Genetic diversity in natural populations of neogastropod, *Babylonia Zeylanica* (Bruguiere, 1739) along Tamilnadu, southeast coast of India: A molecular approach through microsatellite markers

Mootapally Chandra Shekar¹,²*, Sabapathi Arularasan¹, Galib Uz Zaman², Pandian Krishnan³ & Eswaran Suresh⁴

¹Centre of Advanced Study in Marine Biology, Annamalai University, Parangipettai-608 502, India
²Department of Animal Genetics and Breeding, College of Veterinary Science, Assam Agricultural University, Guwahati-781022, India
³Conservation of Costal and Marine Resources Division, National Center for Sustainable Coastal Management, Anna University, Guindy-600025, India
⁴National Bureau of Fish Genetic Resources (ICAR), Lucknow - 226002, India

*E-mail: chandu.avi@gmail.com*

Received 30 May 2014; revised 27 November 2014

Genetic diversity and population structure of six populations of commercially important whelk, *B. zeylanica* from Southeast coast of India were studied. A total of 187 individuals were genotyped at 12 loci and differences in the genetic diversity between populations were correlated with known population histories. Study identified 475 alleles and all the studied loci were highly polymorphic. The number of alleles ranged between 4 and 12 with a global mean of 6.597. Global mean of observed and expected heterozygosities were 0.547 and 0.794 respectively. Within population inbreeding estimate (FIS) value (0.311) indicated shortfall of heterozygosity in the populations. Microsatellite analysis revealed less genetic diversity in all studied genetic groups. Analysis of Molecular Variance (AMOVA) showed that 4% of the total variation was due to differences between genetic groups.

**Keywords:** *Babylonia Zeylanica*; Microsatellites; Heterozygosity; Polymorphic Information Content

**Introduction**

Whelks (*B. zeylanica*) are important food species in Indo-pacific region¹. Production of *B. zeylanica* in India increased considerably from 30,499 t in 1950 to 1,21,657 t in 2011². High demand for export of *B. zeylanica* meat has lead to overexploitation of undersized whelk which results in the reduction of the natural stock. Recently, due to over exploitation, some species have been listed as endangered³. In 2001, the gastropods like trochus, turbo and large number of other ornamental species have been listed under schedule I of the Wild Life Protection Act, 1972⁴. Literatures on population genetics of marine molluscs are very scanty⁵. Moreover, recent developments in gastropod fishery and its utilization indicate the need to have scientific data on the genetic diversity of selected gastropods for planning their conservation and management⁶.

*B. zeylanica* has a very smooth shell with high spire, rounded whorls, slightly impressed sutures and a large ovate body whorl. Though, the shell bears distinctive brownish patches on white background, the major characteristic is the violet staining at the fasciole (Fig. 1). This species is distributed in southeast and southwest coasts of India and also in Andaman and Nicobar and Lakshadweep Island waters.

**Fig. 1**— a typical *B. zeylanica* from Tamilnadu coastal waters
Information on genetic diversity of a particular species under natural conditions will provide inputs for their domestication. Knowledge about genetic diversity levels and population differentiation through microsatellite analysis on natural populations will be useful for formulating management measures for sustainable exploitation and conservation of this commercially important species.

### Table 1—Summary of sampling locations and number of whelks sampled

<table>
<thead>
<tr>
<th>Species</th>
<th>Sampling sites</th>
<th>Acronyms</th>
<th>Latitude and longitude</th>
<th>Number of samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. zeylanica</td>
<td>Mudasal Odai</td>
<td>MUD</td>
<td>11°29’07.74” N 79°46’28.10” E</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>Nagapattinam</td>
<td>NAG</td>
<td>10°45’37.94” N 79°50’57.82” E</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>Rameshwaram</td>
<td>RAM</td>
<td>9°16’49.46” N 79°19’02.44” E</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>Punnaikayal</td>
<td>PUN</td>
<td>8°38’15.20” N 78°07’13.63” E</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>Arogyapuram</td>
<td>ARO</td>
<td>8°07’10.76” N 77°33’32.25” E</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>Colachel</td>
<td>COL</td>
<td>8°10’20.67” N 77°14’56.42” E</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td></td>
<td></td>
<td>187</td>
</tr>
</tbody>
</table>

Population genetic studies of commercially important marine molluscs based on microsatellite markers are useful for the analysis of population structure and relationships as demonstrated by various studies viz., genetic diversity in *Mytilus galloprovincialis* of Iberian Peninsula, marine black nerite, *Nerita atraentosa*, swan mussel, *Anodonta cygnea*, spotted *Babylonia areolata* and parental assignment in the eastern oyster as well as other marine molluscs species from other countries. However, the population structure of *B. zeylanica* from Indian seas has not been assessed yet using molecular markers.

