% genetic variance, whereas among populations within groups variance 9.81% (Table 6).

Discussion

Genetic Diversity and Divergence

The current study reveals the information of genetic diversity of *S. serrata* from the coastal waters of Pakistan. It has concluded through the genetic analyses in combination with morphological characters that, the mud crab *S. serrata* (Forskål, 1775) is a complex of four

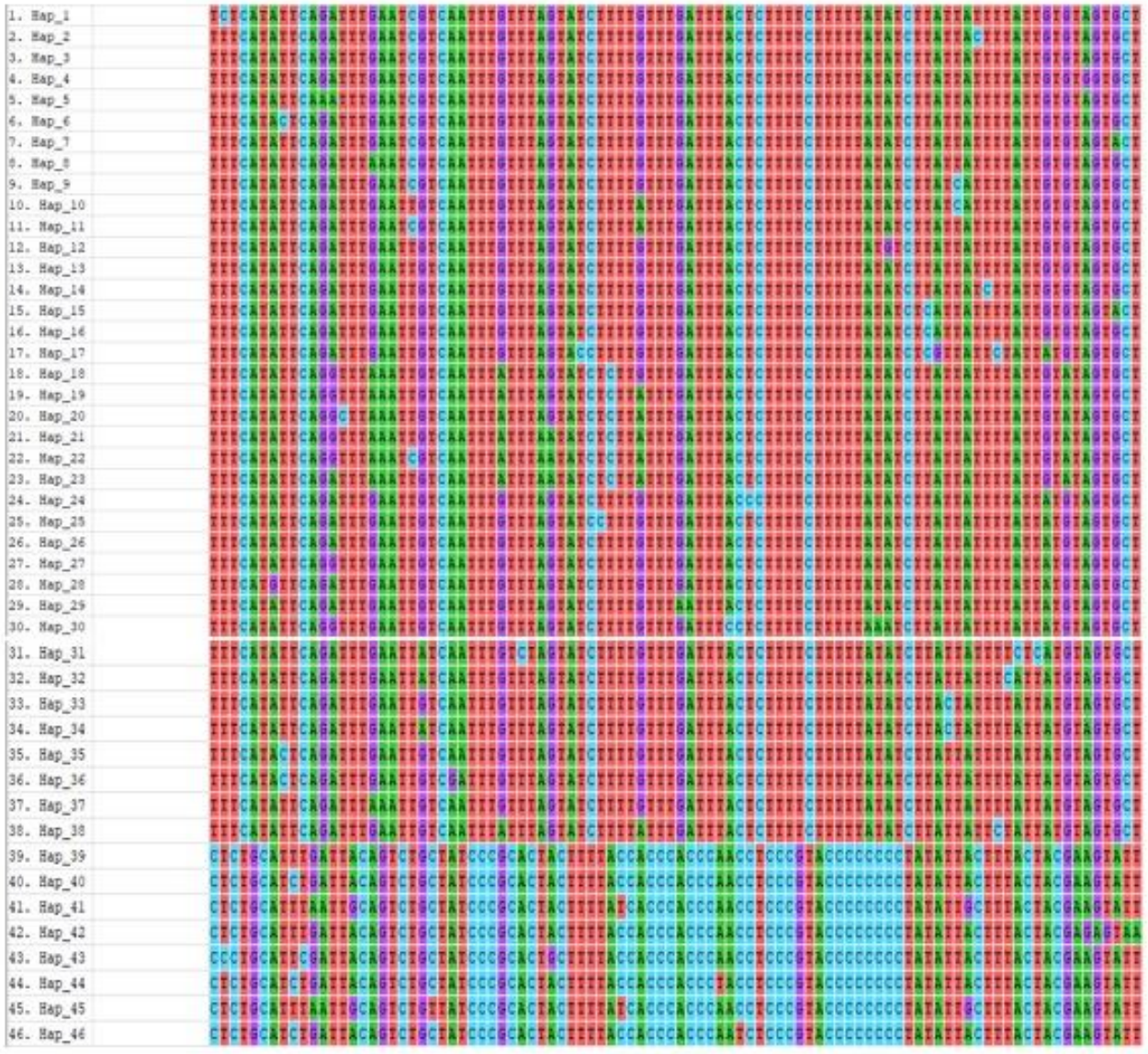

Figure 3. The sequence alignment of major haplotypes for *Scylla serrata.*

species *S. olivacea*, *S. paramamosain*, *S. serrata*, *S. tranquebarica* (Keenan *et al*., 1998). The various species of *Scylla* already confirmed from different areas as *S. serrata* and *S. tranquebarica* through RFLP by Shekhar *et al*., (2005) and S*. serrata, S. oceanica* and *S. tranquebarica* through RAPD analysis by Klinbunga *et al*., (2000) and observed dissimilarity in genotypes among these three in eastern Thailand. Similarly, a single species of *S. serrata* reported from the coastal waters of Pakistan but now one more species *S. olivacea* conformed and included as the new report (not included in this study). The confirmation of *S. olivacea* indicated the range extension and disperse pattern of the species in the region. The maximum number of the haplotypes in the present study, anticipated the presence of various morphs, these differences assume the morphological variation due to their ecological and environmental responses. According to Bucklin *et al*., (1997); Fratini *et al*., (2002) and Lai *et al*., (2010) Portunid crab shown moderately high haplotype diversity and relatively low sequence divergence (approximately less than 0.5%) as this trend exhibit the similarity to other marine organisms including crustacean with planktonic larvae (Gopurenko *et al*., 1999; Fratini *et al*., 2002). According to Zhou *et al*., (2016) genetic diversity of Sesarmid crab showed the moderate level of haplotype diversity (0.338 to 0.731) and a low level of nucleotide diversity (0.00058 to 0.00278). Klinbunga *et al*., (2000) estimated genetic diversity between *S. serrata, S. tranqubarica* and *S. oceanica* and large genetic differences between species were found (D (ij) = 0.425 to 0.751), whereas those between populations within each species were much lower (D (ij) = 0.171 to 0.199) and revealed the moderate genetic exchange between sympatric and different species rather than a single Panimitic species as exhibit different morph in eastern Thailand.

