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Phylogenetic Position and Genetic Diversity of Neridae - Polychaeta Based on Molecular Data from 16S r RNA Sequences

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Abstract: Molecular phylogenetic analysis and genetic diversity distance among two species of marine polychaetes relationship was investigated based on 16S rRNA sequences. Data set be analysed by using Clustal W and MEGA likelihood methods. Genetic differentiation (*D*) among populations was higher in the *Pseudonereis variegata* and *Namalycastis indica D:* 0.64. The differences among the *D* values of all populations forms were significant by ANOCOVA; $F_{28.16}$ =0.784, *P* = 0.676). Our investigation showed how such simple statistical summaries of huge DNA sequences data can enable to identify the structural signature of genome as well as to classify phylogenetic relationships amongst different species reflected in the differentiation of genetic diversity distributions within their DNA sequences.

Key words: Annelida · Molecular taxonomy · Polychaetes · Phylogeny · 16S r RNA

INTRODUCTION

Comparative phylogeographic approaches are essential for inferring historical causes leading to congruent patterns of colonization and population processes and in terms of biodiversity; an identifying areas of endemism as a basis for the protection of marine biodiversity and ecosystems [1]. Whereas the above mentioned hypotheses on polychaeta systematics have been obtained by analyzing morphological data, in recent years many attempts have been made to unravel polychaete relationships using molecular data [2]. Several recent reviews have listed cases where genetic analyses have played a critical role in the recognition of species boundaries [3-5]. In many cases, morphological differences are very subtle, although sympatric forms often have clear differences in life history. Despite this progress, lack of resolution of basal nodes is a recurring issue in molecular studies of polychaetes systematics and rooting of the annelid tree remains an outstanding problem. 16S r RNA - this marker is the mitochondrial version of the large ribosomal subunit, In most analyses to date, only a short 450–500 nucleotide fragment of this gene has been examined corresponding primers designed [6]. This region is typically useful for intraspecific and intrageneric level relationships and has limited utility at higher levels [7]. The molecular phylogenetic studies of polychaete relationships that move beyond tree building to infer the evolution of features such as reproductive mode, feeding habit, larval development and heterochrony is building slowly [8]. Here we report the genetic diversity and phylogenetic relationship with in polychaete groups using 16S r RNA sequences.

MATERIALS AND METHODS

Collection of Samples: Pondicherry mangroves, the study area lies within the boundaries of latitudes 11°46’03” to 11°53’40” North and longitudes 79°49’45” to 79°48’00” East. Mangrove exists as fringing vegetation over 168ha distributed along the sides of Ariankuppam estuary, it is seasonally bar-built and semi diurnal type that flows eastwards emptying in to the Bay of Bengal at Veerampatinam on south east coast of India. Vanveen grab were employed to collect the mangrove benthic fauna. Sediment was sieved via 0.5 mm mesh and the benthic polychaete individuals were separated

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and preserved in 95% Ethanol. Fauna were identified to the lowest practical taxonomic level using standard references [9].

DNA Isolation and Sequencing: DNA was extracted from the muscle tissue of each specimen usingQiagen DNA Easy blood and tissue kit. 16S r RNA was subsequently amplified under the following thermal conditions: 2 min at 95°C; 35 cycles of 0.5 min at 94°C, 0.5 min at 52°C and 1 min at 72°C; 10 min at 72°C; held at 4°C. The 12.5 µl PCR mixes included 6.25 µl of 10% trehalose, 2.00 µl of ultra pure water, 1.25 µl PCR buffer [200 mM Tris– HCl (pH 8.4), 500 mM KCl], 0.625 µl $MgCl_2$ (50 mM), using primer forward name: Palumbi F- seq: cgcctgtttatcaaaaacat, reverse-name: Palumbi R -seq: ccggtttgaactcagatcatg". PCR product was then run through a 1 % DNA-grade agarose electrophoresis gel made with 1x TBE and 2 µL of ethidum bromide, using 2 µl of PCR product and 2 µL of loading buffer to load each sample into the gel (BIORAD-USA). After detection by gel electrophoresis the products were purified with PCR Purification Kit (Qiagen). Samples were then sequenced using the same primers used in PCR amplification (5 µM conc.) on an ICM version 3.1 automated sequencer (MegaBace) Bioserve DNA sequencing facility at the BioServe Biotechnologies Pvt. Ltd, of Hyderabad, India.