Twelve well-characterized polymorphic microsatellite loci of widely separated natural populations of *B. zeylanica* from southeast coast of India is examined in the present study to understand their genetic diversity and population structure in the natural populations along Tamilnadu coast using microsatellite markers hence to establish a microsatellite profile.

### Materials and Methods

Fresh specimens of *B. zeylanica* were collected from commercial catch of fish landing centers at different localities in Tamilnadu (Fig. 2). 30 to 32 specimens were collected from each location, with the total collection of 187 (Table 1). Foot tissue of each specimen was fixed in TNES-buffer and stored at 4°C until use.

Genomic DNA was isolated from foot tissue of *B. zeylanica* following the phenol-chloroform method with minor modifications. DNAzol (Invitrogen) reagent was used instead of SDS and proteinase K. The DNA was observed on 0.8% agarose gel containing ethidium bromide. The quantification of DNA was done using ND-1000 spectrophotometer (Thermo scientific). A total of 12 well-characterized microsatellite markers (Table 2) developed by Longo *et al.* for black murex, *Hexaplex nigritus* were used based on their level of polymorphism, allele size range and reliability of allele to evaluate genetic structure in *B. zeylanica*.

### Table 2—Details of microsatellite markers used in present study

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Locus name</th>
<th>Primer sequences (5’→ 3’)</th>
<th>Repeat motif</th>
<th>Labeled dye</th>
<th>T&lt;sub&gt;r&lt;/sub&gt; (°C)</th>
<th>Allele size range (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>HNI_A3</td>
<td>F:CCATTGCTGAGAGACTGAAGAA R:ACATTTCGCGTTGTTTATGGC</td>
<td>(CA)&lt;sub&gt;22&lt;/sub&gt;</td>
<td>6 FAM</td>
<td>58</td>
<td>238–268</td>
</tr>
<tr>
<td>2</td>
<td>HNI_A12</td>
<td>F:AGTAGGGCCGCTTTTCACTTC R:CACGAAAACCTGCAAAAGACG</td>
<td>(CA)&lt;sub&gt;17&lt;/sub&gt;</td>
<td>ROX</td>
<td>58</td>
<td>136–216</td>
</tr>
<tr>
<td>3</td>
<td>HNI_A5</td>
<td>F:CTGTGGCAACATCTCTCATTTT T:ATTGTTTCATGCTTTACAAAGATGG</td>
<td>(TAA)&lt;sub&gt;7&lt;/sub&gt;</td>
<td>Tamra</td>
<td>57</td>
<td>164–182</td>
</tr>
<tr>
<td>4</td>
<td>HNI_A120</td>
<td>F:CTAGCCCACGTGATGGTCT R:GGTGTCAGTCTCATTGG</td>
<td>(CA)&lt;sub&gt;21&lt;/sub&gt;</td>
<td>HEX</td>
<td>57</td>
<td>202–282</td>
</tr>
<tr>
<td>5</td>
<td>HNI_A10</td>
<td>F:GAATCCATCTGGTTTTCACAG R:AAAGAGAGGGAAGAAGAAATAGG</td>
<td>(CA)&lt;sub&gt;31&lt;/sub&gt;</td>
<td>6 FAM</td>
<td>56</td>
<td>133–237</td>
</tr>
<tr>
<td>6</td>
<td>HNI_B9</td>
<td>F:GGGTCTCACAACACGGTG T:GATGGASTGATGTTG</td>
<td>(CATC)&lt;sub&gt;19&lt;/sub&gt;</td>
<td>Tamra</td>
<td>56</td>
<td>121–161</td>
</tr>
<tr>
<td>7</td>
<td>HNI_B120</td>
<td>F:GCAAACACACTCACACACTT R:CATTCAAGTAAGCAGGAAGAC</td>
<td>(CTAC)&lt;sub&gt;26&lt;/sub&gt;</td>
<td>ROX</td>
<td>57</td>
<td>240–286</td>
</tr>
<tr>
<td>No.</td>
<td>Primer Set</td>
<td>Forward Primer</td>
<td>Reverse Primer</td>
<td>Microsatellite Repeat</td>
<td>Allele Range</td>
<td>FAM/HEX/Tamra</td>
</tr>
<tr>
<td>-----</td>
<td>------------</td>
<td>----------------</td>
<td>----------------</td>
<td>----------------------</td>
<td>--------------</td>
<td>---------------</td>
</tr>
<tr>
<td>8</td>
<td>HNI_A117</td>
<td>F: GGCAGAACGCCATTAACTATG</td>
<td>R: CAGGGATCGACAGAAGAATCAG</td>
<td>(TCTG)$_8$</td>
<td>6 FAM</td>
<td>57</td>
</tr>
<tr>
<td>9</td>
<td>HNI_C12</td>
<td>F: TGTGCAATACGATGGAGAGTG</td>
<td>R: GGTCTGTCTTACATGGAAG</td>
<td>(TACA)$_{23}$</td>
<td>HEX</td>
<td>58</td>
</tr>
<tr>
<td>10</td>
<td>HNI_B12</td>
<td>F: CACGCACCGTATACATACAC</td>
<td>R: CTTATCTTCCCCCTCTTCCTTT</td>
<td>(CA)$_{51}$</td>
<td>Tamra</td>
<td>58</td>
</tr>
<tr>
<td>11</td>
<td>HNI_B104</td>
<td>F: ATCGAAGAAGTGGCGATATTG</td>
<td>R: ACTGTTAAAGATGGGTTTGTG</td>
<td>(CATC)$_{14}$</td>
<td>HEX</td>
<td>57</td>
</tr>
<tr>
<td>12</td>
<td>HNI_C102</td>
<td>F: TGAGGCTTCGTGGTGAAG</td>
<td>R: CGTCATAAAATGCAACATAGTG</td>
<td>(TACA)$_{21}$</td>
<td>6 FAM</td>
<td>57</td>
</tr>
</tbody>
</table>