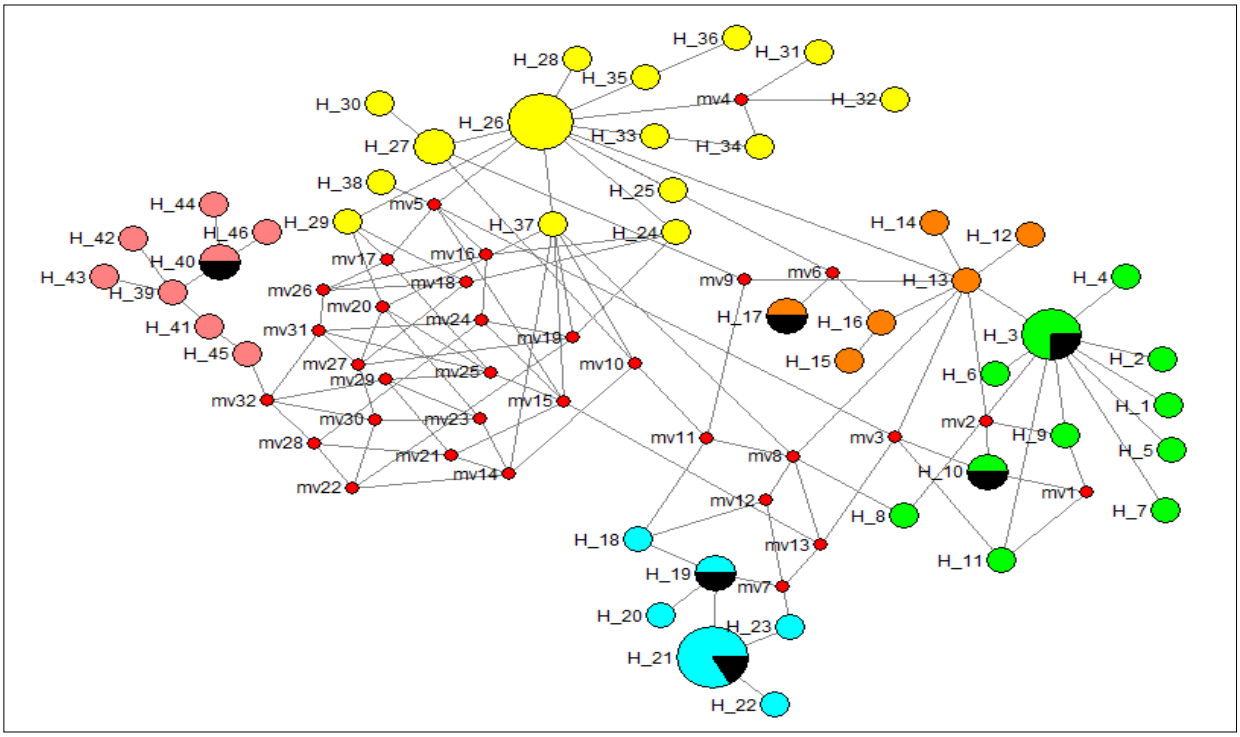

Figure 4. The phylogenetic relationship of haplotypes for *Scylla serrata* (clades I, II and III): MJ network and steps of over two substitutions between the haplotypes for MJ network. The distributional regions of haplotypes in networks are distinguished using different colors with dark purple for Northwest Australia, yellow from Arabian Sea, cyan for West Pacific, pink for Red Sea-South China Sea, and green for West Indian Ocean.

Table 5. Tajima's *D* and Fu's *F,* SSD and Raggedness Index for COI by geographic groups of *Scylla serrata*

	1A	1B	1C	11	111
Haplotype	20	7	15	12	9
Tajima's D	-2.07591	-0.10944	-1.49598	-1.12253	-1.14965
P-value	0.002*	0.55300	0.064	0.188	0.139
Fu's FS test	-12.41699	-1.99294	-8.55732	-2.89747	-1.09284
P-value	0.000*	0.056	0.000*	0.006*	0.044
SSD	0.00341	0.03036	0.03431	0.00909	0.02467
P-value	0.6200	0.600	0.1300	0.6300	0.6900
Raggedness Index	0.05620	0.07483	0.19166	0.10468	0.05633
P- value	0.4100	0.88	0.0600	0.5600	0.7300

The ability to the identification of these species has numerous applications like distribution range of larvae and environmental parameters that affect the survival and growth of juveniles and hybridizing breeding studies, phylogenetic relationships and genetic identity of *Scylla* species from the coastal waters of Pakistan. The minimum nucleotide diversity inherent character of crustacean (Shubart *et al*., 2006) whereas haplotype diversity of S*. serrata* in tropical Africa similar to the other Indo-Pacific marine species with the planktonic larval stage (Brasher *et al*., 1992; Lavery *et al*., 1996; Palumbi *et al*., 1997; Williams and Benzie, 1997, 1998). Genus *Scylla* has shown the highest divergence rate (0.102 ± 0.009) within species. Ma *et al*., (2012) observed