Data Analysis: Sequences were aligned with CLUSTAL W [10] using the default parameters for gap opening and gap penalty and subsequently manually edited by using Bio Edit [11]. Gap positions and regions that could not be aligned unambiguously were excluded from the analysis. Sequence data were submitted to the Nucleic acid sequence database [(http://ncbi.nlm.nih.gov, see (Satheeshkumar *et al.*, 2009)] and to GenBank (Accession numbers in Table 1). The organism chosen in this case of 26 Polychaetes, the source of data is NCBI Data Bank. Phylogeny of polychaete species using the 16S r RNA gene maximum-likelihood phylogenetic analyses of 16S r RNA sequence data were performed using MEGA 4.1 Version.

RESULTS AND DISCUSSION

Genetic Diversity: Genetic distance (*D*) was calculated for each group of polychaetes, based on the *D*- values dendrogram was drawn by wards method clustering (Fig. 1). The dendrogram showed two major clusters clearly separately the four groups between the polychaetes group *D* values ranged from 0.3-0.66 (average 0.632), the same result which confirm to the matrix plot (Fig. 2). These differences seem to be attributable to the differences in life history strategies between the

Table 1: List of taxa used in the analysis with 16S r RNA sequences accession numbers

Species Name	Family	Author	Accession No
Lumbrinereis magnidendata	Lumbrinereidae	Rousset *et al.,* 2006	DQ779621
Thelepus cincinnatus	Terebellidae	Rousset *et al.,* 2006	DQ779636
Chaetopterus sarsi	Chaetoperidae	Rousset *et al.,* 2006	DQ779607
Marphysa belli	Eunicidae	Rousset *et al.,* 2006	DQ779623
Chrysopetalum depile	Chrysopetalidae	Wiklund *et al.,* 2008	EU555046
Pseudonereis variegata	Nereidae	Satheeshkumar *et al.,* 2009	GU230890
Namalycastis indica	Nereidae	Satheeshkumar *et al.,* 2009	GU230891
Polygordius lacteus	Polygordiidae	Rousset *et al.,* 2006	DQ779633
Paramphinome jeffreysii	Amphinomidae	Rousset *et al.,* 2006	DQ779629
Dipolocirrus glaucus	Flabeeligeridae	Osborn 2009	FJ944504
Cirratulus cirratus	Cirratalidae	Rousset *et al.,* 2006	DQ779609
Protodorvillea kefersteini	Dorvilleidae	Rousset *et al.,* 2006	DQ779634
Glycera alpa	Glyceridae	Rousset *et al.,* 2006	DQ779615
Marenzelleria wireni	Spionidae	Blank & Bastrol 2007	EF431980
Marenzelleria bastropi	Spionidae	Blank & Bastrol 2007	EF431964
Malacoceros fuliginosus	Spionidae	Blank & Bastrol 2007	EF431962
Polydora giardi	Spionidae	Rousset *et al.,* 2006	DQ779632
Caulleriella sp	Spionidae	Rousset *et al.,* 2006	DQ779606
Macrochaeta sp	Acrocirridae	Osborn and Rouse 2008	EU694114
Dysponetus caecus	Chrysopetalidae	Wiklund *et al.,* 2008	EU555047
Dysponetus sp	Chrysopetalidae	Wiklund *et al.,* 2008	EU555048
Notophyllum foliosum	Phyllodocidae	Rousset *et al.,* 2006	DQ779627
Paleanotus sp	Chrysopetalidae	Wiklund *et al.,* 2008	EU555050
Bhawania reyssi	Chrysopetalidae	Wiklund *et al.,* 2008	EU555045
Bhawania heteroseta	Chrysopetalidae	Wiklund *et al.,* 2008	EU555044
Lepidonotus squamatus	Polynoidae	Rousset *et al.,* 2006	DQ779620
Nereimyra punctata	Hesionidae	Rousset *et al.,* 2006	DQ779626
Vrijenhoekia balennophila	Hesionidae	Pleijel *et al.,* 2006	DQ513305

