Available online at: www.mbai.org.in doi:10.6024/jmbai.2022.64.1.2295-01

Population genetics of Indian white shrimp, *Penaeus indicus* (H. Milne Edwards, 1837) from Indian waters

J. Raymond Jani Angel*, K. K. Vinaya, T. N. Vinay, M. S. Shekhar, A. Panigrahi, C. P. Balasubramanian, G. Gopikrishna and K. K. Vijayan
ICAR-Central Institute of Brackishwater Aquaculture, Chennai-600 028, Tamil Nadu, India.

*Correspondence e-mail: raymond.angel@icar.gov.in

Received: 23 July 2021 Accepted: 08 Mar 2022 Published: 30 Mar 2022

Original Article

Abstract

The Indian white shrimp, *Penaeus indicus* (H. Milne Edwards, 1837), is a commercially important marine crustacean species, widely distributed along the Indo-Pacific region. The population genetic structure and genetic diversity of *P. indicus* were studied using the mitochondrial 16S rRNA gene. A total of 121 individuals representing seven populations from distant parts of India were used for the study. Results indicated that there were seventeen haplotypes and the mean haplotype diversity (Hd) was relatively moderate when there was a low level of nucleotide diversity (π). AMOVA analysis indicated that variation within populations contributed to 69.32% of the total variation. All the populations, except Kanyakumari, showed high polymorphism and haplotype diversity, indicating genetically varied stocks in the wild. The pair-wise F_{ST} value ranged from as low as -0.03587 between Kanyakumari and Kakinada to as high as 0.68650 between Kakdwip and Kakinada populations with an overall F_{ST} value of 0.30684 ($p<0.05$). The pattern of the haplotype network and phylogenetic tree revealed two major groups. The Tajima's D statistic showed the presence of a recent expansion of the population. Our study exhibits a polyphyletic relationship in *P. indicus* between Kakdwip (West Bengal) and other populations and the high haplotype diversity and genetic divergence of Indian white shrimp can be utilized for selective breeding programs.

Keywords*: Indian white shrimp, mt DNA, population genetics, genetic diversity*

Introduction

The penaeid shrimps (Super family: Penaeoidea and Family: Penaeidae) comprise of an ecologically diverse group of marine decapods inhabiting estuarine and marine environments throughout the world oceans (Anne and Theresa, 2004; de Croos and Pálsson, 2010) with about 17 extant genera and about 200 extant species (Ma *et al*., 2011). Penaeid shrimps are one of the economically most important groups among the decapod crustaceans (Chan, 1998). Four species, including *Penaeus vannamei, Penaeus monodon, Penaeus indicus* and *Penaeus japonicus* are widely used in shrimp aquaculture across the world and the global trade of shrimp and prawns is estimated at US$ 28 billion annually (Globefish, 2015). High diversity of penaeid shrimps is found in the Indo-West Pacific region (Chan *et al*., 2008), which includes the Indian peninsula.

The shrimp fauna of the Indian subcontinent, including the Andaman and Nicobar and the Lakshadweep archipelagos accounts for about 437 species. The family Penaeidae with 73 species is the most speciose group among the penaeoids, and many species form commercially important fisheries that are of interest to aquaculture (Radhakrishnan *et al*., 2012). Indian brackishwater aquaculture sector was dominated by a single species, the giant tiger shrimp, *P. monodon* until the outbreak of the disease caused by white spot syndrome virus (WSSV) during the early 1990s, causing substantial loss to the shrimp farming industry (Kumar *et al*., 2007). The introduction of SPF *P. vannamei* in 2009 has substantially changed the shrimp culture scenario in India. The export of frozen shrimp reached 1.29 million tons in 2019-20 fetching export earnings of about Rs. 46663 crores (US$ 6679 million) (MPEDA, 2020). Shrimp production in India presently is 90% dependent upon a single species, the Pacific White leg Shrimp, *P. vannamei*. Rapid expansion along with intensification without the consideration of carrying capacity brings with it disease outbreaks. Disease incidences in *P. vannamei* have already been reported in many parts of India (Rajendran *et al*., 2016; Remany *et al*., 2018). Against this backdrop, the native Indian White Shrimp, *P. indicus*, which has been used in the Indian farming sector since the 1980s, is a

natural choice for carrying out a selective breeding programme for improved growth and other better traits.

The Indian white shrimp, *P. indicus* is one of the commercially important cultured shrimp in India, considered along with *P. vannamei* and *P. monodon*. Identification of existing stocks and comparative growth performance evaluation of these stocks under commercial conditions are the first crucial steps towards domestication and selective breeding (Benzie, 1994; Kumar *et al.*, 2007). For effective management of fisheries and sustainable harvesting of fishery populations, it is essential to assess the population genetic structure and diversity (Thorrold *et al.*, 2002; de Croos and Pálsson, 2010). An assessment of intraspecific genetic diversity and population genetic structure augments the existing information for taxonomic and evolutionary history studies (Benzie *et al.*, 1995) and culture of candidate species and their conservation (Valles-Jimenez, 2006; de Croos and Pálsson, 2010). Given the stable maternal inheritance, lack of recombination, and rapid evolutionary rate, mitochondrial DNA (mtDNA) markers have been widely used in studies of intraspecific population genetics (Tzeng *et al.*, 2004; De Bruyn *et al.*, 2005; Tsoi *et al.*, 2007; Alam *et al.*, 2014; Yang and Li, 2017; Sharma *et al.*, 2018). The mitochondrial genes are highly conserved (Singh *et al.*, 2015) and shows a high rate of evolution and is used to distinguish between haplotypes even in a small sample size (Sharma *et al.*, 2018). The 16 S ribosomal RNA (16 S rRNA), which is a component of the 30 S small subunit of the vertebrate mitochondrial ribosome, is reported to be highly conserved in fish (Naock *et al.*, 1996) and is used to analyse the inter and intra-specific divergence in fish (Singh *et al.*, 2015). Among the various mtDNA markers, 16S rRNA exhibits a low rate of evolution (Calo-mata *et al.*, 2009) due to a slower mutation rate in the conserved region (Jahromi *et al.*, 2018). The mitochondrial 16S rRNA gene is considered to be well suited for population genetic studies and species delineation methods (Hellberg, 2009; Baeza and Fuentes, 2013; Baeza and Prakash, 2019). To characterise the different stocks of *P. indicus* in Indian waters, the present investigation focussed on the genetic diversity, population structure, and phylogenetics of *P. indicus* from India using sequence analysis of the mitochondrial DNA 16S rRNA gene.