Fig. 2—Distribution of sampling localities for natural population of *B. zeylanica* for present study (Politeness to Google earth map).
The forward primer of each marker was fluorescently labeled with FAM, ROX, TAMRA or HEX dye. All microsatellite markers were first checked under single locus amplification conditions to evaluate their performance in the multiplex. Multiplex PCR was used for multicolor fluorescence genotyping following Henegariu et al.\textsuperscript{16} and Loffert et al.\textsuperscript{17} for setting up the initial parameters. The basic PCR reaction mixture (15 µl) containing 20-50 ng of template DNA; 1.5 mM MgCl\textsubscript{2}; 5 picomoles each of forward and reverse primers; 1 unit of taq DNA polymerase and 200 mM dNTPs was prepared. Amplification was carried out with initial denaturation at 95°C for 2 min followed by 30 cycles of denaturation (95°C for 30 sec), annealing (52°C to 58°C for 30 sec) and extension (72°C for 45 sec) using Applied Biosystems Veriti\textsuperscript{TM} 96-well thermal cycler.

Genotyping was carried out on an automated DNA Sequencer (ABI HITACHI 3500) and data were analyzed using Gene Mapper v. 4.0 (Applied Biosystems) to generate genotype calls for each locus by using GS 500 (- 250) LIZ as size standard. GenAlex v. 6.5\textsuperscript{18} was used to estimate genetic diversity and differentiation parameters. Parameters estimated include number of alleles (N\textsubscript{a}), allele frequencies, effective number of alleles (N\textsubscript{e}), Private alleles, observed (H\textsubscript{o}) and expected (H\textsubscript{e}) heterozygosity, F-statistics, Shanon’s information index (I), Hardy-Weinberg equilibrium (HWE), Principal component analysis (PCA) and Analysis of molecular variance (AMOVA). Polymorphic information content (PIC) was calculated using Excel Microsatellite Toolkit 3.1\textsuperscript{19}. Expected frequencies of null alleles at the 12 loci were calculated using Micro-Checker v. 2.2.1\textsuperscript{20}. Phylogenetic and molecular evolutionary analyses were conducted using MEGA v. 5.05\textsuperscript{21}. F-statistics were estimated following Weir and Cockerham\textsuperscript{22}. Wright’s F\textsubscript{ST} summarizes the effects of non random mating within subpopulations on average individual heterozygosity. F\textsubscript{ST} characterizes the reduction in individual heterozygosity expected within subpopulations relative to a total population as a result of genetic drift, effectively measuring the extent of population subdivision and the counteracting evolutionary processes of drift on the one hand and gene flow on the other. Finally, F\textsubscript{IT} summarizes the extent to which average individual heterozygosity deviates from Hardy-Weinberg expectations due to both nonrandom mating within subpopulations and population subdivision\textsuperscript{23}. 
## Table 3—Microsatellite analysis in natural populations of *B. Zeylanica*