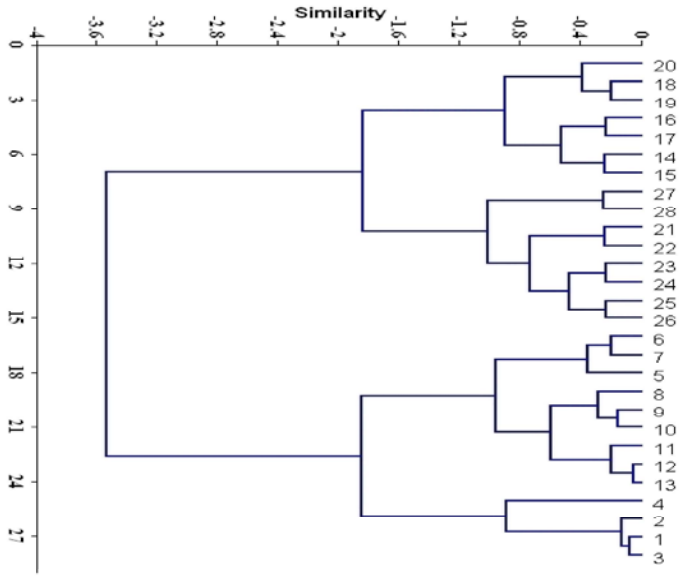

Fig. 1: Cluster analysis of polychaetes genetic diversity
[1] *Marenzelleria wireni*; [2] *Malacoceros fuliginosus*; [3] *Marenzelleria bastropi*; [4] *Pseudonereis variegata*; [5] *Namalycastis indica*; [6] *Diplocirrus glaucus*; [7] *Vrijenhoekia balenophila*; [8] *Paleanotus* sp; [9] *Dysponetu*s sp; [10] *Dysponetus caecus*; [11] *Chrysopetalum debile*; [12] *Bhawania heteroseta*; [13] *Bhawania reyssi*; [14] *Macrochaeta* sp; [15] *Thelepus cincinnatus*; [16] *Protodorvillea kefersteini*; [17] *Polydora giardi*; [18] *Polygordius lacteus*; [19] *Paramphinome jeffreysii*; [20] *Notophyllum foliosum*; [21] *Nereimyra punctata*; [22] *Marphysa bellii*; [23] *Lumbrineris magnidentata*; [24] *Lepidonotus squamatus*; [25]*Glycera alba*; [26] *Cirratulus cirratus*; [27] *Chaetopterus sarsi*; [28] *Caulleriella* sp

