RESEARCH ARTICLE

Genetic variation of *Parapenaeopsis sculptilis* (Decapoda, Penaeidae) and reassessment of the phylogenetic relationships within the genus *Parapenaeopsis* based on mitochondrial DNA variation

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Abstract – The genus *Parapenaeopsis* is an important group of marine shrimps for wild capture in the Indo-West Pacific region. Phylogenetics of penaeid shrimps is still a debatable issue. This study focuses on the phylogenetic relationships among seven species within the genus *Parapenaeupsis*, the population genetic variation of *Parapenaeupsis sculptilis* along Bangladesh coastline of the Bay of Bengal and the phylogeography of *P. sculptilis* in the Indian Ocean region by analysing cytochrome oxidase subunit 1 barcode (CO1) sequence. No population structure was detected in *P. sculptilis* collected from two sampling sites along the Bangladesh coastline (AMOVA and $\Phi_{ST} = -0.014$, $p > 0.05$; $F_{ST} = 0.061$, $p = 0.04$), which expanded first around 73 (CI: 36−119) kyr ago. The genealogical relationships in Bangladesh *P. sculptilis* population are shallow with haplotype diversity (h) of 0.58 and nucleotide diversity (π) of 0.0014. The different *P. sculptilis* samples from Bangladesh, India and Mozambique of the Indian Ocean revealed connectivity between western Indian Ocean and the Bay of Bengal. The phylogeny within the genus *Parapenaeupsis* showed a polyphyletic relationships for *P. hardickwii* and its taxonomy needs to be reevaluated. The study will help for genetic upgradation in aquaculture and monitoring of the population genetic diversity of *P. sculptilis*.

Keywords: Bay of Bengal / population genetics / historical demography / effective population size / management / aquaculture

1 Introduction

The genus *Parapenaeopsis* (Alcpck, 1901), which is synonymized to Mierspenaeopsis (Sakai and Shinomiya, 2011), Alcockpenaeopsis (Sakai and Shinomiya, 2011), Batepenaeopsis (Sakai and Shinomiya, 2011), *Ganjampenaeopsis* (Sakai and Shinomiya, 2011), *Kishinouyepenaeopsis* (Sakai and Shinomiya, 2011), constitutes an important group of penaeid shrimps in the Indo-West Pacific (IWP) region (Chanda, 2016; Chowdhury *et al.*, 2019; ITIS, 2016; WoRMS, 2020). The rainbow shrimp *Parapenaeopsis sculptilis* (Heller, 1862) is a penaeid shrimp distributed throughout the Indo-West Pacific region, including north-eastern Australia, northern Bay of Bengal, west coast of India and south-eastern Africa (De Grave, 2015; ITIS, 2020). It is a commercially important marine shrimp for wild capture in the Indo-West Pacific region, including Bangladesh (Silvestre and Pauly, 1997; Department of Fisheries Bangladesh, 2013; BOBLME, 2013; WoRMS, 2020).

The Indo-Pacific region is considered as a hotspot of marine biodiversity in terms of species richness and unique ecosystems (Crandall and Riginos, 2014; Keyse *et al.*, 2014). The marine biodiversity, including genetic diversity, of this diverse biogeographic region is insufficiently studied and unevenly sampled (Crandall and Riginos, 2014; Keyse *et al.*, 2014). Bangladesh possesses an enriched diversity due to its extensive mangrove forests and large flow of freshwater, and harbours a wide varieties of crustaceans (Quader, 2010; Hossain, 2014; IUCN Bangladesh, 2014). The crustaceans shrimps and prawns are the among the dominant ones, and have considerable commercial and nutritional valuesfor

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millions of coastal people through direct engagement in the shrimp industry (DoF, 2013). In Bangladesh waters, 24 species of freshwater prawns and 36 saline water shrimps including 24 penaeid shrimps have been reported (DoF, 2013, 2019). Overexploitation, fishing with banned gears and during banned period, water pollution, destruction of mangrove and wetlands due to shrimp farming, cyclones, escaping of farmed shrimp to nature are among the major threats to diversity of shrimp in Bangladesh (Quader, 2010; DoF, 2013; Alam, 2016). Thus, it is important to unveil the phylogeographic and phylogenetic relationships, population differentiation and demographic history of shrimp for their proper management and conservation.