<table>
<thead>
<tr>
<th>POP</th>
<th>Panel 1</th>
<th>Panel 2</th>
<th>Panel 3</th>
<th>Panel 4</th>
<th>Panel 5</th>
<th>Panel 6</th>
<th>Average across loci</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M1</td>
<td>M2</td>
<td>M3</td>
<td>M4</td>
<td>M5</td>
<td>M6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.833±0.423</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.158 5.445 4.019±0.241</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>6.964 6.219 6.125±0.332</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PUN</td>
<td>5.311</td>
<td>6.480</td>
<td>3.532</td>
<td>5.684</td>
<td>5.400</td>
<td>5.378</td>
<td>9.257 6.481 7.621</td>
</tr>
<tr>
<td></td>
<td>7.906 4.765 6.062±0.412</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ARO</td>
<td>5.000</td>
<td>5.357</td>
<td>3.674</td>
<td>4.506</td>
<td>4.173</td>
<td>5.844</td>
<td>8.982 4.592 5.294</td>
</tr>
<tr>
<td></td>
<td>6.081 4.447 3.913 5.515±0.408</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.235 3.562 3.081±0.272</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>POP</td>
<td>7.137</td>
<td>1.905</td>
<td>1.677</td>
<td>1.721</td>
<td>1.809</td>
<td>1.549</td>
<td>1.597 1.701 1.564</td>
</tr>
<tr>
<td></td>
<td>1.759 1.466 1.703±0.483</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PUN</td>
<td>6.250</td>
<td>0.250</td>
<td>0.125</td>
<td>0.222</td>
<td>0.412</td>
<td>0.867</td>
<td>0.500 0.833 0.813</td>
</tr>
<tr>
<td></td>
<td>0.888 0.523±0.077</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ARO</td>
<td>5.791</td>
<td>0.813</td>
<td>0.728</td>
<td>0.778</td>
<td>0.760</td>
<td>0.829</td>
<td>0.899 0.813 0.811</td>
</tr>
<tr>
<td></td>
<td>0.755 0.744 0.795±0.013</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>COL</td>
<td>7.646</td>
<td>0.826</td>
<td>0.753</td>
<td>0.785</td>
<td>0.795</td>
<td>0.721</td>
<td>0.715 0.833 0.773</td>
</tr>
<tr>
<td></td>
<td>0.727 0.699 0.766±0.044</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>POP</td>
<td>5.384</td>
<td>0.332</td>
<td>0.612</td>
<td>0.529</td>
<td>0.492</td>
<td>0.163</td>
<td>0.280 0.428 0.113</td>
</tr>
<tr>
<td></td>
<td>0.075 0.311±0.033</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Significant (P<0.05); **Highly significant (P<0.01); NS: Not significant (P≥0.05); POP: Population; PAR: Parameter; M1: HNI_A3; M2: HNI_A12; M3: HNI_A5; M4: HNI_A120; M5: HNI_A10; M6: HNI_B9; M7: HNI_B120; M8: HNI_A117; M9: HNI_C12; M10: HNI_B121; M11: HNI_B104; M12: HNI_C102; Number of alleles; Ne: Effective number of alleles; PIC: Polymorphic information content; He: Observed Heterozygosity; Ho: Expected Heterozygosity; Fs: Deficit or excess of Heterozygotes; PFW: Probability value of significant deviation from Hardy–Weinberg equilibrium; I: Shannon’s Information Index.
Linkage disequilibrium was tested using a contingency table test for genotype linkage disequilibrium between pairs of loci in a population, based upon the null hypothesis that genotypes at one locus are independent of genotypes at other locus. Relationships based on genetic distance estimates generated from microsatellite data among the six *B. zeylanica* populations were made and a dendrogram was plotted, employing the UPGMA method with arithmetic averages based on Nei's genetic distance estimates, in MEGA. To test the confidence level of each branch in the dendrogram, data were bootstrapped 1000 times. PCA leads to a representation of populations as a cloud of points in a metric space. Comparison between the inertia of single-marker enables to compare the typological value of markers. Inertia can be split up according to axes and/or loci. AMOVA was computed to determine the genetic differentiation between groups through $F_{ST}$ estimations.

