



Sequence based evolutionary relationships among annelids

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Abstract

Annelids are important and diverse group of animals with worldwide distribution. Evolutionary history of this group is still a not resolved. In the current study different coding gene sequences were retrieved and large scale phylogenetic analysis were carried out using multiple methods. The relative study indicates coding genes as phylogenetically informative. The trees showed more or less similar species clustered together but did not form distinct clades as per their lifestyles and morphological similarities. The result also showed that several species appear to be polyphyletic and several unrelated species appear to share the same clade. This may be attributed to adaptive radiation or mutations.

Keywords: annelid, species, coding genes, sequences, monophyletic, polyphyletic, phylogeny, clade

Introduction

Annelids are soft-bodied, bilaterally symmetrical segmented animals distributed throughout the world, from deep ocean bottoms to high mountain glaciers. Despite their success and ecological importance, the evolutionary history of the group is still a mystery. Numerous species of annelids are known and it is likely that many still remain unknown and undescribed. Recent phylogenetic analyses have led to profound changes in the view that the Annelida, as traditionally formulated, is a natural, monophyletic group. The objectives of the present study were survey and retrieval of selected coding gene sequences from worldwide database and phylogenetic study of different species using multiple methods.

Materials and Methods

Retrieval of sequences and taxon sampling

The gene sequences belonging to phylum Annelida were retrieved from NCBI-GenBank database (Benson *et al.*, 2013) [1] using entrez key word search and PERL script. The

sequences were filter searched and sequences were selected referring to specific genes. The sequences were sorted based on gene types using Bioedit software version 7.0.5.3 (Hall, 1999) [3]. Altogether nine gene categories of genes were retrieved and separated. These gene sequences were considered for further analysis.

Multiple sequence alignment and Phylogenetic analysis

The retrieved gene sequences were saved and fasta formatted for multiple sequence alignment. The sequences were aligned using CLUSTAL W (Thompson *et al.*, 1994). For pair wise sequence alignment the gap opening penalty and extension penalties were 15 and 6.66 respectively. The aligned file was exported for phylogenetic analysis. Five different methods (ML, NJ, ME, UPGMA and MP) were adopted to perform phylogenetic analysis using MEGA 7 software (Kumar *et al.*, 2016) [5]. The branch length and consistency, retention and composite indices are shown (Table 1).

Table 1: Branch length and indices of CI, RI and CI

S. No	Gene	Sum of Branch Length					Consistency Index	Retention Index	Composite Index
		ML	NJ	ME	UPGMA	MP			
1	atp6 (ATP synthase 6)	0.122	-995	4.801	4.949	260	0.398	0.901	0.361
2	cox1 (cytochrome c oxidase subunit1)	-5380	2.770	2.770	2.758	1045	0.258	0.864	0.228
3	gap (glyceraldehyde 3-phosphate dehydrogenase)	-3725	0.619	0.6197	0.616	527	0.625	0.589	0.434
4	metallothionein 2	-1135	0.793	0.793	0.694	187	0.694	0.518	0.446
5	ND2 (NADH dehydrogenase subunit2)	-2511	3.462	3.462	3.469	402	0.965	0.965	0.940
6	ND4 (NADH dehydrogenase subunit4)	2090.65	2.828	2.828	2.819	533	0.936	0.979	0.929
7	RAG1 (Recombination Activation Gene1)	-4303	0.494	0.494	0.495	580	0.592	0.468	0.434
8	RAG2 (Recombination Activation Gene2)	-2525	0.131	0.131	0.134	152	0.655	0.631	0.544
9	aprA (alkaline metalloproteinaseA)	-1929	0.912	0.912	0.921	330	0.756	0.756	0.593

ML: Maximum Likelihood, NJ: Neighbour Joining, ME: Minimum Evolution, UPGMA: Unweighted Pair Group Method with Arithmetic Mean, MP: Maximum Parsimony, CI: Consistency Index, RI: Retention Index and CI: Composite Index.

All characters were equally weighted and unordered. Alignment gaps were treated as missing data. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap was 500 replicates. The evolutionary distances were computed.

Results

Maximum Likelihood Tree

The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model (Tamura and Nei, 1993). The trees with the highest log-likelihood are shown (Table 2).

Table 2: Gene sequences with highest log-likelihood values in the ML trees

S. No.	Gene	Highest log-likelihood	S. No	Gene	Highest log-likelihood	S. No	Gene	Highest log-likelihood
1	atp6	-1101.5774	4	MT2	-1135.3252	7	RAG1	-4303.0987
2	cox1	-5380.1513	5	ND2	-2511.4474	8	RAG2	-2528.0024
3	gap	-3725.2005	6	ND4	-2094.1472	9	aprA	-1929.9670

Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value.