Population genetics of several penaeid shrimps *i.e.* *Penaeus indicus* (Milne-Edwards, 1837), *Penaeus monodon* (Fabricius, 1798), *Penaeus semisulcatus* (De Haan, 1844) and *Macrobrachium rosenbergii* (De Man, 1879) with their phylogeography in the IWP region have revealed high genetic variation within the species (Alam *et al.*, 2015; Alam *et al.*, 2016a, 2016b; Alam *et al.*, 2017; Alam and Pálsson, 2018). A clear population structure was detected in *M. rosenbergii* and *P. monodon* within Bangladesh and distinct evolutionary lineages in *P. indicus*, *P. monodon* and *P. semisulcatus* in the IWP region, using mitochondrial DNA markers, microsatellites, genotyping of SNPs and RADseq of the whole genome (Alam *et al.*, 2015, 2016a, 2016b, 2017; Alam and Pálsson, 2018). Inconsistencies between the taxonomic assignment and the mtDNA variation were observed in several genera and speceis of penaeid shrimps.Cryptic diversity or distinct lineages in the IWP region were detected in several species which have been, diverging for million of years, predating even the onset of Ice Age. Unresolved phylogenetic patterns may in some cases reflect ancestral polymorphism shared by different species (Cayuela *et al.*, 2020), hybridization (Bouchemousse *et al.*, 2016) or even wrong taxonomic assignments and genebank errors (Meiklejohn *et al.*, 2019). Several studies have been performed on population genetics and phylogenetic relationships among different species under the genus *Parapenaeopsis*, based on morphology of different species in the Indian waters (Chanda, 2016), morphology and Randomly amplified polymorphic DNA (RAPD) (Rajakumaran *et al.*, 2014), variation in the mitochondrial control region to infer population differentiation in *Parapenaeopsis hardwickii* (Miers, 1878) sampled from eastern part of the China Sea (Tzeng, 2007), and on mitochondrial 16SrDNA and CO1 to unveil phylogenetic relationship among Chinese species of *Parapenaeopsis* (Xinzheng *et al.*, 2014). Even though *P. sculptilis* contribute to a large catch in Bangladesh, no studies have been devoted to unveil its population genetic variation. *Parapenaeopsis sculptilis* has commercially and nutritionally great importance in the Indian and western Pacific Ocean regions. Thus, the study of its population genetic diversity with the phylogeography is required to assist the authority for the management of the shrimp.

The main objectives of the study were to assess the population genetic variation of *P. sculptilis* within Bangladesh and the phylogeographic patterns of *P. sculptilis* in the Indian Ocean region based on mtDNA variation. For comparison we assess the phylogenetic relationships within the genus *Parapenaeopsis*.
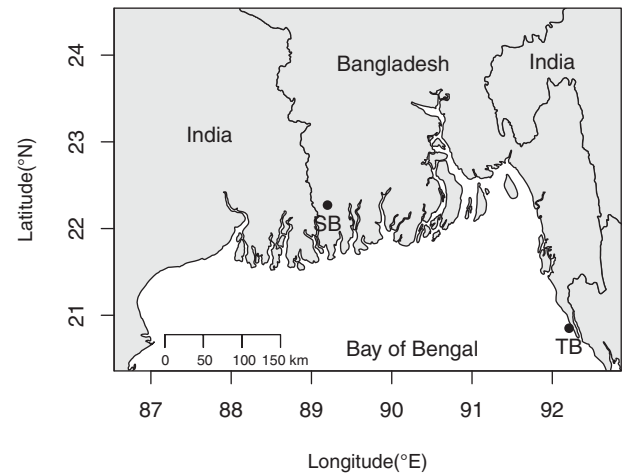

Fig. 1. *Parapenaeopsis sculptilis* sampling sites along the coastline of Bangladesh. Capital letters indicate sites: SB- Sundarban at Satkhira in Bangladesh and TB- Teknaf Beach at Cox's Bazar in Bangladesh.

2 Materials and methods

2.1 Sample collection

Twenty three *P. sculptilis* were collected from Sundarban (SB) mangrove forest of Satkhira district and 17 from Teknaf Beach (TB) of Cox's Bazar district of the Bay of Bengal (BoB) in 2013 (Fig. 1). A pleopod was collected from each individual and preserved in 96% ethanol.

2.2 DNA extraction

Genomic DNA was extracted from approximately 1 mg pleopod tissue using Chelex and proteinase K, following the recipe and the protocol as described in Alam *et al.* (2016a).

2.3 Polymerase chain reaction (PCR) and sequencing

PCR of mitochondrial cytochrome oxidase subunit 1 barcode (CO1b) was performed utilizing 30–150 ng DNA in a final volume of 10 μL, following the recipe and the protocol as described in Alam *et al.* (2016b), using the primers from Folmer *et al.* (1994). The PCR product (5 μL) was purified using ExoSAP clean up, in a final volume of 10 μL, including 1% Exo- I, 2% SAP and 10% SAP-Buffer, at 38 °C for 30 minutes (1 Hold), then 80 °C for 15 min (1 Hold) and finishing at 12 °C (1 Hold). The DNA template was sequenced, utilizing 3 μL DNA template in a final volume of 10 μL, including Big Dye Terminator kit 3.1 (Applied Biosystems) and the forward primer, precipated in ethanol and sequenced in a 3500 xL Genetic Analyzer (AB).