**Results**

**Genetic variability within populations**

A total of 475 alleles were detected over all twelve microsatellite loci, with the number of alleles per locus ranging from four at HN1_A12, HN1_A5, HN1_A120, HN1_B9, HN1_B12 and HN1_B104 to 12 at HN1_B120 (Table 3). All natural populations exhibited relatively low genetic variation and were dissimilar with average number of alleles per locus varying from 5.000 (NAG) to 7.917 (PUN) and average number of effective alleles varying from 4.019 (NAG) to 6.125 (RAM) with the global mean of 6.597 and 5.177 respectively. Observed heterozygosities across loci ranged from 0.438 (PUN) to 0.676 (MUD) and expected heterozygosities ranged from 0.742 (NAG) to 0.830 (RAM) with the global mean of 0.547 and 0.794 respectively. Observed heterozygosities were lower than expected in the all studied populations. Both effective alleles and expected heterozygosities differed significantly across all populations. PIC value revealed that all of the loci studied were highly polymorphic in nature with a global mean of 0.764±0.040 (Table 3).

Wright’s fixation index ($F_{IS}$) a measure of heterozygote deficiency or excess (inbreeding coefficient), and significance values for each locus in six populations are given in Table 3. Significant departures from Hardy–Weinberg expectations were observed in 2 of 72 (12 loci×6 populations) single locus exact tests after applying a sequential Bonferroni correction. Even though, Shannon’s information index (I) which measures the level of diversity, was sufficiently high with a global mean of 1.710±0.030 (Table 3), $F_{IS}$ values greater than zero indicating a deficiency of heterozygotes was evident in these cases. Microsatellite loci exhibiting $+F_{IS}$ values were tested for presence of null alleles. Estimated null allele frequencies assessed with MICROCHECKER were not significant (P>0.05) indicating the absence of null alleles and false homozygotes at any locus. Therefore, for population genetic analysis, information from all twelve microsatellite loci was considered. A total of eleven private alleles were observed in MUD (at loci HN1_A3, HN1_A10, HN1_C12 and HN1_B12), NAG (at loci HN1_B104 and HN1_C102), RAM (at loci HN1_A12 and HN1_A117) and COL (at loci HN1_A10 and HN1_A117) populations (Table 4).

<table>
<thead>
<tr>
<th>Locus</th>
<th>Size (bp)</th>
<th>MUD</th>
<th>NAG</th>
<th>RAM</th>
<th>COL</th>
</tr>
</thead>
<tbody>
<tr>
<td>HN1_A3</td>
<td>254</td>
<td>0.021</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>HN1_A10</td>
<td>201</td>
<td>0.105</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>HN1_C12</td>
<td>289</td>
<td>0.037</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>HN1_B12</td>
<td>301</td>
<td>0.125</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>HN1_B12</td>
<td>305</td>
<td>0.050</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>HN1_B104</td>
<td>211</td>
<td>-</td>
<td>0.071</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>HN1_C102</td>
<td>187</td>
<td>-</td>
<td>0.139</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>HN1_A12</td>
<td>134</td>
<td>-</td>
<td>-</td>
<td>0.147</td>
<td>-</td>
</tr>
<tr>
<td>HN1_A117</td>
<td>118</td>
<td>-</td>
<td>-</td>
<td>0.231</td>
<td>-</td>
</tr>
<tr>
<td>HN1_A10</td>
<td>143</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.222</td>
</tr>
<tr>
<td>HN1_A117</td>
<td>126</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.200</td>
</tr>
</tbody>
</table>