The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated (Fig. 1).

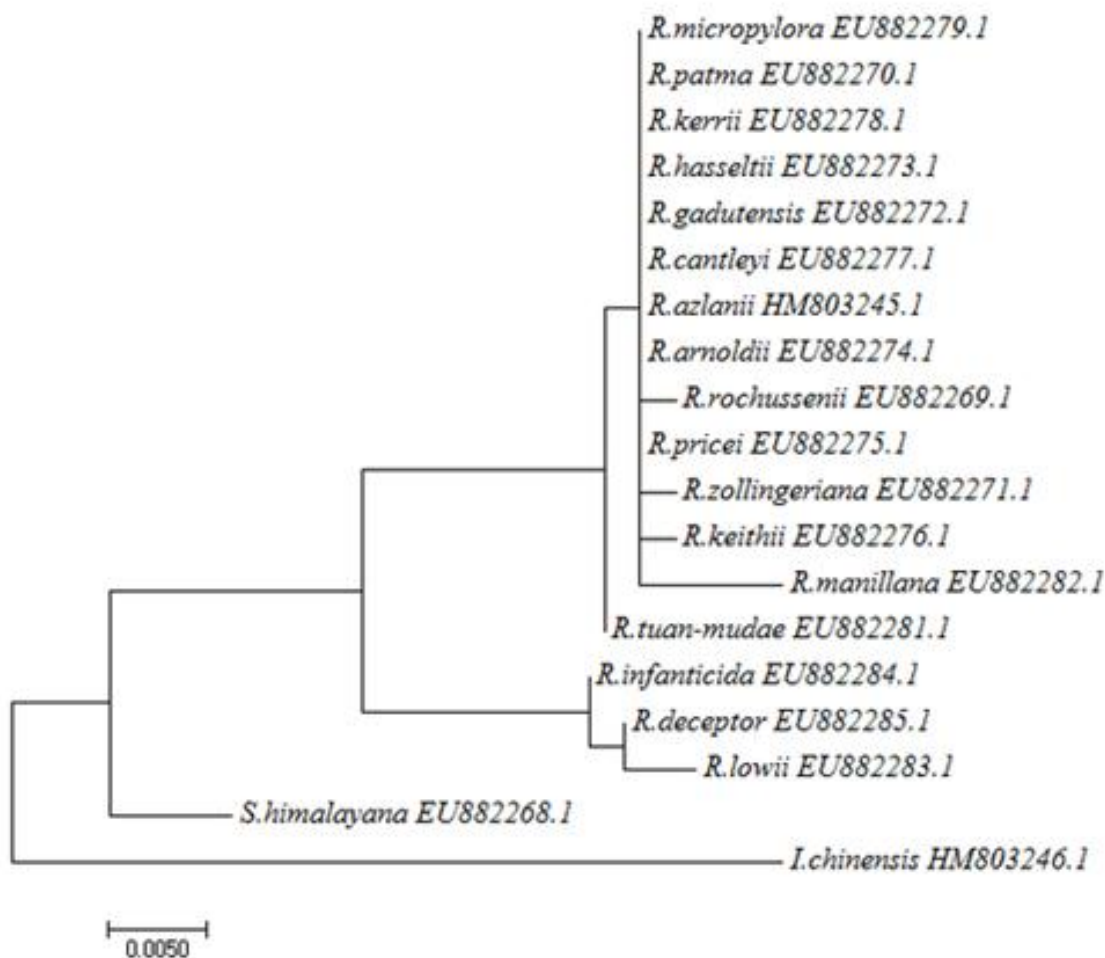


Fig 1: ML tree based on atp6 gene

Neighbor Joining Tree

The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei, 1987) [6]. The optimal trees were drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the

phylogenetic trees. The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura et al., 2004) [7] and are in the units of the number of base substitutions per site.