2.4 Sequence analysis

A total of 112 CO1 sequences at a length of 517 bp from seven species (*P. sculptilis* − 12, *Mierspenaeopsis sculptilis* (Heller, 1862) − 6, *Parapenaeopsis aff. Sculptilis* − 1,

Parapenaeopsis coromandelica (Alcock, 1906) − 23, *Parapenaeopsis stylifera* (H. Milne Edwards, 1837) − 24, *Parapenaeopsis tenella* (Spence Bate, 1888) − 2, *Parapenaeopsis hungerfordi* (Alcock, 1905) − 2, *Parapenaeopsis cornuta* (Kishinouye, 1900) − 1, *P. hardwickii* − 40 and *Parapenaeopsis sp.* – 1) under the genus *Parapenaeopsis* (see Tab. 1) were downloaded from GenBank, and subsequently aligned with the newly generated sequences (40) in this study. Bioedit Sequence Alignment Editor was used to edit and align the sequences (Hall, 1999). In order to minimize the loss of useful information in the phylogenetic reconstruction, eight GenBank sequences were omitted from the downstream analyses when noticing that their inclusion in sequence comparison resulted in shorter total CO1b alignment.

2.5 Genetic diversity and demography

Genetic diversity of *P. sculptilis* from Bangladesh was analysed for each sampling site. The software ARLEQUIN v3.5 (Exoffier and Lischer, 2011) was used to calculate haplotype diversity (*h*), nucleotide diversity and number of segregating sites (S). To compare the number of haplotypes among samples, haplotype richness considering the different sample sizes, was estimated using the function allele.richness in the R package Hierfstat (Goudet, 2005; R Core Team, 2020). An unrooted network was constructed using the software Network v4.6.1.3 (Bandelt *et al.*, 1999) in order to assess the evolutionary relationships among the recorded CO1 haplotypes for *P. sculptilis* from Bangladesh, India and Mozambique.

The differences among samples of *P. sculptilis* within Bangladesh and in the Indian Ocean region (Bangladesh, India and Mozambique) were studied by analysing molecular variation (AMOVA) for the CO1 sequences, considering both pairwise differences among sequences (Φ_{ST}) and solely the haplotype frequencies (F_{ST}), and tested with 1000 permutations, using ARLEQUIN v3.5 (Excoffier and Lischer, 2011) and by analysing genetic distances between the populations. Genetic distances within and between the samples from the tree countries (Tab. 1), based on CO1 marker from our study and available GenBank sequences, were estimated with Kimura two parameter model (K80) using the APE package in R (Paradis, 2006). Net pairwise genetic distances between populations were calculated following the equation of Nei and Li (1979) as $D = dxy − [(dxx + dyy)/2]$, where *dxy* is the mean difference between populations *x* and *y,* and *dxx* and *dyy* are the mean distances within population *x* and *y,* respectively. Significance of the pairwise differences were estimated by 1000 permutations of sequences among the populations compared.

Forty CO1 sequences of *P. sculptilis* were analyzed to reconstruct its historical demography within Bangladesh, by comparing the fit of the distribution of pairwise nucleotide differences (mismatch distribution) with the expected values of a demographic expansion, following the methods developed by Rogers and Harpending (1992). The fit was estimated by the sum of squared deviation (SSD) and the Regardness Index (Harpending, 1994), and tested using Arlequin v3.5 (Excoffier and Lischer, 2011). Fu's F (Fu, 1997) and Tajima's D

(Tajima, 1993) tests were performed, to further estimate the demographic changes, or possible deviation from neutrality, using Arlequin v3.5. The time since expansion of *P. sculptilis* population was estimated as $t = \tau/2 \mu L$, where τ is the median of the mismatch distribution = 0.85696, μ is the mutation rate = 1% per site per Myr for the CO1 (Knowlton *et al.*, 1993; Knowlton and Weight, 1998) assuming one generation per year, and L is the length of the sequence = 589 bps.

The changes in effective population size (N_e) of *P. sculptilis* were calculated through construction of Bayesian Skyline Plot (BSP) in BEAST v1.7.5 (Drummond *et al.*, 2007). The MCMC simulations in BEAST were run for 6.0×10^7 times with a sample per 1000 generations, following the "GTR + I" substitution model, selected after comparison of likelihood of different models using PhyML (Guidon *et al.*, 2010) implemented in Ape R package (Paradis, 2006; R Core Team, 2020). A strict molecular clock model was set as priors in BEAST, using the molecular clock of 1% per million year (Myr) for the CO1 as above. Effective sample sizes (ESS) of the Markov Chain sampled model parameters were investigated using TRACER v1.5 (Rambaut and Drummond, 2009) and deemed sufficient if >200. The BEAST log files were read in TRACER software (Rambaut and Drummond, 2009) to construct Skyline Plot which excluded 10% sample skylines as burn-in. The Skyline plot was finally redrawn in R (R Core Team, 2020) using the ape package (Paradis, 2006).

