

## Genetic variation of reef-building polychaete *Sabellaria chandraae* in the South-Eastern Arabian Sea based on mitochondrial COI gene sequences

Periasamy Rengaiyan\* and Baban Ingole\*\*

Biological Oceanography Division

CSIR-National Institute of Oceanography, Dona Paula, Goa-403004, India

(\*corresponding to: vasanthperiaswamy@gmail.com)

\*\*Present Address: ESSO-National Centre for Polar & Ocean Research  
Headland Sada, Vasco-da-Gama, Goa - 403804, INDIA

### Abstract

The honeycomb worm *Sabellaria chandraae* is the most common reef-building polychaete in the intertidal area along the South-Eastern Arabian Sea between the Ratnagiri and Kanyakumari. We used the mitochondrial cytochrome c oxidase subunit I (mtCOI) gene to examine the levels of genetic variation among population. The samples were collected from seven intertidal locations along the South-Eastern Arabian Sea. The hierarchical Analysis of Molecular Variance (AMOVA) and conventional population statistics ( $F_{ST}$ ) showed a low level of genetic differentiation ( $F_{ST} = 0.0864$ ,  $p < 0.05$ ) among the population of *Sabellaria chandraae* distributed over a distance of  $\geq 1200$  kms. Our results suggests that there was no geographical patterning among the seven populations of honeycomb worm. The present study therefore is able to provide a reference for the single stock population of the reef building *S. chandraae* along the South-Eastern Arabian Sea.

**Key words:** Mitochondrial DNA; Cytochrome c oxidase subunit I; Genetic diversity; reef building; *Sabellaria chandraae*; West Indian Coastal Current; Eastern Arabian Sea.

The reef-building polychaete worms of the genus *Sabellaria* are known as honeycomb worms. They form large reef-like aggregations in many parts of the world. *Sabellaria chandraae* de Silva, 1961 is a broadly distributed polychaete species along the intertidal zone of the Indian and Sri Lanka (Nishi et al. 2010). The habitat selection by its larvae and massive construction of reefs in the intertidal rocky area, require the strong flow of water for the transport of the tube-building material (Achari 1974). In the Indian coastal system, the boundary currents of Western India Coastal Current (WICC) and Eastern India Coastal Current (EICC) plays an important role in the larval dispersal pattern along with the India coast. The WICC flows toward the equator during the Indian summer monsoon season. That is major recruitment period for many benthic organisms along the Indian coast ( Ingole & Parulekar 1998; Sivadas and Ingole 2016). The benthic polychaetes showed 34% of the species being common between the western and eastern basin which was also confirmed by the genetic analysis (Kumar et al. 2012; Kunal et al. 2013) and larval dispersal studies (George et al. 2011). The polychaete distribution is generally influenced by water circulation pattern, availability of suitable habitat, population fecundity and recruitment success (Dawson et al. 2014). Since the origins of the reef-building worms yet remain unclear, therefore we hope that the molecular study will finally bring some clarity in the true evolutionary relationship (Telford 2004). We used the mtCOI gene to investigate the genetic diversity and population variation of 62 individuals of *S. chandraae* collected along the ~1260 km coastline of the South-Eastern Arabian Sea.

Specimens of *S. chandraae* were collected during November 2014 and August 2015 using a hand-held acrylic core from the reefs and preserved in 95% ethanol until DNA extraction. The species was identified based on the key morphological characteristic described in Nishi et al. (2010). After sorting, all the specimens were stored at room temperature in the National Institute of Oceanography (CSIR-NIO), Goa, India with individual voucher numbers. (Table 1). Total genomic DNA was extracted using the commercial QIAamp Tissue Kit (QIAGEN). The partial sequences of mtCOI fragments were amplified using the universal primers (Carr et al. 2011). The PCR reactions were carried out in a total volume of 50 µl that consisted of 50–100 ng genomic DNA, 1 × PCR buffer, 2.5 mM MgCl<sub>2</sub>, 0.2 mM dNTPs, 10 µM primers, and 5U Taq DNA polymerase (Sigma). The temperature profile was as followed: 94°C for 3 min; 30 cycles of denaturation at 94°C for 30 s, annealing at 54.5°C for 30s and extension at 72°C for 60s; followed by a final extension at 72°C for 5 min. The PCR products were examined on 1% agarose gels, stained with Genecolour™ (Biotium, USA), and photographed with transmitted illumination. The PCR-amplified DNA fragments were purified using QIAquick gel purification kit according to manufacturer's instructions (QIAGEN). Sequences were produced using the same primers and determined on an Applied Biosystems (ABI) 3730xl automated DNA sequences,