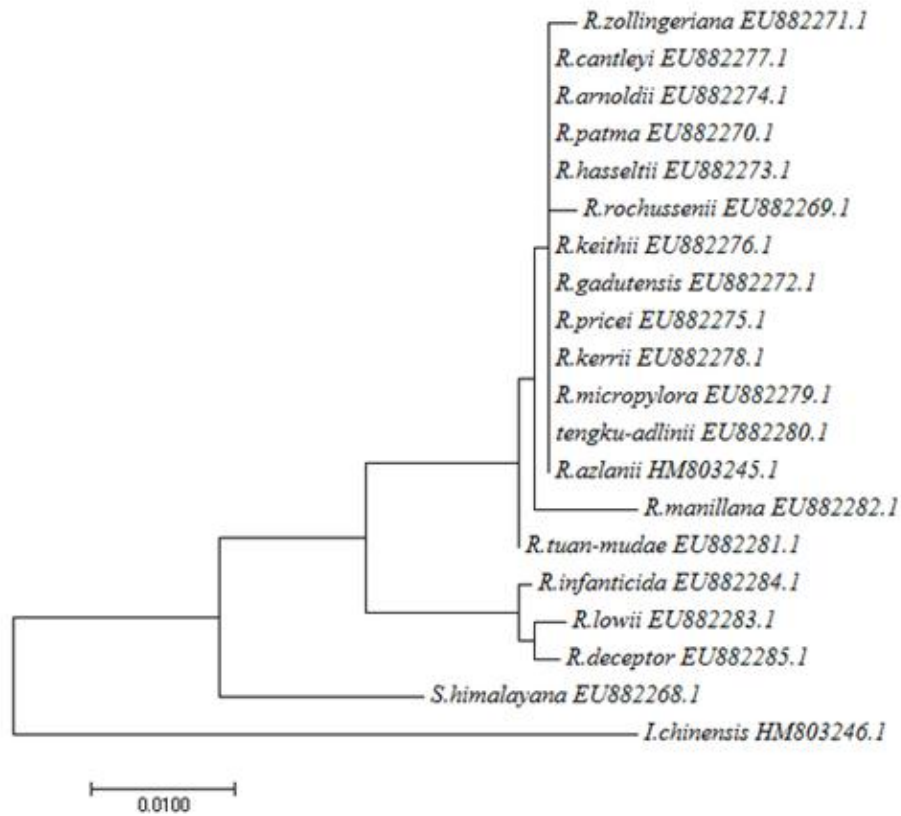


Fig 2: NJ tree based on atp6 gene

Minimum Evolution Tree

The evolutionary history was inferred using the Minimum Evolution method (Rzhetsky and Nei, 1992). The trees are drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic trees. The evolutionary distances were computed using the

Maximum Composite Likelihood method (Tamura *et al.*, 2004) [7] and were in the units of the number of base substitutions per site. The ME trees were searched using the Close-Neighbor-Interchange (CNI) algorithm (Nei and Kumar, 2000) [5].

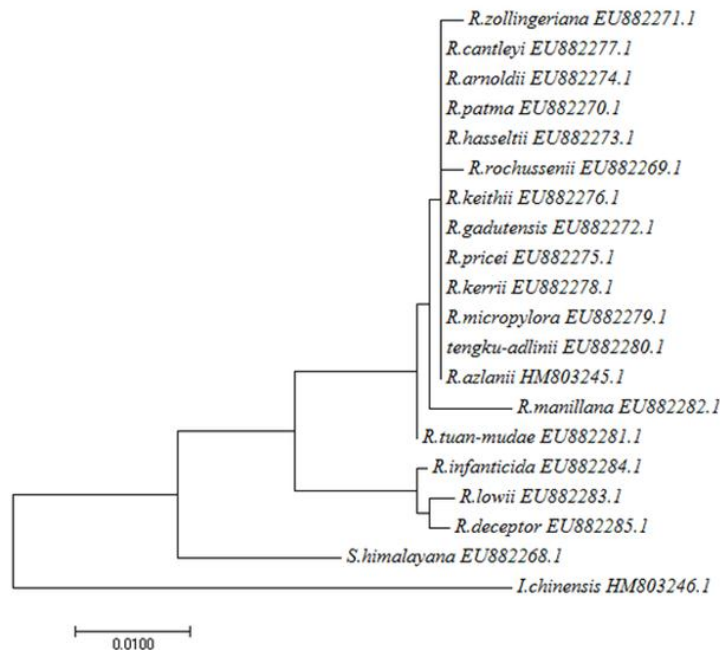


Fig 3: ME tree based on atp6 gene

UPGMA Tree

The evolutionary history was inferred using the UPGMA method (Sneath and Sokal, 1973) [11]. The optimal trees were drawn to scale, with branch lengths in the same units as those

of the evolutionary distances used to infer the phylogenetic trees. The evolutionary distances were computed using the Maximum Composite Likelihood method and were in the units of the number of base substitutions per site.