2.6 Phylogenetic relationships among the species under the genus *Parapenaeopsis*

Phylogenetic relationships among the species under the genus *Parapenaeopsis* in the IWP region were analyzed by constructing a phylogenetic tree, utilizing available sequences (144) of different species *i.e.*, *P. sculptilis*, *Parapenaeopsis aff. sculptilis*, *M. sculptilis*, *P. coromandelica*, *P. stylifera*, *P. tenella*, *P. hungerfordi*, *P. cornuta*, *P. hardwickii* (see Tab. 1). The Bayesian inference tree was constructed in BEAST v1.7.5 (Drummond *et al.*, 2007) using nucleotide substitution model "GTR" derived from PhyML test (Guindon *et al.*, 2010) using APE package (Paradis, 2006) in R (R Core Team, 2020) based on lowest Akaike information criterion. Strict molecular clock model and coalescent diversification model were set as tree priors in BEAST. MCMC simulations were run for 1.0×10^7 generations and sampled every 1000th generations. Effective sample sizes (ESS) of the Markov Chain sampled model parameters were investigated using TRACER v1.5 (Rambaut and Drummond, 2009) and deemed sufficient if >200. Maximum clade credibility tree was constructed using TreeAnnotator v1.7.5 (Rambaut and Drummond, 2013) with a 10% burn-in. The software FigTree v1.4.0 (Rambaut, 2012) was used to observe the divergence time by reading Bayesian inference tree. Divergence time of the lineages based on CO1 was estimated in BEAST v1.7.5, again using the molecular clock of 1% per million year (Myr). *Metapenaeus dobsoni* (Miers, 1878) and *Metapenaeopsis barbata* (De Haan, 1844) were used as outgroup. The tree was redrawn to increase visibility using APE package in R. Clades which diverged with a posterior probability (PP) 90% or above will be considered as well resolved.

Table 1. Information about the sequences for CO1b of the mitochondrial DNA of the genus *Parapenaeopsis* utilized for the study.

Species	Sampling location	Sample size	GenBank accession number	Reference
	Bangladesh	40	MW381251-MW381290	[*0]
		1	MN200398	[*1]
P. sculptilis	India	11	KU341998-KU342000	[*2]
			MK779149-MK779151	[*3]
			MK779166-MK779168	[*3]
			KT898913, KX399423	[*4], [*5]
M. sculptilis	Bangladesh	1	MH429355	[*1]
	Mozambique	5	KP297897-KP297901	[*6]
	Sub-Total	**58**		
P. aff. sculptilis	India	1	KX399432	[*5]
	Sri Lanka	18	HQ180247- HQ180264	De Croos and Pálsson (2010)
P. coromandelica		5	KX139140-KX139141	[*2]
	India		KU341991-KU341993	[*2]
	India	22	KF613003, KU341997	[*7], [*2]
			KX139142, KX139143	[*2]
			KY316145-KY316147	[*2]
			MH712487-MH712489	[*8]
P. stylifera			MH724294	[*9]
			MK779140-MK779142	[*3]
			MK779158, MK779159	
			MK986637, MK986638	
			MK986647-MK986650	
	Iran	2	KR261592, KR261593	Samadi *et al.* (2016)
P. tenella	China	2	MK164420, FJ435655	Meng *et al.* (2019), [*10]
P. hungerfordi	China	2	NC_038069, FJ435656	[*11], [*10]
P. cornuta	China	1	KP072694	Yang *et al.* (2015)
P. hardwickii	India	6	KU341994- KU341996	[*2]
			MK430870, MK430871	[*12], [*13]
			MH577540	[*13]
	China	30	KU899136, KU302814	[*14], [*15]
			KR258740-KR258746	[*16]
			MF632006-MF632025	[*17]
			NC_030277	[*15]
	Bangladesh	4	MF601487, MF614763	[*18]
			MF614765,MN200402	[*18], [*1]
Parapenaeopsis sp.	Unknown	1	LT009696	[*19]
	Sub-Total	**94**		
	Total	**152**		

[*0] Present study.
[*1] M.S. Ahmed *et al.*, Department of Zoology, University of Dhaka, Dhaka, Bangladesh, Unpubl data.
[*2] L.M. Chowdhury *et al.*, National Bureau of Fish Genetic Resources, Kerala, India, Unpubl data.
[*3] A.R. Dhawade *et al.*, College of Fisheries, Maharashtra, India, Unpubl data.
[*4] G.T. Mehetre and M.S. Dharne, CSIR-National Chemical Laboratory, Pune, Maharashtra, India, Unpubl data.
[*5] R. Ram *et al.*, ICAR-Central Institute of Fisheries Education, Maharashtra, India, Unpubl data.
[*6] L. Simbine, Universidade Federal de Sao Carlos, Sao Paulo, Brazil, Unpubl data.
[*7] P.S. Bhavan *et al.*, Bharathiar University, Tamil Nadu, India, Unpubl data.
[*8] A. Dhawade *et al.*, College of Fisheries, Maharashtra, India, Unpubl data.
[*9] T.M. Sawant, G.N. Khalsa College, Mumbai, Maharashtra, India, Unpubl data.
[*10] W.J. Mai, and C.Q. Hu, South China Sea Institute of Oceanology, Guangdong, China, Unpubl data.
[*11] S. Zhong, Guangxi Institute of Oceanology, Guangxi, China, Unpubl data.
[*12] N. Mandal and D. Mondal, Bose Institute, Kolkata, West Bengal, India, Unpubl data.
[*13] A. Mandal *et al.*, Rajiv Gandhi Centre for Aquaculture, Karaimedu, Sirkazhi, India, Unpubl data.
[*14] Y. Yuan *et al.*, Chuanqiao, Shanghai, China, Unpubl data.
[*15] P. Liu and Z. Mao, Chinese Academy of Fishery Sciences, Yellow Sea Fisheries Research Institute, Shandong, China, Unpubl data.
[*16] P. Liu and Z.C. Mao, Chinese Academy of Fishery Sciences, Yellow Sea Fisheries Research Institute, Shandong, China, Unpubl data.
[*17] M. Yang, Department of Marine Organism Taxonomy & Phylogeny, Institute of Oceanology, Chinese Academy of Sciences, Qingdao, China, Unpubl data.
[*18] K.A. Habib *et al.*, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh, Unpubl data.
[*19] M. Staats *et al.*, RIKILT, WUR, Akkermaalsbos 2, 6708 WB Wageningen, Netherlands, Unpubl data.

