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 *Parapenaeopsis*, the population genetic variation of *Parapenaeopsis 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 $F_{ST} = -0.014, p > 0.05; F_{ST} = 0.061, p = 0.04$), 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 *Parapenaeopsis* 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* (Alcock, 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 values for...
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), Peneaus monodon (Fabricius, 1798), Peneaus 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 species of penaeid shrimps.Cryptic diversity or distinct lineages in the IWP region were detected in several species which have been, diverging for millions 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 (Bouchemouss 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.

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, precipitated 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 (Excoffier 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 (\(\Phi_{ST}\)) 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 \(D = d_{xy} - [(d_{xx} + d_{yy})/2]\), where \(d_{xy}\) is the mean difference between populations \(x\) and \(y\), and \(d_{xx}\) and \(d_{yy}\) 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 \(\tau = \tau/2 \mu\), where \(\tau\) is the median of the mismatch distribution = 0.85696, \(\mu\) 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 \(6.0 \times 10^7\) times with a sample per 1000 generations, following the “GTR + I” substitution model, selected after comparison of likelihood of different models using PhyML (Guindon 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 \(1.0 \times 10^7\) 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.

<table>
<thead>
<tr>
<th>Species</th>
<th>Sampling location</th>
<th>Sample size</th>
<th>GenBank accession number</th>
<th>Reference</th>
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<tr>
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<td>Bangladesh</td>
<td>40</td>
<td>MW381251-MW381290</td>
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<td></td>
<td>Bangladesh</td>
<td>1</td>
<td>MN200398</td>
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<tr>
<td></td>
<td>India</td>
<td>11</td>
<td>KU341998-KU342000</td>
<td><em>2</em></td>
</tr>
<tr>
<td><strong>Sub-Total</strong></td>
<td></td>
<td><strong>58</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>M. sculptilis</em></td>
<td>Bangladesh</td>
<td>1</td>
<td>MH429355</td>
<td><em>3</em></td>
</tr>
<tr>
<td></td>
<td>Mozambique</td>
<td>5</td>
<td>KP297897-KP297901</td>
<td><em>4</em></td>
</tr>
<tr>
<td><strong>Sub-Total</strong></td>
<td></td>
<td><strong>58</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>P. aff. sculptilis</em></td>
<td>India</td>
<td>1</td>
<td>KX399432</td>
<td><em>5</em></td>
</tr>
<tr>
<td></td>
<td>Sri Lanka</td>
<td>18</td>
<td>HQ180247-HQ180264</td>
<td>De Croos and Palsson (2010)</td>
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<tr>
<td><em>P. coromandelica</em></td>
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<td>5</td>
<td>KX39140-KX39141</td>
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<tr>
<td></td>
<td>India</td>
<td>22</td>
<td>KF613003-KU341997</td>
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<td><strong>Sub-Total</strong></td>
<td></td>
<td><strong>58</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>P. tenella</em></td>
<td>China</td>
<td>2</td>
<td>KM164420</td>
<td>Meng et al. (2019), <em>10</em></td>
</tr>
<tr>
<td><em>P. hungerfordi</em></td>
<td>China</td>
<td>2</td>
<td>NC_038069, FJ345655</td>
<td></td>
</tr>
<tr>
<td><em>P. cornuta</em></td>
<td>China</td>
<td>1</td>
<td>KP072694</td>
<td>Yang et al. (2015)</td>
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<tr>
<td><em>P. hardwickii</em></td>
<td>India</td>
<td>6</td>
<td>KU341994-KU341996</td>
<td></td>
</tr>
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<td></td>
<td>China</td>
<td>30</td>
<td>KU899136, KU302814, M1577540</td>
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<tr>
<td><em>Parapeneaopsis sp.</em></td>
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<td>1</td>
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<td></td>
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<tr>
<td><strong>Sub-Total</strong></td>
<td></td>
<td><strong>94</strong></td>
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</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td><strong>152</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*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. Dhwawde *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. Dhwawde *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, Karamedua, Sirkkazi, 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.
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. *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 \((\Phi_{ST} = -0.014, p > 0.05)\) 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, \(\Phi_{ST}\) 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 non-significant \((p > 0.05; \text{Appendix 2})\).

