BIOINFORMATION
Discovery at the interface of physical and biological sciences

open access www.bioinformation.net **Hypothesis**
Volume 10(9)

The conserved mitochondrial gene distribution in relatives of *Turritopsis nutricula*, an immortal jellyfish

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Received August 14, 2014; Accepted August 16, 2014; Published September 30, 2014

Abstract:
Turritopsis nutricula (T. nutricula) is the one of the known reported organisms that can revert its life cycle to the polyp stage even after becoming sexually mature, defining itself as the only immortal organism in the animal kingdom. Therefore, the animal is having prime importance in basic biological, aging, and biomedical researches. However, till date, the genome of this organism has not been sequenced and even there is no molecular phylogenetic study to reveal its close relatives. Here, using phylogenetic analysis based on available 16s rRNA gene and protein sequences of Cytochrome oxidase subunit-I (COI or COX1) of *T. nutricula,* we have predicted the closest relatives of the organism. While we found *Nemopsis bachei* could be closest organism based on COX1 gene sequence; *T. dohrnii* may be designated as the closest taxon to *T. nutricula* based on rRNA. Moreover, we have figured out four species that showed similar root distance based on COX1 protein sequence.

Keywords: *Turritopsis nutricula,* immortal jellyfish, trans-differentiation, phylogeny, relativeness

Background:

Gerontologists and biologists reached a consensus “evolutionary theory of aging,” **[1, 2]** embedding aging research into the mainstream of biological research. *T. nutricula* is the one of the known hydrozoan in the animal kingdom that can revert back into the immature polyp stage after reaching sexual maturity, designating itself as the only immortal animal **[3].** *T. nutricula* interplay with the polyp and sexual maturity stages by virtue of trans-differentiation process **[4].** Theoretically, this process can go on indefinitely therefore, the organism can be considered as biologically immortal and does not experience aging. Hence, in the basic biology of aging research, the organism has found itself great importance **[5].** If a cell or organism undergoes aging, there are two vital biological processes viz. negative regulation of cell division or proliferation and /or positive regulation of apoptosis may occur **[6].** Furthermore, aging and neurodegeneration diseases are directly associated with these two biological processes **[7].** While in cancer, aging does not happen and these two biological processes are inversely associated **[8].** Thus, the organism can be a great tool in cancer, aging, and neurodegenerative related disorders. Although the trans-differentiation mechanism has been documented at physiological and cellular level **[3, 4]**; the molecular mechanism behind the *T. Nutricula's* trans-differentiation is not yet elucidated.

Although 16S rRNA based phylogenetic analysis is well established, COX 1 based analysis is not much reported. COX1 is one of the most conserved genes in almost all eukaryotes. It belong to the cytochrome oxidase subunit I protein family, which also includes mitochondrial encoded COX2 and COX3 those combine to form respiratory complex IV, a final enzyme of electron transport chain in mitochondrial oxidative phosphorylation **[9]**. COX1 exhibits the characteristics of a "housekeeping" gene and is constitutively expressed in almost all tissues. COX1 appears to be responsible for the production of prostaglandins **[10]**. Studies suggest that COX1 has its own importance in phylogenetic analysis. Its sequence is conserved among conspecifics and addition to this its mutation rate is also rapid enough to distinguish closely related species **[11]**. Additionally, in phylogenetic study, invasion patterns can be inferred from COX1 sequences **[12]**. Phylogenetic study on *T. nutricula* is not so far reported. Since the genome sequences or gene or protein expression profile or any kind of molecular data of *T. nutricula* is not available, we focused to identify its close relatives that may have similar physiology of immortality and may have molecular data so that, the organisms can be used to carry out aging and disease research. With these aims, at the very first step, here we tried to identify close relatives of *T. nutricula* using its only available Cytochrome Oxidase Subunit I (COI or COX1) and 16S rRNA sequences and bioinformatics based robust phylogenetic analysis.