Table 2. Genetic diversity of *Parapenaeopsis sculptilis* from Bangladesh based on mitochondrial CO1. N = No. of individuals, N_h = No. of haplotypes, H_R = Haplotype richness, h = haplotype diversity, π = Nucleotide diversity, S = No. of segregating sites, SE = standard error.
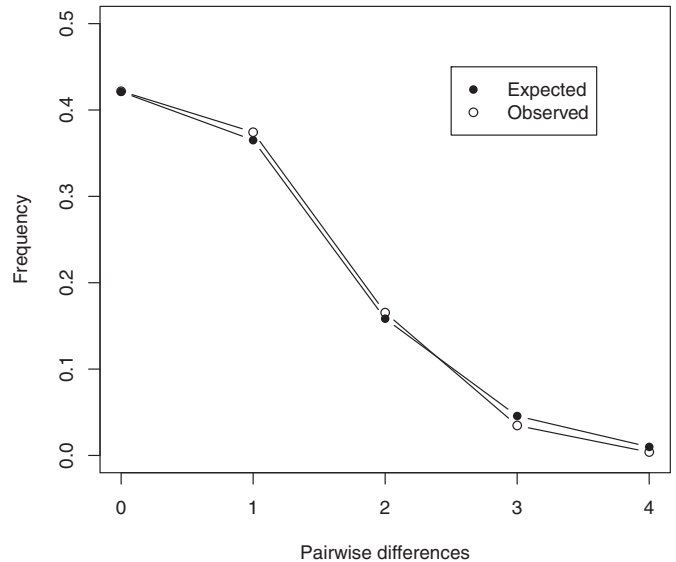
Gene	Sampling Location	N	N_h	H_R	$h \pm$ SE	$\pi \pm$ SE	S
	Sundarban, Satkhira	23	6	4.7	0.40 ± 0.13	0.0009 ± 0.0009	5
CO1	Teknaf, Cox's Bazar	17	9	9	0.79 ± 0.10	0.0022 ± 0.0016	9
Total		40	12	11.3	0.58 ± 0.09	0.0014 ± 0.0011	11
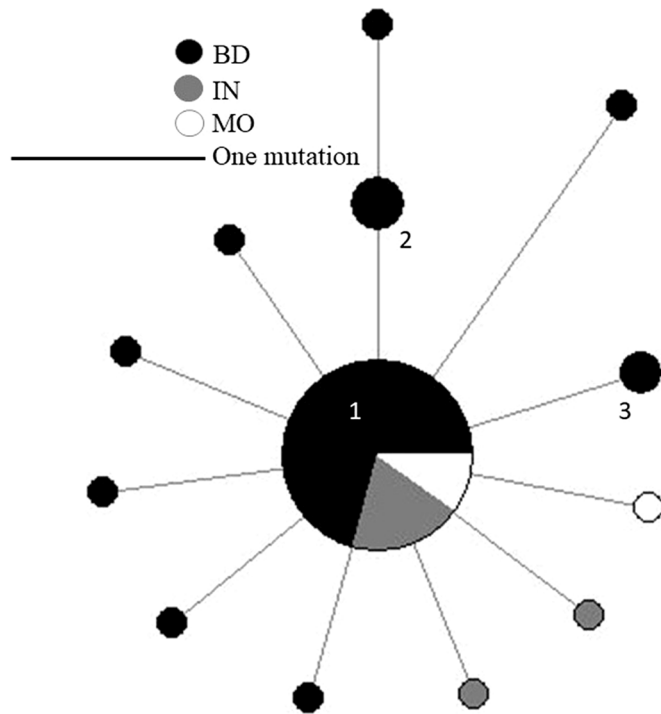
Fig. 2. Median-joining haplotype network based on 56 sequences of mitochondrial CO1 barcode of *Parapenaeopsis sculptilis* sampled from Bangladesh, India and Mozambique. Each pie is a haplotype and its size presents the frequency of samples. Distances between pies represents to mutational differences between the haplotypes. Shadings denote different sampling locations (BD- Bangladesh, IN- India and MO- Mozambique).

Fig. 3. Mismatch Distributions, based on 40 sequences (589 bps) of mitochondrial CO1 barcode of *Parapenaeopsis sculptilis* from Bangladesh. The Sudden Expansion Model was used to obtain the expected values.