Material and methods

Sample collection

Specimens of *P. indicus*, for the study, were collected from 7 distant locations in the Indian peninsula: Mangalore (Karnataka State), n=17; Quilon (Kerala State), n=17; Kanyakumari (Tamil Nadu State), n=11; Chennai (Tamil Nadu State), n=23; Kakinada (Andhra Pradesh State), n=22; Puri (Orissa State), n=19; and Kakdwip (West Bengal State), n=12, through local fish markets as well as landing centres. The sampling sites were selected as per the State landing data available (CMFRI, 2019). The species were morphologically identified following published literature (Pérez Farfante and Kensley 1997; Chan, 1998). Muscle tissue from pleopods was collected and preserved in 95% ethanol for DNA extraction until further studies. The details of sample collection sites of *P. indicus* are given in Table 1.

DNA extraction and PCR amplification

Total genomic DNA was extracted from each sample using 300 μl TEN buffer (10 mM Tris-HCl, pH 8.0, 1 M NaCl, 1 mM EDTA), 15 μl of 1% sodium dodecyl sulfate (SDS) and 6 μl of proteinase K (50 μg/μl final concentration). The mixture was digested overnight at 37°C. The DNA was extracted with an equal volume of phenol-chloroform-isoamyl alcohol and precipitated with isopropanol. The DNA pellet was suspended in TE buffer and preserved at -20°C.

A fragment of mitochondrial 16S rRNA gene was amplified from total genomic DNA extract by the polymerase chain reaction (PCR), using universal primers for mtDNA 16SrRNA region; 16Sar and 16Sbr (Palumbi *et al.*, 1994). The PCR amplification condition included; initial denaturation at 94°C for 5 min, 30 cycles consisting of denaturation at 94°C for 30 s, annealing at 50°C for 45 s and extension at 72°C for 45 s and a final extension at 72°C for 7 min.

Table 1. The sampling locations and NCBI accession numbers of Indian White Shrimp (*P. indicus*) stocks

State	Collection site	Sample code	Coordinates	Sequence
Karnataka	Mangalore	MANG	12°51'46.4"N 74°50'13.0"E	MH094333- MH094349
Kerala	Quilon	QR	8°56'12.8"N 76°32'43.2"E	MH094369- MH094387
Tamil Nadu	Kanyakumari	KR	8°04'53.9"N 77°33'05.4"E	MH094398- MH094408
Tamil Nadu	Chennai	CH	13°07'24.0"N 80°17'50.8"E	MH094310- MH094332
Andhra Pradesh	Kakinada	KAKI	16°56'10.3"N 82°14'05.5"E	MH094288- MH094309
Odisha	Puri	PR	19°48'48.3"N 85°49'53.1"E	MH094350- MH094368
West Bengal	Kakdwip	WR	21°51'52.9"N 88°10'26.5"E	MH094386- MH094397

Alignment and molecular analysis of DNA sequence

The gene sequences were edited and aligned using BioEdit Sequence Alignment Editor Ver. 7.2. (Hall, 1999) and the sequences were analyzed using Arlequin ver. 3.5 (Excoffier and Schneider, 2005). The same program was used to determine the haplotype and nucleotide diversity, the genetic differentiation including the analysis of molecular variance (AMOVA), molecular diversity indices, and F_{ST} values. A median-joining network was also constructed using PopART ver. 1.7 (Bandelt *et al*., 1999). The phylogenetic relationship among individuals of different populations and different haplotypes based on the best-predicted model (HKY+G, bootstraps support-1000) were constructed by implementing the maximum likelihood tree method (MLM) in MEGA ver. X (Kumar *et al*., 2018).

Results

Sequence composition and variation

The amplified size of the mitochondrial 16S rRNA gene was 484bp. The 16s rRNA sequences amplified from 121 individuals of *P. indicus* have been submitted in GenBank under accession numbers MH094288 to MH094408 (Table 1). The average nucleotide composition of all samples were observed to be C = 12.66%; T = 34.24%; A = 33.48%; G = 19.62%.The transition to transversion ratio (R) was 14.99. In the present study, a total of 17 haplotypes were observed out of the total 121 samples analyzed, representing 7 populations. The dominant haplotype h 1 was shared by 96 sequences of six populations, except in the Kakdwip population. Chennai and Kakdwip stocks had the maximum number of haplotypes (5) followed by Mangalore (4), Quilon and Puri (3), Kakinada (2) and Kanyakumari (1). Sharing of haplotypes was observed among the groups, except in the case of Kakdwip stock (Table 2). The intrapopulation nucleotide diversity (π) within the seven stocks was observed to be high in Kakdwip (0.001659 $\pm$ 0.001445) and low in Kanyakumari (0) and the intraspecific haplotype diversity (h) varied from 0 (Kanyakumari) to 0.6667 $\pm$ 0.1409 (Kakdwip) (Table 3). The haplotype diversity of Chennai and Mangalore stocks was also found to be higher compared to other stocks. The maximum nucleotide and haplotype diversities were observed in Kakdwip stock, which also shows a maximum degree of polymorphism within its population with 5 haplotypes (Table 3).

Population structure

The phylogeographic inference was drawn by using a median-joining network (Fig. 1), which gives two major groups and one median vector with a total of 22 mutations. Kakdwip stock

Table 2. Relative haplotype frequencies and haplotype distribution in different stocks (values in parenthesis indicates the number of individuals falling in respective haplotype) for mt 16S rRNA gene in different stocks of *P. indicus* from India

Haplotype	Mangalore	Quilon	Kanyakumari	Chennai	Kakinada	Puri	Kakdwip
	n=17	n=17	n=11	n=23	n=22	n=19	n= 12
h1	0.824 (14)	0.882 (15)	1 (11)	0.7830 (18)	0.9550 (21)	0.8950 (17)	0
h2	0	0	0	0	0.0455 (1)	0	0
h3	0	0	0	0.0435 (1)	0	0	0
h4	0	0	0	0.0435 (1)	0	0	0
h5	0	0	0	0.0870 (2)	0	0	0
h6	0	0	0	0.0435 (1)	0	0	0
h7	0.0588 (1)	0	0	0	0	0.0526 (1)	0
h8	0.0588 (1)	0	0	0	0	0	0
h9	0.0588 (1)	0	0	0	0	0	0
h10	0	0.0588 (1)	0	0	0	0	0
h11	0	0.0588 (1)	0	0	0	0	0
h12	0	0	0	0	0	0.0526 (1)	0
h13	0	0	0	0	0	0	0.5830 (7)
h14	0	0	0	0	0	0	0.0833 (1)
h15	0	0	0	0	0	0	0.0833 (1)
h16	0	0	0	0	0	0	0.1670 (2)
h17	0	0	0	0	0	0	0.0833 (1)
Number of haplotype present	4	3	1	5	2	3	5