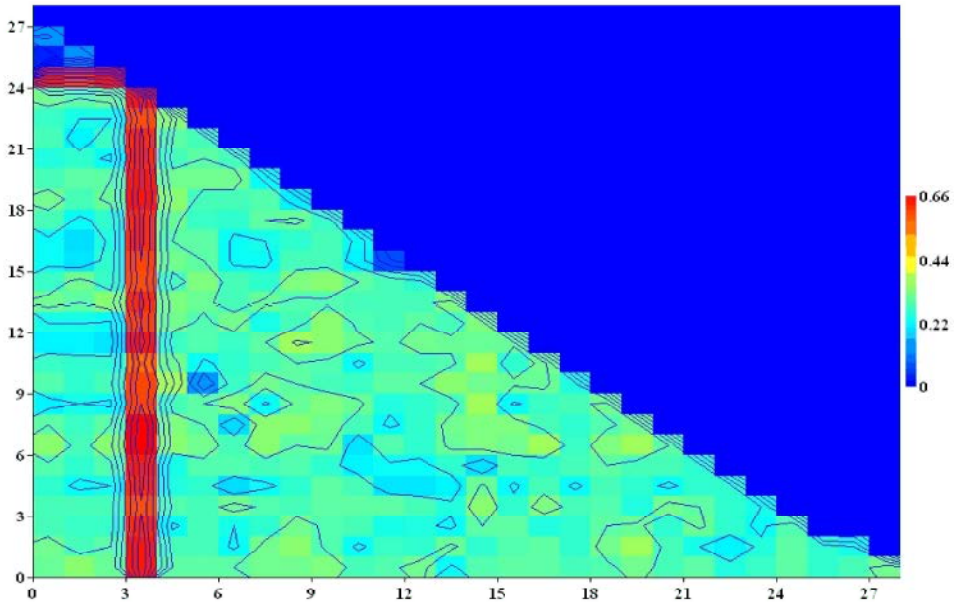

Fig. 2: Matrix plot analysis of polychaetes genetic diversity

polychaetes group. The differences among the *D* values of all populations of both forms were significant by ANOCOVA; $F_{28.16}$ =0.784, *P* = 0.676). *D*-values between the family Neridae species *P. variegata* and *N. indica* (0.64) and also similar characteristic levels among congeneric species in various taxa *D:* 061 [12] and among sibling polychaete taxa *D:* 0.5 [13], however not as high among the *Nereis acuminate D:* 1-36 to 1.76 [14]. An

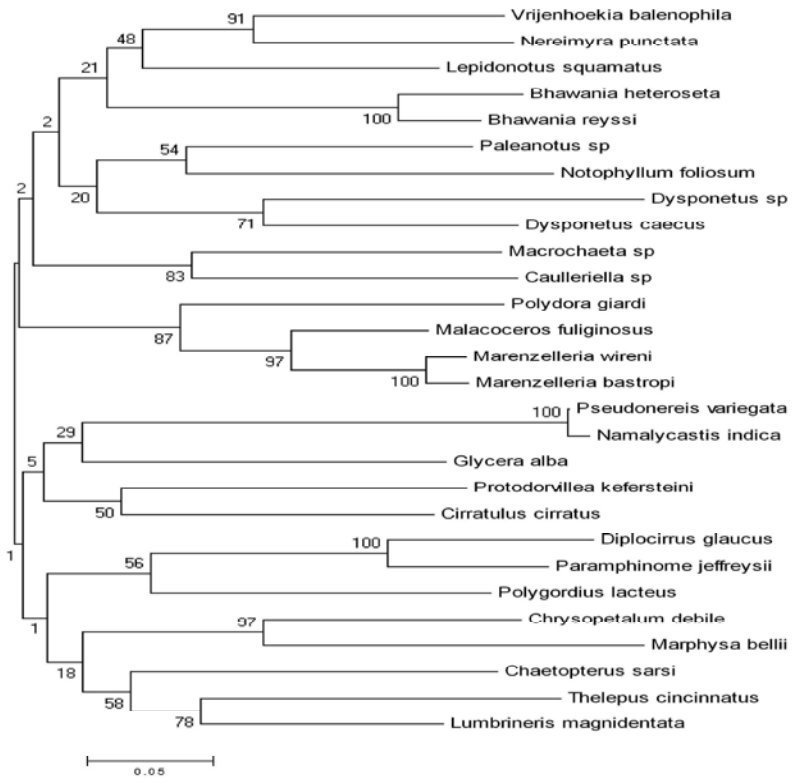

Fig. 3: Phylogenetic analysis of marine Polychaetes

extremely lowest genetic variability such as 0.3 was observed in the *Marenzelleria* populations may be caused by a population size bottle neck.