3 Results

3.1 Mitochondrial DNA diversity of *P. sculptilis*

Intra-population genetic diversity of the *P. sculptilis* from Bangladesh was characterized with low haplotype diversity ($h = 0.58 \pm 0.09$) in comparison with studies on other penaeids from Bangladesh *e.g.* in *Metapenaeus monoceros* (Alam, 2016) where h was close to 1 and also lower nucleotide diversity ($\pi = 0.0014 \pm 0.0011$), and thus, reflected by the shallow genealogy (Fig. 2), (Tab. 2). Twelve unique haplotypes were found in Bangladesh, of which 9 were singletons. Haplotypes 1, 2 and 3 were common in both locations which represented 26, 32 individuals, respectively (Appendix 1). The population differentiation between the two sampling sites was non-significant (AMOVA and $\Phi_{ST} = -0.014$, $p > 0.05$) or marginally significant considering the haplotype frequencies

($F_{ST} = 0.061$, $p = 0.04$), indicating single population within Bangladesh or a weak structure.

A medium-joining haplotype network of the CO1 sequences from Bangladesh, India and Mozambique formed a starlike network, reflecting similar haplotypes in the three countries, where haplotype 1 represented 29 sequences from Bangladesh, 8 from India and 4 from Mozambique, haplotype 2 and 3 represented 3 and 2 individuals respectively from Bangladesh (Fig. 2). The rest 10 haplotypes were singletons (Bangladesh – 7, India – 2 and Mozambique – 1), and the majority maintained a single mutation distance from the common haplotype 1. The population differentiation among Bangladesh, India and Mozambique populations was non-significant (AMOVA, Φ_{ST} and F_{ST}; $p > 0.05$), indicating connectivity or recent split between the Bay of Bengal and western Indian Ocean (Appendix 2). The net genetic distances between the *P. sculptilis* samples from the three countries were also nonignificant ($p > 0.05$; Appendix 2).

The mismatch distribution based on the CO1 sequences from Bangladesh followed the sudden expansion model both for the SSD – 0.004 ($p > 0.685$) and the raggedness index 0.124 ($p > 0.551$), in line with the shallow genealogies observed (Fig. 3). Time since expansion of the Bangladesh *P. sculptilis* population, based on the median of the mismatch
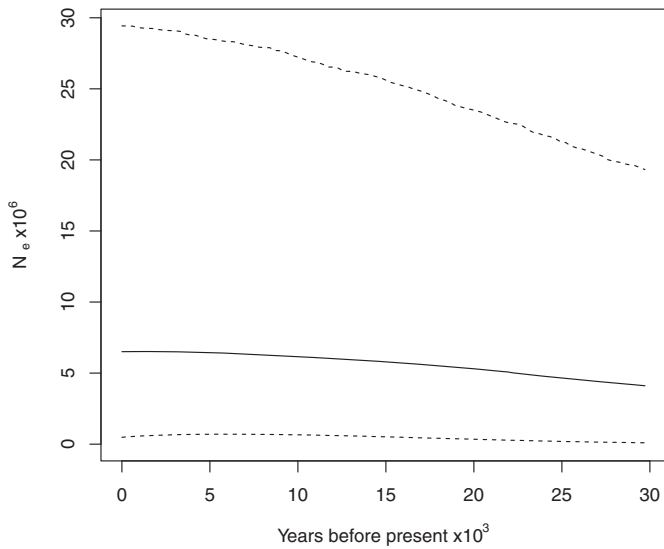
Fig. 4. Bayesian Skyline Plot of *Parapenaeopsis sculptilis* in Bangladesh. The past population dynamics in the plot was estimated from 40 (589 bps) sequences of CO1 barcode. The 95% confidence intervals were shown using dotted lines. Effective population size ($N_e \times 10^6$) per generation is traced back in time from the present to the past.

Fig. 5. Bayesian inference tree for the genus *Parapenaeopsis*. A 517 bp fragment of CO1 barcode for 147 sequences were utilized under 'GTR' model following a mutation rate of 1% per million year. Numbers at the nodes represent divergence in million years and shadings represent Bayesian posterior probabilities (PP, %). *Metapenaeus dobsoni* (GenBank accession number: MK779148) and *Metapenaeopsis barbata* (GenBank accession number: NC_040139) were used as outgroup. Numbers following the names of countries (BD- Bangladesh, IN- India, IR- Iran, CH- China, SL- Sri Lanka, MO- Mozambique) represent the number of individuals.

distributions was around 73 (CI: 36–119) Kyr ago assuming one generation per year. Deviation from the equilibrium was also observed with the Tajima's D and Fu's Fs (Tajima's D = −2.06716 with P = 0.004 and Fu's Fs = −10.78778, P = 0), suggesting the expansion of Bangladesh population from a bottleneck or a selective sweep.

The Skyline plot for the Bangladesh population showed slow expansion for at least 30 Kyr (Fig. 4) with a current effective population of 6.51×10^6 (CI: 0.48×10^6–29.43×10^6), but the confidence interval is large as expected by a single marker with little variation.