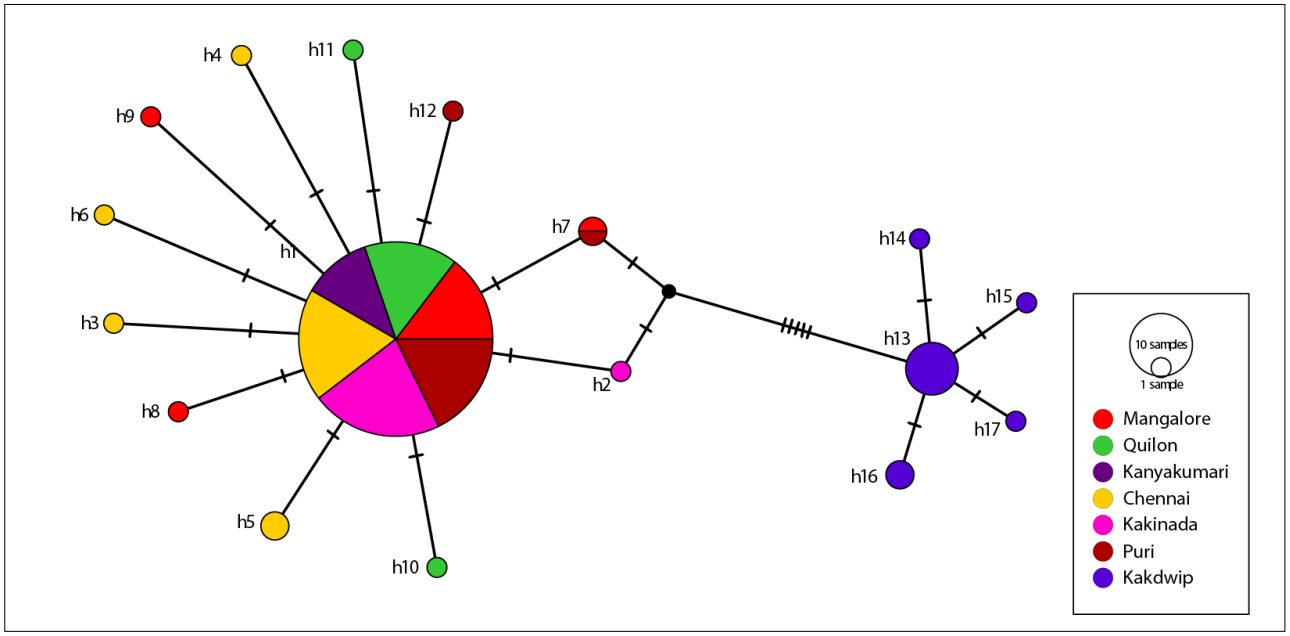

Fig. 1. The median-joining network of 17 haplotypes observed in Indian white shrimp (*P. indicus*) populations

Table 3. Intra-population nucleotide diversities (π), haplotype diversities (h), and Tajima's D for mt 16S rRNA region of *P. indicus*

Location	Nucleotide diversity	Haplotype diversity	Tajima's D
Mangalore	0.000729±0.000836	0.3309 +/- 0.1426	-1.70573
Quilon	0.000486±0.000661	0.2279 +/- 0.1295	-1.50358
Kanyakumari	0.000000±0.000000	0.0000 +/- 0.0000	0
Chennai	0.000882±0.000923	0.3913 +/- 0.1251	-1.67904
Kakinada	0.000188±0.000388	0.0909 +/- 0.0809	-1.16240
Puri	0.000435±0.000616	0.2047 +/- 0.1191	-1.51077
Kakdwip	0.001659±0.001445	0.6667 +/- 0.1409	-1.38479

was found to be monophyletic and all the remaining sites were observed to be paraphyletic containing samples from other populations. The dominant haplotype (h 1) was shared by 96 sequences of six populations except for Kakdwip. Haplotype h 13, which originated from median vector 1 separating all the six populations from the Kakdwip population, exhibits specific haplotypes (h 13, h 14, h 15, h 16 and h 17). It was noted that all the haplotypes were connected by a minimum of one mutational event. Also, the highest mutation appeared to exist between haplotypes h 13 (Kakdwip) and h 2 (Kakinada), which shows that genetic variation may be associated with geographical distance/ environment. The pair-wise F_{ST} matrix for 16s rRNA gene-based genetic relatedness among 7 stocks of *P. indicus* is presented in Fig. 2. The samples of Kakdwip show a very high degree of genetic differentiation with other stocks (>0.25). The F_{ST} values of Mangalore, Quilon, Kanyakumari, Chennai, Kakinada and Puri stocks were observed to be less than 0.05, which indicates that there exists very little genetic differentiation among these stocks. In the present study, 6

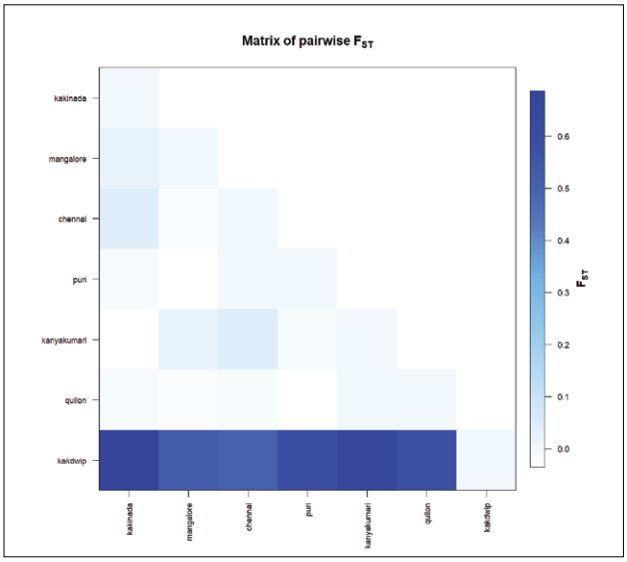

Fig. 2. Genetic differentiation (FST) among Indian white shrimp (*P. indicus*) populations

out of 49 pairwise comparisons showed very high genetic differentiation, whereas all the other comparisons revealed very low levels of genetic differentiation (<0.05).