**Genetic differentiation between populations**

Wright’s $F_{ST}$ characterizes the reduction in individual heterozygosity expected within subpopulations relative to a total population as a result of genetic drift, effectively measuring the extent of population subdivision and the counteracting evolutionary processes of drift on the one hand and gene flow on the other. Pairwise $F_{ST}$ estimates between population pairs varied significantly (P<0.01) from zero for all the pairs along the Tamilnadu coast of India (Table 5).
The overall $F_{ST}$ value of 0.106 indicated that 10.6% of the detected variation arises from between population differences and 89.4% from within population differentiation. Genetic distance ($D_A$) tended to be the least (0.243) between PUN and RAM and the widest (1.687) between COL and NAG (Table 5). The genetic differentiation between different pairs of populations was significantly different from zero. Further, an AMOVA analysis was carried out to analyze the variation within and between populations which revealed that percentage of variation among and within populations were 4 and 96 respectively (Table 6).

| Table 5—Pairwise $F_{ST}$ (above diagonal) and $D_A$ (below diagonal) among six populations |
|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| MUD  | NAG  | RAM  | PUN  | ARO  | COL  |
| MUD   | -    | 0.041** | 0.061** | 0.068** | 0.083** | 0.093** |
| NAG  | 0.315 | -    | 0.071** | 0.076** | 0.098** | 0.110** |
| RAM  | 0.742 | 0.801 | -    | 0.021** | 0.059** | 0.054** |
| PUN  | 0.873 | 0.888 | 0.243 | -    | 0.045** | 0.053** |
| ARO  | 1.073 | 1.266 | 0.775 | 0.505 | -    | 0.064** |
| COL  | 1.399 | 1.687 | 0.680 | 0.663 | 0.770 | -   |

| Table 6—AMOVA analysis in six populations |
|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| Source of variation         | Degree of freedom           | Sum of squares              | Variance component          | Percentage of variation     |
| Among Pops                  | 5                           | 164.879                    | 0.614                      | 4%                          |
| Within Pops                 | 181                         | 2503.923                   | 13.834                     | 96%                         |
| Total                       | 186                         | 2668.802                   | 14.448                     | 100%                        |

The evolutionary history was inferred using the UPGMA method based on Nei’s genetic distances ($D_A$) (Fig. 3). Optimal tree with the sum of branch length = 2.03727854 is shown. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree.

Clustering pattern of the whelk populations showed their geographical origin. To supplement, principal component analysis (PCA) was performed using the $D_A$ values (in Fig. 4). The first two principal components explained 52.37% of the total variation. The first axis contributed about 36.46% of the inertia and distinguished the all studied populations from each other. Second axis contributed 15.19% of the inertia, as a result, these two axes revealed a pattern of association.

![Fig. 3—UPGMA clustering using Nei’s unbiased genetic distance (1978) of the B. zeylanica sampling locations based on twelve microsatellite loci](image)

![Fig. 4—Coordinates of the six populations plotted in dimensions 1 and 2 of the PC](image)
Discussion

Twelve polymorphic microsatellite loci developed for *Hexaplex nigritus* by Longo et al.\textsuperscript{15} were used to evaluate genetic diversity and genetic variation in natural population of *B. zeylanica* collected from four different geographical locations of Tamil Nadu coastal waters of India (Fig. 2). Microsatellite markers used for genetic diversity studies should have more than four alleles in order to have a precise estimate of genetics distance\textsuperscript{27}. In the present investigation all markers had more than four numbers of alleles indicating that the markers used are appropriate to analyse diversity in the whelk genetic groups of Tamilnadu coastal waters.

Allelic and gene diversities are considered as responsible indicators of genetic variation within the populations\textsuperscript{28}. All the investigated populations in the present study have shown low genetic variability based on their estimates of effective number of alleles and observed heterozygosity. The global mean number of alleles observed (6.597) in the present study is lower than the mean number (13.125) reported for *B. areolata* \textsuperscript{11} and *H. nigritus* (19.385), marine gastropods\textsuperscript{15}. Global mean of effective alleles (5.177) is lower than the observed number of alleles which might be due to very low frequency of most of the alleles at each locus and few alleles might have contributed to the major part of the allelic frequency. However, contrary to the present finding, lower mean number of effective alleles (4.664) was reported in *B. spirata*\textsuperscript{29}.