interspecific distances higher than 0.02. Viswanathan *et al*., (2012) perform the analysis of mitochondrial COI in *S. olivacea* and the observed genetic distance 0.093 between four species of Genus *Scylla*. According to Stephenson (1968b), previous taxonomic reviews considered these variations as meagre geographic variants, whereas (Stephenson 1972a; Fratini Vannini, (2002); Ragionieri *et al*., 2009) measured that this regional inconsistency may be the consequence of speciation processes suggested that the evolutionary history of speciation across the Indo-West Pacific region remain complex for genus *Scylla*according to earlier consideration. The further detailed genetic studies helpful to resolve the genetic diversity and speciation of species in future.

Demography of *Scylla serrata*

Climatic and geological changes in the environment on large scales play a significant role in shaping the rates and patterns of diversification (Oaks, 2014) and also influence the evolutionary history of whole communities of co-distributed species and segregate groups or populations of an organism and cause a temporal cluster of speciation. Genetic bottlenecks (colonization events) followed by demographic expansions strengthen the contribution to genetic diversity and our results of Tajima's D test and Fu's Fs-test indicate that *S. serrata* might have undergone a rapid demographic growth. According to Lavery *et al*., (1996) *Scylla serrata* underwent a rapid demographic growth in the recent past. Traces of prehistoric demographic expansion observed in species experiencing population turn down in the present and the population structuring indicates reduced gene flow between geographically secure sites, in spite of the elevated potential for *S. serrata* dispersal.