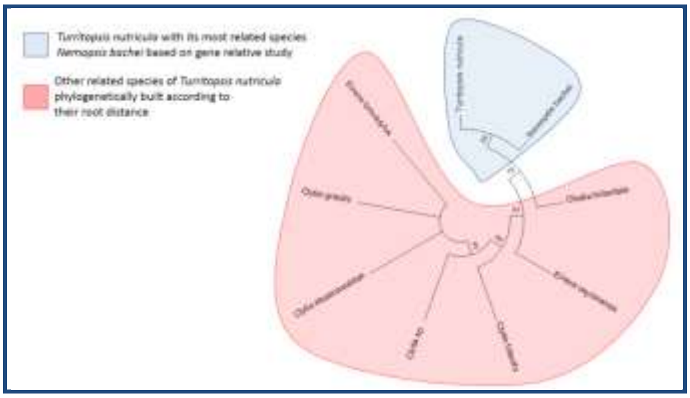

Figure 1: Phylogram representing *Turritopsis nutricula* and its COX1 nt sequence based relatives according to specific root distance. *Turritopsis nutricula* and *Nemopsis bachei* are emerging from the same node.

Methodology:

Gene and protein sequences

All-over, only six nucleotide (nt) sequences (COI/COX1 from four different isolates and 16S rRNA form two different isolates) of *T. nutricula* are available in NCBI database. All the 4 isolates of COI/COX1 and 2 16s rRNA partial sequences have shown similar sequence comparatively. We used all the sequences for our phylogenetic analysis. However, COI/COX1 nt sequence of 621 bp from the isolate-1 (GenBank: JQ716082.1) and partial 16s rRNA sequence of the isolate WHO_1 (GenBank: EU624348.1) based analysis are represented here. In UniProt, only two COX1 protein sequences are available and both have similar homology. The COI/COX1 protein (UniProt: K4P7S9) of 207 amino acid (aa) based phylogenetic analysis is represented here.

Phylogenetic analysis

We have started our analysis to identify the sequence homologs using NCBI BLASTn to identify nt sequence homologs and BLASTp to identify protein or aa based homologs with cut off values 97% identity (for aa) and 84% (for nt). Based on these cut off values, the species specific homologs were selected for multiple sequence alignment. Clustal Omega and ClustalW (http://www.clustal.org) were used for multiple sequence alignment. Molecular Evolutionary Genetic Analysis tool (MEGA) version 5.1 **[13]** was used for the construction of phylogeny. In MEGA 5.1, Neighbourhood joining method **[14]** was used for construction of nucleotide based phylogenetic trees whereas Poisson model **[15]** was used to build protein based phylogeny to estimate the number of amino acid replacements when species are closely related. Pairwise distances are calculated with scope as pairs of taxa using Maximum Composite likelihood method **[16]**. Rates among the sites were considered uniform, pattern of lineages were regarded as homogeneous, and gaps or missing data were treated as deletions.

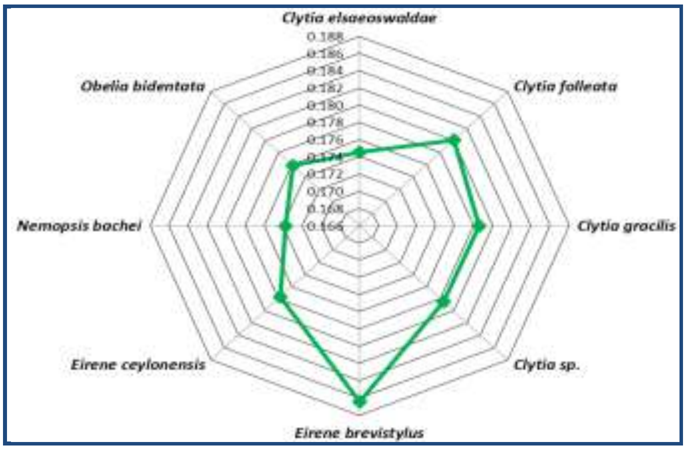

Figure 2: Web Graphical view of root distances of relative's species verses *Turritopsis nutricula*. Root distance of *Nemopsis bacheis* showing low root distance to *Turritopsis nutricula* than other relatives.