The average number of pairwise differences between and within the stocks and Nei's standard genetic distance (d) are presented in Fig. 3. The average number of pairwise difference matrix also agrees with the pair-wise F_{st} matrix showing high levels (1.00000) of an average number of pairwise differences between Kakinada and all other populations. A low level (0.04545) of pairwise differences was observed between Kakinada and Kanyakumari stocks. The lower and higher values of an average number of pairwise differences within stocks were found within Kanyakumari (0.00000) and Kakdwip (0.66667), respectively. Nei's standard genetic distance is used for estimating gene differences per locus between populations (Katada *et al.*, 2004). The Nei's standard genetic distance between Kakdwip and Kanyakumari stocks (0.66667) were high, followed by Kakdwip and Kakinada (0.62121). The AMOVA of the 16s rRNA gene exhibited only 30.68% variation among the population, whereas the differentiation within the group is attributed to 69.32% (Table 4).

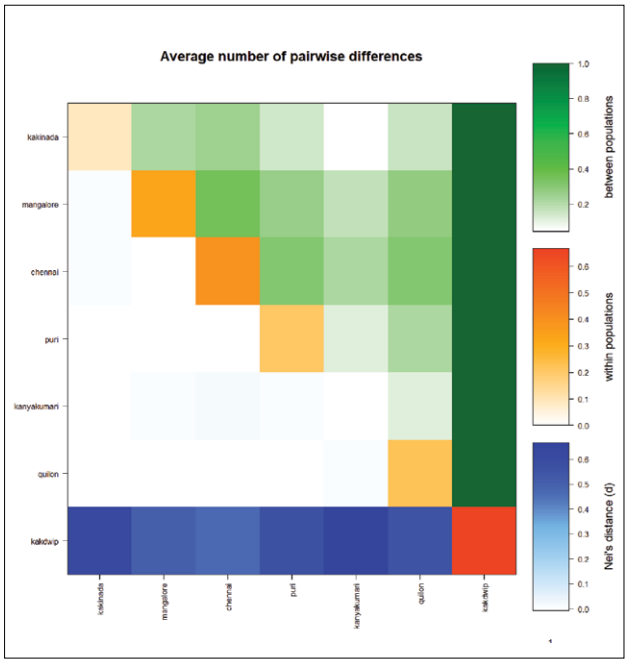

Fig. 3. Genetic distance (d) among Indian white shrimp *(P. indicus)* populations

Phylogenetic and evolutionary relationship

The Maximum likelihood method was used to infer the evolutionary history. Phylogenetic tree using mitochondrial 16s rRNA gene (Fig. 4) and their haplotypes (Fig. 5) showed

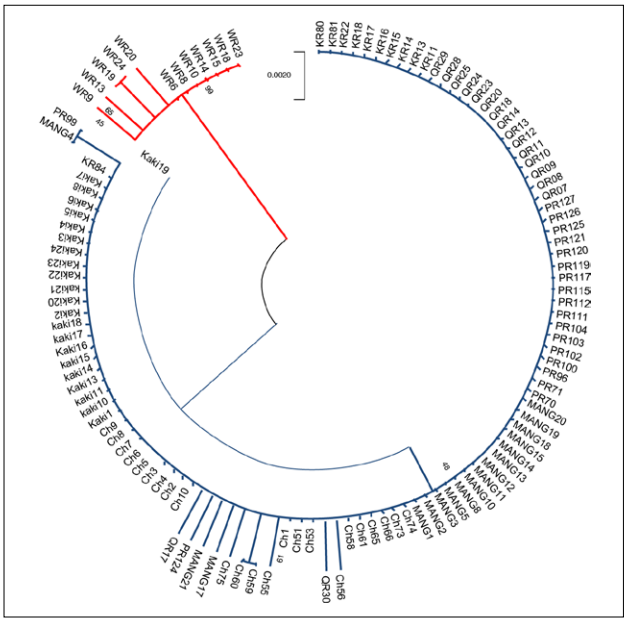

Fig. 4. Maximum likelihood tree of 16S rRNA gene of Indian white shrimp (*P. indicus*) showing two major groups with one paraphyletic intermediate group formed by Kaki19, PR99 and MANG4 (WR-Kakdwip; MANG-Mangalore; PR-Puri; KR- Kanyakumari; Ch-Chennai; Kaki-Kakinada; QR- Quilon)

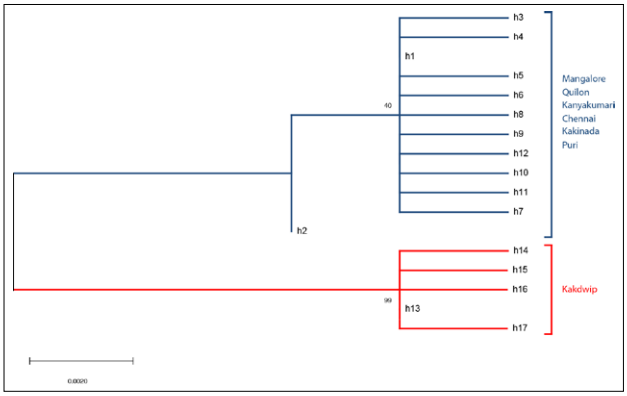

Fig. 5. Maximum likelihood tree of haplotypes of Indian white shrimp (*P. indicus*)

Table 4. AMOVA of mt 16S rRNA gene in *P. indicus*

Source of variation	d.f.	Mean sum of squares	Variance components	Percentage of variation
Among population	6	6.877	0.05917 Va	30.68
Within-population	114	15.238	0.13367 Vb	69.32
Total	120	22.116	0.19284	
Fixation index F_{ST}: 0.30684				

that the sequences were clustered into two major groups with one paraphyletic intermediate group formed by Kaki19, PR99 and MANG4. The Tajima's D, negative for all the stocks except Kanyakumari for which it is 0, indicates that *P. indicus* population across the Indian coasts had experienced population expansion, except in Kanyakumari.