3.2 Phylogenetics of the genus *Parapenaeopsis*

The phylogenetics of the species within the genus *Parapenaeopsis* showed mostly a well resolved tree with five distinct clusters (Fig. 5). All taxa formed monophyletic groups, except *P. hardwickii.* Cluster 1 represented a monophyletic group comprising of *P. sculptilis* from Bangladesh, India and Mozambique except for an individual of *P. hardwickii* from India, which diverged from cluster II comprising of *P. hardwickii* from Bangladesh, India and China for 6.15 (CI: 4.77–7.50) Myr ago (PP > 90) and shared a common ancestor with *P. tenella*. Cluster 4 presented a monophyletic group of *P. coromandelica* from Sri Lanka except for an individual of *P. stylifera* from India, which diverged from cluster 3 representing a monophyletic group of *P. stylifera* from India and Iran except for an unconfirmed specimen of *P. aff. sculptilis* from India for 3.96 (CI: 2.88–4.94) Myr ago (PP > 90) and share a common ancestor with *P. cornuta* and *P. hungrdordi*. Cluster V composed of two *P. hardwickii* (GB accession numbers: NC_030277 and KU302814) which showed a closest similarity with *M. barbata and M. dobsoni*
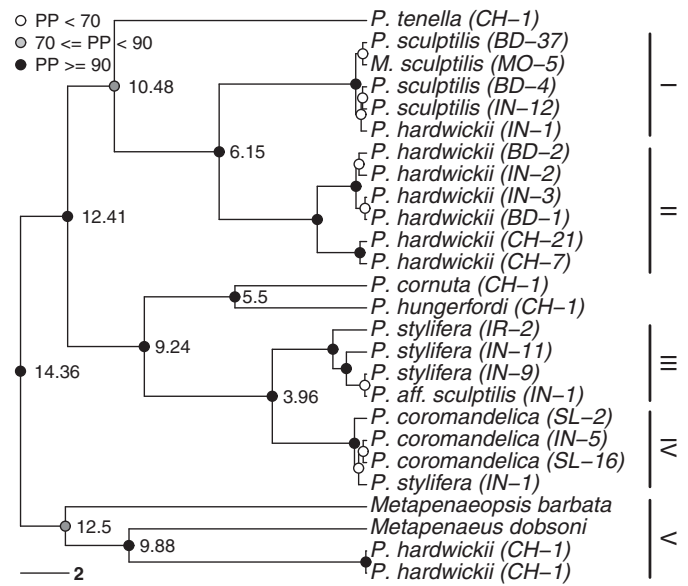
and shared a common ancestor with the other species of the genus *Parapenaeopsis* 14.36 (CI: 12.75–16.04) Myr ago (PP > 90).

4 Discussion

4.1 Population genetics and phylogeography of *P. sculptilis*

The haplotypes of *P. sculptilis* population from Bangladesh were similar and no population structure was unveiled, based on mitochondrial CO1 gene. The genetic variation was smaller than those reported for Bangladesh *P. semisulcatus*, *P. monodon, P. indicus* and *M. rosenbergii* (Alam *et al.*, 2015, 2016a, 2016b, 2017). A genetic homogeneity was also reported in Bangladesh for *P. monodon, P. indicus* and *P. semisulcatus*, based on mitochondrial DNA variation (Alam *et al.*, 2015, 2016a, 2016b, 2017). The *S*lack of differentiation could reflect gene flow or a recent divergence within Bangladesh as the *P. sculptilis* samples were collected from two farthest locations along Bangladesh coastline. A genetic homogeneity was also observed between Bangladesh, west India and the remote sample from Mozambigue. Whether this indicates a recent split or some connectivity, possibly mitigated by human transport is unknown but it is clear that more markers are needed to assess possible differentiation between these areas. This finding differs from the studies on other penaeids reported for

P. semisulcatus, *P. indicus* and *P. coromandelica* from Sri Lanka (De Croos and Pálsson, 2010, 2011; Alam et al., 2016a) and *M. rosenbergii* from Bangladesh (Hurwood *et al.*, 2014; Alam *et al.*, 2017), which showed large differences between western Indian Ocean (east African coast) and eastern Indian Ocean (Bay of Bengal). The databases on penaeids (Holthuis, 1980; WoRMS, 2020) reported that *P. sculptilis* was not naturally distributed in south south-eastern Africa. In the present study the sample from Mozambique showed a close similarity with *P. sculptilis* from Bangladesh or India, which might have been transported from Bangladesh or India through historical currents, colonization routes or recent anthropogenic introductions from other regions purposely for aquaculture or accidentally through transfer of ballast water (Alam *et al.*, 2015).