Phylogenetic Analysis: In the parisomany tree, two large clades emerge. The first, for concision here called A, is further divided into two clades A1, A2 and A3. Clade A1 consists of Vrijenhoekia balenophila, *Nereimyra punctata, Lepidonotus squamatus*, *Bhawania heteroseta* and *Bhawania reyssi*; A2 comprises *Paleanotus* sp, *Notophyllum foliosum, Dysponetus* sp and *Dysponetus caecus.* Clade A3 *Caulleriella* sp and *Macrochaeta* sp; A4 consists *Marenzelleria wireni, Marenzelleria bastropi, Malacoceros fuliginosus, Polydora giardi*. The second major clade, here called B, includes *Pseudonereis- variegata, Namalycastis indica, Glycera alba, Cirratulus cirratus, Diplocirrus glaucus, Paramphinome jeffreysii, Polygordius lacteus, Lumbrineris magnidentata* and etc., (Fig. 3). To place our new sequences from polychaete organisms within a phylogenetic framework, we have combined with 26 sequences from the database. Phylogenetic analysis of 16S r RNA sequences revealed strongly supported taxonomic units with 100 % bootstrap values whether estimated by maximum parsimony analysis. Among the other polychaetes bootstrap support was weaker (1-97%) than for the other nodes.

Phylogenetic relationships among different organisms are of fundamental importance in biology and one of the prime objectives of DNA sequence analysis is phylogeny reconstruction for understanding evolutionary history of organisms [15]. A monophyletic Errantia including Nereidae (represented by *P. variegata* and *N. indica*) yields high support (100%), whereas Nereidae appear paraphyletic in regard to Eunicidae. Spionidae appear paraphyletic in maximum likelihood (ML) analysis in view to the Sabellida, but a monophyletic Spionidae is weakly supported through the phylogeny analysis (54 %), *N. foliosum* join with *M. wireni* and *M. bastropi* (100%). A close relationship between *M. fuliginosus* (97 %) and *P. giardi* is also strongly supported (87 %), remaining Chrysopetalidae cluster together in a strongly supported clade (100 %). Monophyly of the Chrysopetalidae as represented by *B. heteroseta* and *B. reyssi* is also supported (100 %). The Chrysopetalidae are joined by the

undescribed species *V. balennophila* and *Nereimyra punctata* from Hesionidae, which clusters together with the three considered *Hesionidae* species (91%). Further relationships between polychaete families are recovered by ML as follows: a sister group relationship between Nereidae and Siponidae (97 %); Errantia cluster together with the Eunicidae (100%). Our result suggested that, in the vast majority of the taxa examined here (97 %), genetic gap was observed and current case 28 species would have been undetected by using a 1% threshold. Providing that seven species share polymorphism, 13 species with monophyletic 16S rRNA lineages would have been overlooked with a 1% threshold.

Using 16S rRNA gene sequence data, [16] carried out parsimony, distance to reconstruct relationships among 22 taxa, including 18 assigned to *Ophryotrocha*. The largest barcoding study conducted so far on marine polychaeta to date the average observed distance between conspecifics was 1% while the average divergence reached 16.9% between congeneric species [17]. However, the average distance between conspecifics and congeneric species reached 0.3% and 8.3%, respectively, in this present study, pattern strikingly similar to that incorporate the previous reports [16, 18]. Again, this study is so limited in the scope of taxonomic sampling that few conclusions can be drawn from the results. Our analysis has thoroughly demonstrated that distribution of 16S rRNA sequences can capture biologically significant structural patterns in large DNA sequences and it can be used as a tool for phylogenetic analysis may position to a root of the annelid tree. Furthermore, the data suggest that the Nereidae may be closely associated with some Eunicidae groups among the Siponidae. However, additional taxa need to be analyzed to establish a more resolved phylogeny of the Hesanidae assemblage within the Chrysopetalidae.

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