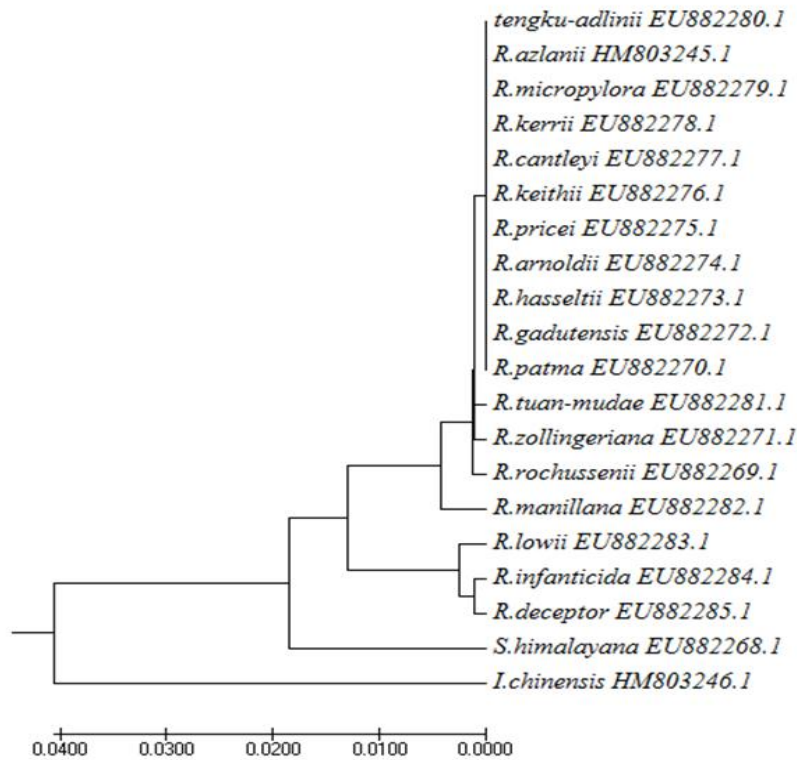


Fig 4: UPGMA tree based on atp6 gene

MP Tree

The evolutionary history was inferred using the Maximum Parsimony method. The MP trees were obtained using the Subtree-Pruning-Regrafting (SPR) algorithm with search level

0 in which the initial trees were obtained by the random addition of sequences (10 replicates). All positions containing gaps and missing data were eliminated.

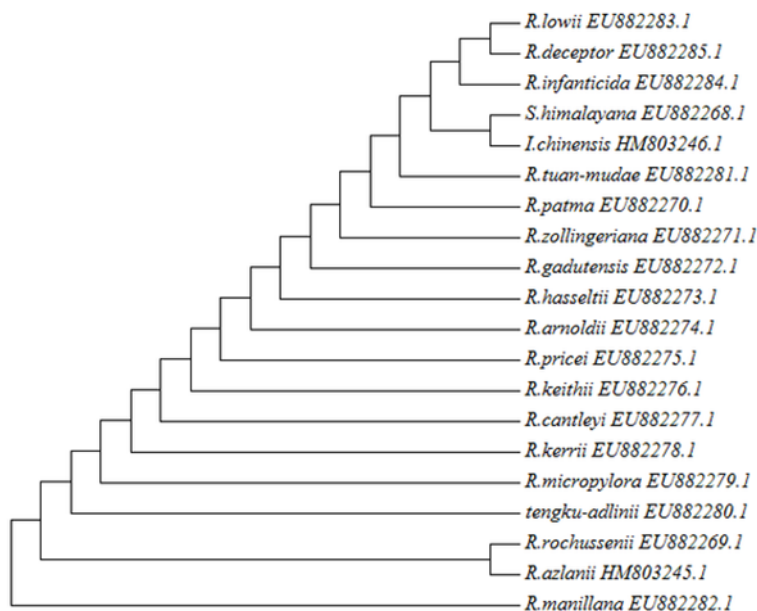


Fig 5: MP tree based on atp6 gene

Conclusion

Identification of unknown species by means of morphology and non-coding genes only may result in unconvicted specimen identifications resulting in to false negatives or positives. In the current study the coding genes were preferred to inspect comprehensive phylogeny in Annelids. The relative study reveals coding genes seem to be phylogenetically informative at the species level. Phylogenetic trees were investigated by different methods to infer evolutionary relationships. The trees showed more or less similar species clustered together but did not form distinct clades as per their lifestyles and morphological similarities. The result also indicated that several species appear to be polyphyletic and several unrelated species appear to share the same clade. This may be due to wrongly named species submitted to GenBank as in some cases (Cai, *et al.*, 2009) ^[2]. Moreover, primary sequences often contain insertions and deletions (indels) making alignment difficult beyond intraspecific levels (Kruger and Gargas, 2008) ^[4]. But still it can be assumed here that phylogenetic analyses using coding gene sequences could be a very productive approach in understanding annelid evolution. Some trees showed similar species remain clustered together with few alterations and this may be assumed by possible adaptive radiation or mutations. With development of new algorithms, efforts should be targeted to increase taxonomic representation in coding genes to analyze the patterns of change through time in morphological, behavioral and developmental characters.

References

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