Discussion

Owing to the ecological and morphological similarity of Penaeid shrimps, the application of molecular genetic techniques is imperative to study their population genetics and systematics (Shakil *et al.*, 2010; Mkare *et al.*, 2014; Mwakosya *et al.*, 2018). Marine organisms show low levels of genetic differentiation among geographical regions due to higher levels of dispersal and the absence of physical barriers (Palumbi, 1994; Hewitt, 2000; Yang and Li, 2017). However, among the *P. indicus* stocks studied along the coastal seas of the Indian subcontinent, Kakdwip stock showed high genetic divergence compared to those from other parts of the country, having no obvious oceanographic or geographic barriers. A similar pattern of divergence was also observed in the *P. indicus* population from Bangladesh (Alam *et al.*, 2014). The effects of ice ages on organisms varied with latitude and topography (Hewitt, 2004; Alam *et al.*, 2014) reported that the genetic diversity may have been more affected by climatic and glacial fluctuations in places near the Himalayan Mountains. During the cold periods of the last ice ages between 40 and 80 kyr ago, Bangladesh may have experienced a series of abrupt oscillations (Schmidt and Hertzberg, 2011). Although the *P. indicus* present in Kakdwip forms a distinct population-based on mtDNA variation, further studies are warranted based on other markers to reveal a finer genetic structure in Indian waters.

Genetic variation

The present work reports the sequence analysis of the mitochondrial 16S rRNA gene of the wild stocks of Indian white shrimp, *P. indicus* from Indian waters. The genetic variation of *P. indicus* populations in India using polymorphic microsatellite loci and mitochondrial COI gene was reported by Sajeela *et al.* (2019). The lack of genetic differentiation in *P. indicus* observed among the Indian peninsular coasts (Mangalore, Quilon, Kanyakumari, Chennai, Kakinada and Puri) may be due to high gene flow, migration and mixing of adult *P. indicus*, between the south-west and south-east Indian coasts as reported by Sajeela *et al.* (2019). However, the *P. indicus* population present in Kakdwip, near Bangladesh is divergent from all other populations in the country, which is in agreement with the report of Alam *et al.* (2014). Genotypic and phenotypic variations can also be observed in species living in heterogenous environmental habitats, as observed in *Etroplus suratensis* representing different tropical climatic zones in India (Sebastian *et al.*, 2020). The divergent Kakdwip stock has been collected from the Sundarbans Estuarine System which is the largest monsoonal, macro-tidal, delta-front, estuarine system in India and the most complex of the 100-odd estuaries that exist along the Indian coast (Chatterjee *et al.*, 2013). The water quality of these regions is influenced by the semi-diurnal tides at their mouths and the freshwater received as local runoff, predominantly by the monsoon rainfall, ranging between 1500 and 2500 mm/year (Attri and Tyagi, 2010). The genetic variation in the Kakdwip stock of *P. indicus* can be attributed to the adaptive selection due to geographic variability and genetic drift, which requires further investigation.

In the present study, the nucleotide composition showed higher A+T (67.72%) content as observed in *P. chinensis* (82.49%) and other penaeid shrimps like *P. aztecus*: 79%; *P. setiferus*: 83%; *P. notialis*: 79%; *P. duorarum*: 82% by Kong *et al.* (2010). A similar composition was also observed in other shrimps (Calo-Mata *et al.*, 2009) and fishes (Cantatore *et al.*, 1994; Luhariya *et al.*, 2014; Sharma *et al.*, 2018). The higher transition to transversion ratio indicates that the larger part of the nucleotide variation was due to the transition than transversion. The maximum number of transitions and substitutions were observed in Chennai (4 and 4, respectively) and Kakdwip (3 and 4, respectively) populations, while no mutation was observed in the stock from Kanyakumari. The 16S rRNA gene sequence analysis of *P. indicus* resulted in 17 haplotypes among 7 distant stocks of varying haplotype diversity (Hd) values ranging from 0 (Kanyakumari) to 0.6667 (Kakdwip), showing the diverse structure and evolutionary history in its population. The low nucleotide diversity (π) and high haplotype diversity (Hd) in Mangalore, Chennai, Quilon and Puri stocks may partly be attributed to their expansion after a period of small effective population size (Avise *et al.*, 1988; Rogers and Harpending,1992; Sharma *et al.*, 2018). Low values of both π and Hd as in the Kakinada population can be attributed to the population undergoing a reduction in size or recent colonization events that generated few mitochondrial lineages (Rosetti and Remis, 2012; Sharma *et al.*, 2018). The null nucleotide or haplotype diversity in Kanyakumari stock may be due to low allelic frequency and shallow genetic structure (Grant and Cheng, 2012; Sharma *et al.*, 2018). Kakdwip stock showed high nucleotide and haplotype diversity when compared to others, indicating a high level of divergence between haplotypes pointing to a long historical evolutionary pattern as well as introgression between earlier allopatric populations (Grant and Bowen, 1998; Sharma *et al.*, 2018). Population genetic studies of *P. indicus* carried out using the mitochondrial COI gene in Tanzania (Mwakosya *et al.*, 2018), Srilanka (de Croos and Pálsson, 2010), Bangladesh (Alam *et al.*, 2014) and India (Sajeela *et al.*, 2019) showed that the nucleotide diversity was low and haplotype diversity was high in Bangladesh and

moderate in Tanzania and India, whereas, the nucleotide and haplotype diversity was contrastingly high in Srilanka.

Among all, six stocks (Mangalore, Quilon, Kanyakumari, Chennai, Kakinada and Puri) shared the ancestral 'h1' haplotype with high connecting nodes and the remaining haplotypes (h 13-h 17) were exclusively present in Kakdwip stock probably as a result of subsequent mutational events. None of the haplotypes observed in Kakdwip stock was found to be shared among others indicating genetic uniqueness in the *P. indicus* of Kakdwip. The presence of isolated haplotypes in Kakdwip stock may be due to significant population differentiation and a lack of intermixing among the stocks (Sharma *et al.*, 2018).