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

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**Table 2.** Genetic diversity of *Parapenaeopsis sculptilis* from Bangladesh based on mitochondrial CO1. \(N = \text{No. of individuals}, N_h = \text{No. of haplotypes}, H_R = \text{Haplotype richness}, h = \text{haplotype diversity}, \pi = \text{Nucleotide diversity}, S = \text{No. of segregating sites}, \text{SE} = \text{standard error.} \)

<table>
<thead>
<tr>
<th>Gene</th>
<th>Sampling Location</th>
<th>(N)</th>
<th>(N_h)</th>
<th>(H_R)</th>
<th>(h) ± SE</th>
<th>(\pi) ± SE</th>
<th>(S)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO1</td>
<td>Sundarban, Satkhira</td>
<td>23</td>
<td>6</td>
<td>4.7</td>
<td>0.40 ± 0.13</td>
<td>0.0009 ± 0.0009</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Teknaf, Cox’s Bazar</td>
<td>17</td>
<td>9</td>
<td>9</td>
<td>0.79 ± 0.10</td>
<td>0.0022 ± 0.0016</td>
<td>9</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>40</td>
<td>12</td>
<td>11.3</td>
<td>0.58 ± 0.09</td>
<td>0.0014 ± 0.0011</td>
<td>11</td>
</tr>
</tbody>
</table>
showed a closest similarity with (GB accession numbers: NC_030277 and KU302814) which P. hungrdordi = C2 effective population of 6.51 slow expansion for at least 30 Kyr (Fig. 4) with a current bottleneck or a selective sweep.

Mozambique except for an individual of P. hardwickii. except P. hardwickii India, which diverged from cluster II comprising of P. stylifera (SL−2) P. coromandelica (SL−16) P. stylifera (IN−1) P. coromandelica (SL−5) Metapenaeopsis barbata Metapenaeus dobsoni P. hardwickii (CH−1) P. hardwickii (CH−7) P. stylifera (IN−9) P. stylifera (IN−11) P. stylifera (IR−2) P. coromandelica (IN−5) P. coromandelica (IN−8) P. stylifera (IR−2) P. coromandelica (CH−1) P. hardwickii (BD−1) P. hardwickii (IN−2) P. hardwickii (BD−2) P. hardwickii (CH−21) P. cornuta (CH−1) P. stylifera (IR−2) P. stylifera (IN−11) P. stylifera (IN−9) P. aff. sculpitlis (IN−1) P. coromandelica (SL−2) P. coromandelica (CH−1) P. stylifera (IR−2) P. coromandelica (IN−5) P. coromandelica (SL−16) P. stylifera (IN−1) Metapenaeopsis barbata Metapenaeus dobsoni P. hardwickii (CH−1) P. hardwickii (CH−1) P. sculptilis (BD−37) M. sculptilis (MO−5) P. sculptilis (BD−4) P. sculptilis (IN−12) P. hardwickii (IN−1) P. hardwickii (BD−1) P. hardwickii (IN−3) P. hardwickii (CH−21) P. cornuta (CH−1) P. stylifera (IR−2) P. stylifera (IN−11) P. stylifera (IN−9) P. aff. sculpitlis (IN−1) P. coromandelica (SL−2) P. coromandelica (IN−5) P. coromandelica (SL−16) P. stylifera (IN−1) Metapenaeopsis barbata Metapenaeus dobsoni P. hardwickii (CH−1) P. hardwickii (CH−1)

\[ N_e x \times 10^6 \] years before present x10^3 0 5 10 15 20 25 30

Fig. 4. Bayesian Skyline Plot of Parapenaeopsis sculpitlis 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 (Ne x 10^6) per generation is traced back in time from the present to the past.

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 \( 6.51 \times 10^6 \) (CI: \( 0.48 \times 10^6–29.43 \times 10^6 \)), 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. sculpitlis 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. sculpitlis 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. sculpitlis

The haplotypes of P. sculpitlis 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 slack of differentiation could reflect gene flow or a recent divergence within Bangladesh as the P. sculpitlis 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 Mozambique. 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 Fs. 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 phyogeography 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 CO1. 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 seem 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. styliifera (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 Parapeneaopsis. 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 (Leaché 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 mtDNA, 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 (FST) and F-statistics on haplotype frequencies (FST) between P. sculptilis populations from Bangladesh, India and Mozambique.
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