The global mean of observed and expected heterozygosity (0.547 and 0.794) in the present study is lower than the observed (0.700) and expected (0.854) heterozygosity in *B. areolata* \textsuperscript{11}. However, the present findings of observed and expected heterozygositys observed in the present investigation are in accordance with the findings of Longo *et al.*\textsuperscript{15} in *H. nigritus* populations viz., Punta Chueca (PCH) (0.608 and 0.742); El Borrascoso (EBO) (0.632 and 0.747); Isla San Jorge (ISJ) (0.679 and 0.775); San Luis Gonzaga (SLG) (0.7 and 0.762). Genetic markers showing PIC values higher than 0.5 are normally considered as informative in population genetic analyses\textsuperscript{30}. Consequently, all the loci in the present investigation possessed high PIC values (above 0.50) signifying once again that these markers are highly informative for characterization of *B. zeylanica* genetic groups of South East coast of India. Global mean PIC value (Table 3) in the *B. zeylanica* populations under study (0.764) corroborated with the mean PIC (0.727) in *B. spirata*\textsuperscript{29}.

The deviation from HWE (Table 3) and moderately higher global mean $F_{IS}$ (0.311) observed in the present study could be attributed to several factors viz., non-random mating, non-amplifying alleles or the genetic drift. A similar observation of heterozygosity shortfall (32.3 %) has also been reported in *B. spirata* population\textsuperscript{29}. Inbreeding detected in these populations could possibly be attributed to increased exploitation rate in recent past and fishing of undersized whelks, owing to the market demand. Generally, gastropods are having slow migration rate and small breeding territories which lead to their genetic death. Altogether the effective population size is curtailed and breeding between relatives stimulates inbreeding and genetic drift. Micro-Checker analysis revealed that absence of null alleles in *B. zeylanica* population sampled here and also supports to nonexistence of stutter bands\textsuperscript{31}. The fixation coefficient of populations ($F_{ST}$) had a global mean of 0.106, showing that 10.6% of the genetic variation was explained by differences between populations. In addition, AMOVA indicated the genetic variation between populations to be 4%, confirming moderately higher within population diversity in the investigated genetic groups. However, the measures of population differentiation indicated trivial differences between groups.

There was no evidence of significant population sub-structuring among all samples from natural waters. The PCA (Fig. 4) analysis using Nei's\textsuperscript{25} unbiased minimum distance clearly supported the above population differentiation analysis based on pair-wise $F_{ST}$ values. Moreover, an unrooted UPGMA diagram (Fig. 3) is based on a dissimilarity matrix of genetic distances that are symmetrical and unsigned, and as indicated that populations were grouped according to their geographic locations.

The present finding of genetic divergence of microsatellite allele frequencies among sampled *B. zeylanica* populations suggests that Indian
southeast coast populations are drawn from the same panmictic, randomly mating gene pool. Whether each of the six sub-populations studied should therefore be treated as separate management units cannot be determined with the information available now. A natural extension of the present study will be to examine life history traits in this species at the population level to assist in formulating strategies for managing and supplementing wild stocks. Whatever management strategies are eventually developed, evaluation and monitoring of genetic diversity and population structure will be critical for their success. The information would also be required for successful domestication programme. In the long term, the extent of genetic gain under selection is proportional to the available genetic variance and hence, the study calls for further investigation to identify the factor(s) that influence genetic heterogeneity in B. zeylanica populations.

Conclusion
The PIC values observed in the present study is indicative of the fact that the markers used are highly informative for characterization of whelk germplasms of India. With the actual genetic diversity and the population structure of these B. zeylanica species evaluated, it was possible to clarify the importance as well as to propose some management strategies for this genetic resource. This fact, coupled with evident environmental adaptation, emphasizes the importance of genetic regulation and conservation of these indigenously evolved germplasm and for sustainable utilization. The results show that levels of genetic diversity in natural populations of specific genetic group are moderately low. Estimates of differentiation and genetic structure confirm the geographical origin of individual populations.

Acknowledgement
Authors are thankful to the authorities of Annamalai University for constant support and encouragement for this study and to authorities of Network Project on Animal Genetic Resources- Core Laboratories from Assam, Gujarat and Chennai for providing necessary facilities, technical support and valuable guidance during the period of study. We also take the opportunity to acknowledge the help rendered by fisherman in the field throughout sample collections.

References