Wright (1938, 1951) assumed that F_{ST} <0.05, 0.05-0.15, 0.15-0.25 and >0.25 represents a low, moderate, high and very high genetic differentiation, respectively. The pair-wise F_{ST} value ranged from as low as -0.03587 between Kanyakumari and Kakinada to as high as 0.68650 between Kakdwip and Kakinada stocks with an overall F_{ST} value of 0.30684, showing significant genetic differentiation (p<0.05) between Kakdwip and other populations. The significant and high pairwise F_{ST} values between Kakdwip and other stocks reveal no recent gene flow between these populations, which are separated by at least five mutational steps. Similar observations were also reported by Tsoi *et al.* (2014) in *P. japonicus.* The result of the present study implies that Nei's genetic distance data were consistent when compared to an average number of pairwise genetic differences between and within the stocks. The average number of pairwise differences between the populations and the Nei's standard genetic distance (d) revealed similarity with the mean pairwise F_{ST} values. Analysis of genetic distances also revealed that from all the other stocks, the Kakdwip population is clustered separately. AMOVA analysis indicated that variation within stocks contributed to 69.32% of the total variation at a 95% confidence level, which was also observed in *P. chinensis* (Kong *et al.*, 2010), indicating that the stocks did not differ between geographic regions. This essentially means that although there are differences among the stocks, there is considerable genetic variability within a stock.

Phylogenetic and demographic history

Phylogenetic analysis revealed two major groups and one paraphyletic intermediate group. The populations from Kakdwip showed distinct genetic and cladistics separation from the rest of the populations due to their geographical isolations. In a recent study by Sajeela *et al.* (2019), phylogenetic analyses using the mitochondrial COI gene of *P. indicus* resulted in four distinct clades. This may be due to variation among the mtDNA markers, which varies from the lowest in the 16S rRNA to the highest in COI region (Jahromi *et al.*, 2018). The median joining network also showed that there were two separate clusters consisting of Kakdwip stock and remaining stocks of India, showing the genealogical history of Indian white shrimp across 17 haplotypes, which is connected by a link of five mutational events between haplotypes of Kakdwip (West Bengal) and Kakinada (Andhra Pradesh) populations. The negative values of Tajima's D imply that the θ_π (an estimation of θ obtained from the mean number of pairwise differences, $\pi\hat{}$) value is less than the θ_k (an estimation of θ obtained from the observed number of alleles k) value. With less average heterozygosity than that of segregating sites, the negative Tajima's D value supported a stable population structure along with the recent size expansion of a few populations (Tajima, 1989; Sharma *et al.*, 2018). This also indicates the presence of rare alleles in high frequency, which may explain the history of rapid expansion after genetic bottleneck event in the populations of *P. indicus* (Tajima, 1989; Kathirvelpandian *et al.*, 2014). Negative Tajima's D with lower nucleotide and haplotype diversity values suggests a recent population expansion after a bottleneck under the neutral evolution of mtDNA (Saitoh *et al.*, 2016; Sharma *et al.*, 2018).

Conclusions

The present study indicated that *P. indicus* stocks in India can be clustered into two major groups and that most of the genetic variation is present within the stocks. The stock from Kakdwip appeared to be genetically distinct from the rest. The within stock variation in all the stocks is high indicating a very rich and diverse genetic pool. Hence it would be appropriate to have a base population that includes the genetic material from all locations to make the selective breeding programme a robust one.

Acknowledgements

This study was funded by research project no. FISHCIBACOP200800400037 entitled "Outreach activity on fish genetic stocks", from the Indian Council of Agricultural Research (ICAR), New Delhi. Grateful acknowledgements are extended to anonymous researchers and their institutions for helping in sample collection and making the present study possible. The authors also thank all reviewers for constructive comments on the manuscript.

References

Alam, M. M. M., K. M. Westfall and S. Pálsson 2014. Mitochondrial DNA variation reveals cryptic species in *Fenneropenaeus indicus. Bull. Mar. Sci.*, 91(1): 15-31.

Anne, L. M. and B. M. Theresa. 2004. Genetic Diversity in the MTDNA Control Region and Population Structure in the Pink Shrimp *Farfantepenaeus duorarum. J. Crustac. Biol.*, 24(1): 101-109.

Attri, S. D. and A. Tyagi, 2010. Climate profile of India. Met Monograph No. Environment Meteorology-01/2010.

Avise, J. C., R. M. Ball and J. Arnold. 1988. Current versus historical population sizes in vertebrate species with high gene flow: a comparison based on mitochondrial DNA lineages and inbreeding theory for neutral mutations. *Mol. Biol. Evol.*, 5: 331-344.

Baeza, J. A. and M. S. Fuentes. 2013. Exploring phylogenetic informativeness and nuclear copies of mitochondrial DNA (numts) in three commonly used mitochondrial genes: mitochondrial phylogeny of peppermint, cleaner and semi-terrestrial shrimps (Caridea: Lysmata, Exhippolysmata, and Merguia). *Zool. J. Linn. Soc.*, 168(4): 699 -722.

Baeza, J. A. and S. Prakash. 2019. An integrative taxonomic and phylogenetic approach reveals a complex of cryptic species in the 'peppermint'shrimp *Lysmata wurdemanni* sensu stricto. *Zool. J. Linn. Soc.*, 185(4): 1018-1038.

Bandelt, H. J., P. Forster and A. Röh. 1999. Median-joining networks for inferring intraspecific phylogenies. *Mol. Biol. Evol.*, 16(1): 37-48.

Benzie, J. A. H., M. Kenway, E. Ballment, S. Frusher and L. Trott. 1995. Interspecific hybridization of the tiger prawns *Penaeus monodon* and *Penaeus esculentus*. *Aquac.*, 133(2): 103-11.

Benzie, J. A. H. 1994. Genetic and reproduction research on giant tiger prawn *Penaeus monodon*: pond reared spawners achieved in Australia. *Aust. Biotechnol.*, 4: 222-224.

Calo-Mata, P., A. Pascoal, Fernández-No I, K. Böhm, J. M. Gallardo and Barros-Velázquez. 2009. Evaluation of a novel 16S rRNA/tRNAVal mitochondrial marker for the identification and phylogenetic analysis of shrimp species belonging to the superfamily Penaeoidea. *Anal. Biochem.*, 391(2): 127-134.

Cantatore, P., M. Roberti, G. Pesole, A. Ludovico, F. Milella, M. N. Gadaletal and C. Saccone. 1994. Evolutionary analysis of cytochrome b sequences in some Perciformes: evidence for a slower rate of evolution than in mammals. *J. Mol. Evol.,* 39(6):589-597.

Chan, T. Y., J. Tong, Y. K. Tam and K. H. Chu. 2008. Phylogenetic relationships among genera of the Penaeidae (Crustacea: Decapoda) revealed by mitochondrial 16S rRNA gene sequences. *Zootaxa*, 1694: 38-50.