Based on the mtDNA variation the population of *P. sculptilis* in Bangladesh started expansion during the late Pleistocene following the sudden expansion model and was supported also by the negative Tajima's *D* and Fu's *F*s. Based on the estimated time since expansion (*t*), more recent expansion was observed in *P. sculptilis* than those reported for *P. monodon*, *P. semisulcatus* and *P. indicus* (Alam *et al.*, 2015, 2016a). Climatic fluctuations affected the demographic history of Asian marine species, where the species may have survived in several Pleistocene refugia (Liao *et al.*, 2010; Stewart *et al.*, 2010; Tsang *et al.*, 2012). The effect of Ice Ages on organisms is considered to have varied both with latitude and topography of the land of organisms (Hewitt, 2004), resulting in expansion of different populations or species with varied times.

The phytogeography of *P. sculptilis*, based on the analysis of mitochondrial CO1b gene, did not reveal any genetic subdivision in the Indian Ocean region. The *P. sculptilis* populations from Bangladesh, Indian and Mozambique should thus be considered as single panmictic population based on mtDNA COI. This lack of population genetic structure in *P. sculptilis* differs from that already reported for other species of shrimps in the region. *Penaeus semisulcatus, P. indicus, P. monodon and M. rosenbergii* showed distinct lineages on the East and West sides of the Sunda-Shelf and between western Indian Ocean and Bay of Bengal (Alam, 2016; Alam *et al.*, 2015, 2016a, 2016b; Alam and Pálsson, 2018). The other penaeids studied seemed to have diverged in isolation in different regions of the IWP Ocean, unlike *P. sculptilis* which appear to have survived only in a single region and may have expanded from that area recently. Further studies are though needed to cover the geographic range and to applying more genomic markers to assess the possible genetic structure.

4.2 Phylogenetic relationships

A polyphyly in different species of the genus *Parapenaeopsis* was revealed from phylogenetic analysis. An individual of *P. hardwickii*, and *P. stylifera* clustered together with *P. sculptilis* (cluster − I), *P. stylifera* (cluster − III) and *P. coromandelica* (cluster − IV) respectively that could be errors, hybridization or an ancestral polymorphism. But two *P. hardwickii* showed more similarity with the outgroup *Metapenaeopsis barbata* and *Metapenaeus dobsoni* and indicated that these two *P. hardwickii* belong to the *Metapenaeopsis* rather than the *Parapenaeopsis*. The patterns

in *P. hardwickii* could have resulted from incomplete lineage sorting (ancestral polymorphism) or widespread hybridization between the species under the genus. As species trees can differ from single gene trees (Pamilo and Nei, 1988) and mtDNA can give different result from other markers *e.g.* in the horned lizard (Leache and McGuire, 2006), it is important to assess variation in other markers. Mitogenomic markers have though repeatedly provided interesting insight into the natural history of different species and especially in case of clear divergence provided valuable markers to distinguish origin of different specimens *e.g.* in tracing harvesting species (Galimberti *et al.*, 2013; Bucklin *et al.*, 2011).

Even though mitochondrial DNA was used here to infer population genetic variation and connectivity of *P. sculptilis* in the Indian Ocean region and the phylogenetic relationships of seven species within the genus *Parapenaeopsis,* we acknowledge the limitations of mitochondrial markers. A single marker may lack the statistical power to detect subtle differentiation and due to lack of recombination it may be vulnerable to selection at any loci within the mtNDA, furthermore, indirect selection could arise from linkage disequilibrium with maternally inherited symbionts (Hurst and Jiggins, 2005; Rokas and Carroll, 2005; Gatesy *et al.*, 2007; Nielsen and Beaumont, 2009; Beaumont *et al.*, 2010; Casillas and Barbadilla, 2017). Thus, to get a complete scenario of the species diversity, phylogeographic relationships and the classification of species within the genus *Parapenaeopsis*, extended studies are required utilizing nuclear and mitochondrial DNA markers.

To conclude, the population genetic diversity of *P. sculptilis* within Bangladesh, based on mitochondrial CO1 barcode, is characterized with low nucleotide and haplotype diversity with shallow genealogy. The mitochondrial DNA variation in *P. sculptilis* did not reveal any distinct population structure within Bangladeshor in larger region covering India and Mozambique. The phylogenetics of the species within the genus *Parapenaeopsis* revealed monophyletic group for almost all taxa except for *P. hardwickii,* which showed a close similarity for some specimens with *M. barbata and M. dobsoni.* Thus the taxonomy of *P. hardwickii* need to be reevaluated. The Bangladesh, Indian and Mozambique populations of *P. sculptilis* should be considered as single evolutionary significant unit (ESU) or conservation unit, which could be managed through regional cooperation. The trend of *Parapenaeopsis sculptilis* population in Bangladesh can be monitored and its harvest could be sustained by using the information of its genetic diversity. Sustainable development of the species could be facilitated through genetic upgradation by selective breeding.

Supplementary Material

The Supplementary Material is available at
https://www.alr-journal.org/10.1051/alr/2020027/olm.

Appendix 1. Median-joining haplotype network based on 40 sequences of mitochondrial CO1 barcode of *Parapenaeopsis sculptilis* sampled along the Bangladesh coastline.
Appendix 2. Pairwise genetic distances (Φ_{ST}) and F-statistics on haplotype frequencies (F_{ST}) between *P. sculptilis* populations from Bangladesh, India and Mozambique.

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