Phylogeography of the *Scylla serrata*

The demography of the *Scylla serrata* revealed that the dispersal of common species throughout the Indo West Pacific (IWP) like; in the coastal areas of East Africa, India, the Indo-Malaysia archipelago, various islands and Australia. The previously phylogeographic pattern of *S. serrata* have been studied based on the coding mitochondrial DNA cytochrome oxidase subunit I gene (COI) (Gopurenko *et al*. 1999; Fratini and Vannini 2002; Gopurenko and Hughes 2002; He *at al*., 2011). According to species range description the Gopurenko *et al*., (1999) defined *S. serrata* population into two distinct clades, clade I, distributed across the Indo Pacific region, whereas clade II, confined to Northern Australia. A particular expansion event from a Western Pacific origin the population of *S. serrata*, colonized in the Indian Ocean during the last Pleistocene period and that infer existing gene flow between populations interrupted by a unique haplotype. Fratini *et al*., (2002) also describes *S. serrata* within the Indian Ocean and revealed that a significant genetic discrimination and low level of gene flow between geographically lock sites, although the elevated prospective for dispersion. Similarly, He, *et al*., (2011) studied the Phylogeography of the mud crab (*Scylla serrata*) in the Indo-West Pacific and concluded that *S. serrata* distributed in two major clades: clade one distributed widely across the entire IWP, whereas the other clade (Clade II) is confined to Western and Northern Australia and reveled the phylogeographic structure of *Scylla serrate* related to four subpopulations: Northwest Australia, West Indian Ocean, Red Sea-South China Sea and West Pacific. In the current study, the neutrality test (to estimate of unique mutations, as evidence of recent population expansion) showed non-significant results except West Indian Ocean (IA). Whereas Fu's F's shown significant variation in West Pacific (IC) Northern Indian Ocean Northern Arabian Sea (III), Mismatch analysis showed a bimodal distribution in Northern Indian Ocean Northern Arabian Sea, therefore consistent distribution with allopatric divergence followed by population growth. The negative distribution indicated a slight population expansion. According to Liao *et al*., (2010) and Rosly *et al*., (2013) negative non-significant value induced by the population extension and restricted to sampling sites of the crab population. In the present study different haplotypes obtained as depicted by He *et al*., (2011) from the gene bank and revealed the existence of four subpopulations: Northwest Australia, West Indian Ocean, Red Sea-South China Sea and West Pacific in

Table 6. AMOVA using the Tamura and Nei distance method from five subpopulations for *Scylla serrata*

Source of Variation	df	Sum of squares	Variance components	Percentage of variance
Among populations	4	557.126	11.33175	90.19
Within population	58	71.461	1.23209	9.81
Total	62	628.587	12.56384	
Fixation Index (FST)		0.90193		

addition sequences form the coastal waters of Pakistan including for the analysis and evaluate the phylogeographic pattern of *S. serrata.* The similar pattern described by He *et al*., (2011), clade one the IWP, Clade II Western and Northern Australia, an additional clade confined to the Pakistan northern Arabian Sea and caused by the allopatric speciation (geographic speciation, vicariant speciation). The similar speciation observed in lobster subspecies *P. homarus megasculptus* and *P. homarusrubellus* attributed to the weaker glacial surface circulation due to the summer south-west monsoon wind in the northwest Indian Ocean (Somali ⁄ Arabian basin). The Agulhas current around the southeast coast of South Africa and tip of South Madagascar weaker oceanic circulation as compared to present (Interglacial) time. Laccadive-Chagos Ridge to the Southwest of the Indian continent would result in increases retention of larvae within the Northern Arabian Sea thereby promoting speciation (Pollock, 1993). This study provides insight towards the preliminary understanding of the different genetic process that regulates community assemblage and leads towards a study of evolutionary biology.

Acknowledgements

This work is supported by a Higher Education Commission (HEC) of Pakistan, through grant No EC No: 20-1673/R and D/10 to NUS which is gratefully acknowledged.

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