Chan, T. Y. 1998. Shrimps and prawns. In: KE. Carpenter & VH. Niem (eds.) FAO Species Identification Guide for Fishery Purposes. The Living Marine Resources of the Western Central Pacific. Rome: FAO, p. 851-971.

Chatterjee, M., D. Shankar, G. K. Sen, P. Sanyal, D. Sundar, G. S. Michael, A. Chatterjee, P. Amol, D. Mukherjee, K. Suprit and A. Mukherjee. 2013. Tidal variations in the Sundarbans estuarine system, India. *J. Earth Syst. Sci.*, 122(4): 899-933.

CMFRI, 2019. Annual Report 2019. Central Marine Fisheries Research Institute, Kochi, 364 pp.

De Bruyn, M., E. Nugroho, M. M. Hossain, J. C. Wilson and P. B. Mather. 2005. Phylogeographic evidence for the existence of an ancient biogeographic barrier: the Isthmus of Kra Seaway. Heredity. 94(3): 370.

de Croos, M. D. S. T. and S. Pálsson. 2010. Mitochondrial DNA variation and population genetic structure of White Shrimp *Fenneropenaeus indicus* along the coastal belt of Sri Lanka. *Aquat. Living Resour.*, 23(3): 315-323.

Excoffier, L., G. Laval and S. Schneider. 2005. Arlequin (version 3.0): an integrated software package for population genetics data analysis. Evolutionary bioinformatics. 117693430500100003.

Globefish, FAO. 2015. Globefish-Information and Analysis on World Fish Trade, towards sustainability in the shrimp industry http://www.fao.org/in-action/globefish/market-reports/resource-detail/en/c/1261310/ (accessed September 2020).

MPEDA. 2020. Marine product exports. https://mpeda.gov.in/MPEDA/marine_products_exports.php# (accessed September 2020).

Grant, W. A. S. and B. W. Bowen. 1998. Shallow population histories in deep evolutionary lineages of marine fishes: insights from sardines and anchovies and lessons for conservation. *J. Hered.*, 89: 415-426.

Grant, W. S. and W. Cheng. 2012. Incorporating deep and shallow components of genetic structure into the management of Alaskan red king crab. *Evol Appl.*, 5: 820-837.

Hall, T. A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. In Nucleic acids symposium series 1999 Jan 1 [London]: Information Retrieval Ltd., c1979-c2000. 41: 95-98.

Hellberg, M. E. 2009. Gene flow and isolation among populations of marine animals. *Annu. Rev. Ecol. Evol. Syst.*, 40: 291-310.

Hewitt, G. M. 2000. The genetic legacy of the Quaternary ice ages. Nature. 405: 907-913.

Hewitt, G. M. 2004. Genetic consequences of climatic oscillations in the Quaternary. *Phil. Trans. R. Soc. Lond., B.*, 359 pp.

Jahromi, S. T., A. S. Othman and R. Rosazlina. 2018. Morphometrics and mitochondrial DNA genes analysis suggest a new species of *Penaeus* (Crustacea: Penaeidae) from the Persian Gulf. *Bioch. Genet.*, 57(2):193-213.

Katada, Y., K. Ohkura and K. Ueda. 2004. The Nei's standard genetic distance in artificial evolution. InProceedings of the 2004 Congress on Evolutionary Computation (IEEE Cat. No. 04TH8753). 2: 1233-1239.

Kathirvelpandian, A, A. Gopalakrishnan,W. S. Lakra, G. Krishna, R. Sharma, P. R. Divya, R. Kumar and J. K. Jena. 2014. Mitochondrial ATPase 6/8 genes reveal genetic divergence in the *Coilia dussumieri* (Valenciennes, 1848) populations of north east and northwest coasts of India. *Mol. Biol. Rep.*, 41(6): 3723-3731.

Kong, X. Y., Y. L. Li, W. Shi and J. Kong. 2010. Genetic variation and evolutionary demography of *Fenneropenaeus chinensis* populations, as revealed by the analysis of mitochondrial control region sequences. *Genet. Mol. Biol.*, 33(2): 379-389.

Kumar, N., W. S. Lakra, K. C. Majumdar, M. Goswami and K. Ravinder. 2007. Genetic diversity in the Indian population of *Penaeus monodon* (Fabricius, 1798) as revealed by mtDNA sequence analysis. *Aqua. Res.*, 38: 862-869.

Kumar, S., G. Stecher, M. Li, C. Knyaz and K. Tamura. 2018. MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Mol. Biol. Evol.,* 35: 1547-1549.

Luhariya, R. K., K. K. Lal, R. K. Singh, V. Mohindra, A. Gupta, P. Masih, A. K. Dwivedi, R. Das, U. K. Chauhan and J. K. Jena. 2014. Genealogy and phylogeography of Cyprinid fish *Labeo rohita* (Hamilton, 1822) inferred from ATPase 6 and 8 mitochondrial DNA gene analysis. *Curr. Zool.,* 60(4):460-471.

Ma, K. Y., T. Y. Chan and K. H. Chu. 2011. Refuting the six-genus classification of *Penaeus* s.l. (Dendrobranchiata, Penaeidae): A combined analysis of mitochondrial and nuclear genes. *Zool. Scr.*, 40: 498-508.

Mkare, T. K., S. von der Heyden, J. C. Groeneveld and C. A. Matthee. 2014. Genetic population structure and recruitment patterns of three sympatric shallow-water penaeid prawns in Ungwana Bay, Kenya, with implication for fisheries management. *Mar. Freshw. Res.*, 65(3): 255-266.

Mwakosya, C. A., Y. D. Mgaya and N. S. Jiddawi. 2018. Genetic connectivity of *Fenneropenaeus indicus* (H. Milne Edwards 1837) among three prawn fishing grounds of Tanzanian coastal waters. *Reg. Stud. Mar. Sci.*, 24: 107-112.

Noack, K., R. Zardoya and A. Meyer. 1996. The complete mitochondrial DNA sequence of the bichir (*Polypterus ornatipinnis*), a basal ray-finned fish: ancient establishment of the consensus vertebrate gene order. *Genetics,* 144(3): 1165-1180.

Palumbi, S. R. 1994. Genetic divergence, reproductive isolation, and marine speciation. Ecology, Evolution and Systematics. 25: 547-572.

Pérez Farfante, I. and B. Kensley. 1997. Penaeoid and Sergestoid shrimp and prawns of the World: Keys and Diagnoses for the families and genera. Editions du Muse´um, Paris.