following the standard cycle sequencing protocol. The sequences were submitted to NCBI GenBank. Allotted sequence number are given in Table 1. The mtCOI gene sequences were aligned using CLUSTAL W (Thompson et al. 1994) implemented in BIOEDIT (Hall 1999). Hierarchical analysis of molecular variance (AMOVA) was used for separating of genetic variance within and among the localities and between the two regions (Northern and Southern). Genetic differentiation was examined by means of pairwise  $F_{st}$  values, using 10,000 permutations to determine significance in Arlequin 3.5 (Excoffier and Lischer 2010).

The Haplotype ( $h$ ) and nucleotide ( $\pi$ ) diversities were estimated using DnaSPv5 (Nei 1987). Total haplotype diversity values of mtCOI gene being 0.21. Total nucleotide diversity of mtCOI gene sequence was 0.0005 (Table 1). The low genetic variation among the *S. chandraae* populations is consistent with the boundary currents of WICC which play an important role in the larval dispersal pattern along the west coast of India. The accurate test of population differentiation (non-differentiation exact P values) showed no significant differences among 62 samples collected from seven different locations. . The AMOVA results of *S. chandraae* shows no difference within the populations ( $F_{CT} = 0.036$ ;  $p > 0.795$ ) and among populations ( $F_{ST} = 0.086$ ;  $p > 0.1$ .) between north and south populations (Table 2). The existence of low genetic variation confirmed a single stock population of *S. chandraae* along the ~1260 km of the west coast of India. Since, the distribution of the species depends on existence of historical factors, reversing WICC. Lacking of oceanic barrier to the larval movement among study areas were further confirmed by the low values of  $F_{ST}$ .

### **Acknowledgements**

We wish to thanks the Director, CSIR-National Institute of Oceanography Goa, for encouragement and facilities. This work was part-funded by the Ministry of Earth Sciences (COMAPS project No.GAP-2741) Govt. of India and Maharashtra Gene Bank (Project No. GAP2871). Our sincere thanks to Shri Ram Murti Meena for sequencing facility. This the contribution no..... of CSIR-NIO Goa.

### **Disclosure statement**

No potential conflict of interest was reported by the authors.

### **Funding**

This work was under COMAPS project No.GAP-2741.

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**Table 1.** Details of nucleotide and haplotyde diversity showed in the COI gene sequences of *Sabellaria chandraae* along the South-Eastern Arabian Sea.

Geographic region	No. of sequences	No. of haplotypes	Haplotype diversity $h$	Nucleotide diversity $\pi$	Lat_Long	Specimen voucher numbers	Genbank accession numbers
Ganapatipule	7	2	0.25	0.0005	17.08 N, 073.16 E	GP0348-GP0355	KX525531-KX525538
Devgad	8	4	0.694	0.001	15.41 N, 073.41 E	DP0339-DP0347	KX525522-KX525530
Vengurla	9	2	0.2	0.0007	15.49 N, 073.38 E	VP0393-VP0402	KX525576-KX525585
Arapol	5	1	0.333	0.0005	15.41 N, 073.41 E	AP0333-AP0338	KX525516-KX525521
Palolem	9	1	0	0	15.00 N, 074.01 E	PP0383-PP0392	KX525566-KX525575
Mangalure	10	1	0	0	13.11 N, 074.44 E	MP0365-MP0375	KX525548-KX525558
Kanyakumari	8	1	0	0	11.52 N, 075.21 E	KP0376-KP0384	KX525539-KX525547

**Table 2.** Analysis of molecular variance (AMOVA) of *Sabellaria chandraae* between the populations

Source of variation	df	Sum of squares	Percentage of variation
Among groups	1	0.095	3.68
Among populations within groups	5	1.058	12.32
Within populations	56	5.386	91.36
Total	62	6.539	100