Radhakrishnan, E. V., V. D. Deshmukh, G. Maheswarudu, J. Josileen, A. P. Dineshbabu, K. K. Philipose, P. T. Sarada, S. Lakshmi Pillai, K. N. Saleela, Rekhadevi Chakraborty, Gyanaranjan Dash, C. K. Sajeev, P. Thirumilu, B. Sridhara , Y. Muniyappa , A. D. Sawant, Narayan G. Vaidya , R. Dias Johny , J. B. Verma, P. K. Baby, C. Unnikrishnan, N. P. Ramachandran, A. Vairamani, A. Palanichamy, M. Radhakrishnan and B. Raju. 2012. Prawn fauna (Crustacea: Decapoda) of India-An annotated checklist of the Penaeoid, Sergestoid, Stenopodid and Caridean prawns. *J. Mar. Biol. Ass. India,* 54(1): 50-72.

Rajendran, K. V., S. Shivam, P. Ezhil Praveena, J. J. Sahaya Rajan, T. Sathish Kumar, Satheesha Avunje, V. Jagadeesan, S.V. A. N. V. Prasad Babu, Ashish Pande, A. Navaneeth Krishnan, S. V. Alavandi and K. K. Vijavan. 2016. Emergence of *Enterocytozoon hepatopenaei* (EHP) in farmed *Penaeus* (*Litopenaeus) vannamei* in India. *Aquac.*, 454: 272-280.

Remany, M. C., R. Kirubagaran, D. Cyriac, O. C. Prem, P. K. Varadaraju, A. K. Panda, J. Kumar and Y. C. Samraj. 2018. Detection of IHHN virus in a Specific Pathogen Free bonafide *Penaeus vannamei* (Boone, 1931) broodstock consignment imported to India. *Curr. Sci.,* 114(1): 196-200.

Rogers, A. R. and H. Harpending. 1992. Population growth makes waves in the distribution of pairwise genetic differences. *Mol. Biol. Evol.*, 9: 552-569.

Rosetti, N. and M. I. Remis. 2012. Spatial genetic structure and mitochondrial DNA phylogeography of Argentinean populations of the grasshopper *Dichroplus elongatus. PLoS One,* 7:e40807.

Saitoh, K., K. Shindo, Y. Fujimoto, K. Takahashi and T. Shimada. 2016. Mitochondrial genotyping of an endangered bitterling *Acheilognathus typus* (Cyprinidae). *Zoo. Keys.*, 623: 131-141.

Sajeela, K. A., A. Gopalakrishnan, V. S. Basheer, A. Mandal, K. K. Bineesh, G. Grinson, and S. D. Gopakumar. 2019. New insights from nuclear and mitochondrial markers on the genetic diversity and structure of the Indian white shrimp *Fenneropenaeus indicus* among the marginal seas in the Indian Ocean. *Mol. Phylogenet. Evol.*, 136: 53-64.

Sebastian, W., S. Sukumaran and A. Gopalakrishnan. 2020. Signals of adaptive mitogenomic evolution in an indigenous Cichlid, *Etroplus suratensis* (Bloch, 1790) from India. *Reg. Stud. Mar. Sci.*, 40: 101546.

Shakil, V., M. C. Yang, R. M. Pillay, S. Said, O. Henriksson, M. Grahn and C. A. Chen. 2010. Genetic connectivity and historical demography of the blue barred parrotfish (*Scarus ghobban*) in the western Indian Ocean. *Mar. Biol.*, 157(7): 1475-1487.

Sharma, L., S. Ali, A. Barat, R. Kumar, V. Pande, M. A. Laskar, P. K. Sahoo and S. Sumer. 2018. Molecular identification and genetic diversity analysis of Chocolate mahseer (*Neolissochilus hexagonolepis*) populations of Northeast India, using mitochondrial DNA markers. Mitochondrial DNA Part A. 30(3): 397- 406.

Schmidt, M. W. and J. E. Hertzberg. 2011. Abrupt climate change during the last ice age. *Nat. Educ. Knowl*., 3 (10): 11.

Singh, A. K., R. Kumar, M. Singh, A. K. Mishra, U. K. Chauhan, V. S. Baisvar, R. Verma, N. S. Nagpure and B. Kushwaha. 2015. Mitochondrial 16S rRNA gene-based evolutionary divergence and molecular phylogeny of *Barilius* spp. *Mitochondrial DNA*, 26(1): 41-47.

Tajima, F. 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics*. 123(3): 585-595.

Thorrold, S. R., G. P. Jones, M. E. Hellberg, R. S. Burton, S. E. Swearer, J. E. Neigel, S. G. Morgan and R. R. Warner. 2002. Quantifying larval retention and connectivity in marine populations with artificial and natural markers. *Bull. Mar. Sci*., 70: 291-308.

Tsoi, K. H., T. Y. Chan and K. H. Chu. 2007. Molecular population structure of the kuruma shrimp *Penaeus japonicus* species complex in western Pacific. *Mar. Biol*., 150(6): 1345-1364.

Tsoi, K. H, K. Y. Ma, T. H. Wu, S. T. Fennessy, K. H. Chu and T. Y. Chan. 2014. Verification of the cryptic species *Penaeus pulchricaudatus* in the commercially important kuruma shrimp *P. japonicus* (Decapoda: Penaeidae) using molecular taxonomy. *Invertebrate Systematics*. 28(5): 476-490.

Tzeng, T. D., S. Y. Yeh and C. F. Hui. 2004. Population genetic structure of the kuruma prawn (*Penaeus japonicus*) in East Asia inferred from mitochondrial DNA sequences. *ICES, J. Mar. Sci.,* 61(6): 913-920.

Valles-Jimenez, R., P. M. Gaffney and R. Perez-Enriquez. 2005. RFLP analysis of the mtDNA control region in white shrimp (*Litopenaeus vannamei*) populations from the eastern Pacific. *Mar. Biol*., 148 (4): 867-873.

Wright, S. 1938. Size of population and breeding structure in relation to evolution. *Science,* 87: 430-431.

Wright, S. 1951. The genetical structure of populations. *Ann. Eugen*., 15: 323-354.

Yang, M. and X. Z. Li. 2017. Population genetic structure of the sword prawn *Parapenaeopsis hardwickii* (Miers, 1878) (Decapoda: Penaeidae) in the Yellow Sea and East China Sea. *J. Crustac. Biol.,* 38(1): 27-33.