Results:

Homologous sequence similarity search provided number of related species from which best homologous sequences were selected according to the cut-off values **Table 1 (see supplementary material).** *Nemopsis bachei* is found to be closest relative of *T. nutricula* in COI/COX1 gene based study that exhibits the shortest root distance of 0.174 **(Figure 1 & 2) (Table 1)**with a bootstrapping percentage of 88. The 16S rRNA based analysis **Table 2 (see supplementary material)** revealed that the immortal jellyfish is genetically close to *T. dohrnii* **[17, 18]** (Root distance: 0.034) followed by *T. rubra* **[17,18]** (Root distance: 0.099) **(Figure 3 & 4) (Table 1)**with the bootstrapping percentages of 100 and 99.In contrast to the nucleotide or gene based analysis, the COI/COX1 protein based analysis **Table 3 (see supplementary material)** showed some different findings. The result demonstrates that *T. lata, Amphinema dinema, Eucheilota menoni, and Eutima levuka* have similar root distance of 0.029 **(Figure 5 & 6) (Table 1).** These species have shown a low bootstrapping percentage of 70 when compared to the gene and 16s rRNA bootstrapping results.

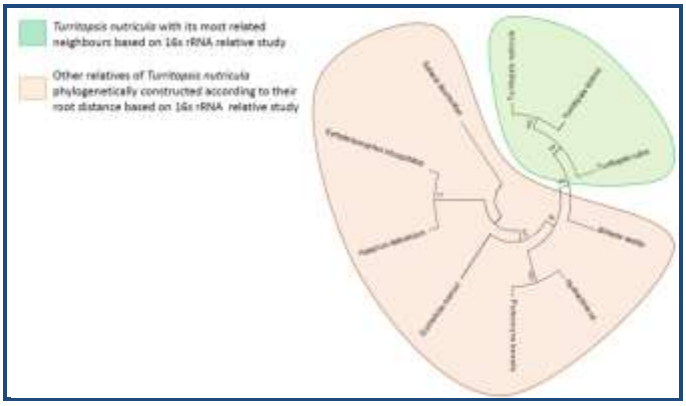

Figure 3: Phylogram representing *Turritopsis nutricula* and its 16s rRNA based relatives according to root distance. In this Phylogram *Turritopsis dohrnii* and *Turritopsis nutricula* are shown to be emerging together.

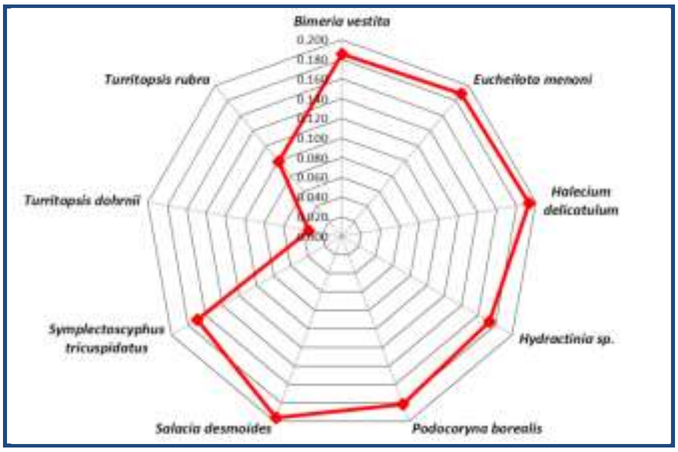

Figure 4: Web Graphical view of root distances showing relative species verses *Turritopsisnutricula*. Centre point of the web refers to *Turritopsis nutricula*. The distance of *Turritopsisdohrnii* followed by *Turritopsis rubra* are nearer to *Turritopsis nutricula* according to their root distances based on 16s rRNA relative study.

Discussion:

Phylogenetic analysis is a powerful tool for precise taxonomic classification as well as predicting origin or relatives of a species **[19].** Attempts have been made in systematic classification of the genus *Turritopsis* **[20, 21].** However, the closest taxa based on these studies are not reported to have features of immortality similar to the *T. nutricula*. Since our aim is to identify closest organism of *T. nutricula* having possible immortality; we discussed the results focusing on this specific aspect. In *T. nutricula* trans-differentiation directly allows functionally differentiated cells to switch to entirely new functions. Theoretically, this process can go on indefinitely therefore, the organism can be considered as biologically immortal and does not experience aging **[8].** Based on the well-established 16S rRNA phylogeny, our predicted close relatives of *T. nutricula* are *T. dohrnii*and *T. rubra* where *T. dohrnii* is reported to undergo reverse development **[17, 18].** COX1 is recently used for phylogenetic analysis **[11, 12].** While considering the association of COX with aging and immortality, recent study on *C. elegans* illustrates that two COX assembly factors are involved in energy metabolism, reproduction, and aging **[22].** Genomic level studies on *Podospora anserina* support the idea that, immortality can be acquired by the lack of active cytochrome c oxidase **[23].** In *Drosophila*, COX is down regulated during aging **[24]** and in male wistar rats an inter-link between muscle-fiber trans-differentiation and mitochondrial respiratory chain was observed **[25].** Therefore, based on these aging and trans-differentiation association of Cytochrome C Oxidase, we used *T. nutricula* available *COI/COX1* gene and protein sequences for this phylogenetic study. Based on the *COI/COX1* gene sequence, our predicted closest taxa of *T. nutricula* is *Nemopsis bachei*. However, we have observed some deviation in COX1 amino acid based analysis. Most of the predicted relatives are from different genus except *T. lata*; although few of these genus are jellyfish and as per our analysis they are close relatives. As per out limited knowledge, the life cycle of these identified possible close relatives of *T. nutricula* are not studied in depth. Therefore, we are not sure if these animals are having the features of immortality like the *T. nutricula*. Since our prediction demonstrates close relativeness of these animals with *T. nutricula*, they might have immortal features and hence the biology of these animals may be explored to find possible identification of new members of immortal animal.

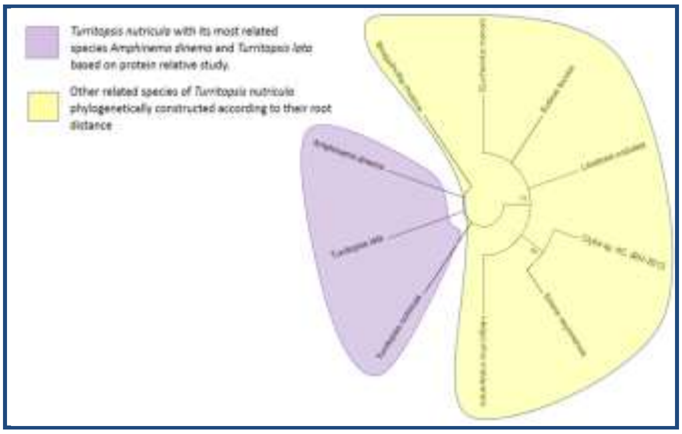

Figure 5: Phylogram representing *Turritopsis nutricula* and its COX1 amino acid based relatives according to specific root distance.

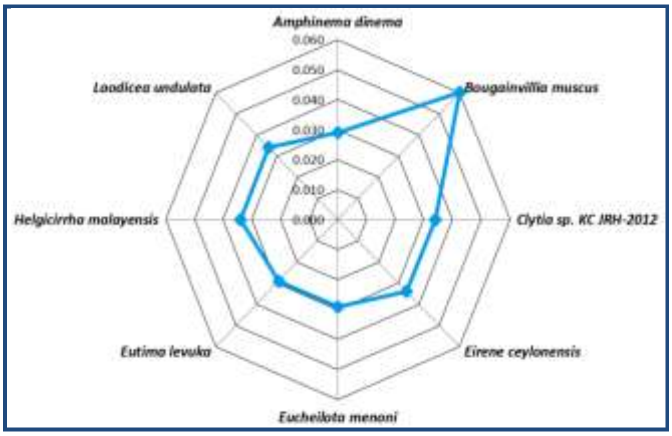

Figure 6: Web Graphical view of root distances of relative's species verses *Turritopsis nutricula*. Except *Bougainvillia muscus*

almost all species are have shown similar root distance of ~0.030 towards *Turritopsis nutricula*.

Conclusion:

Predicted seven species to be closely related to immortal jellyfish *T. nutricula* based on COX1 and 16S rRNA sequences. Among these seven, three (*T. dohrnii, T. rubra*and *T. lata*) belongs to same genus *Turritopsis*, two (*Nemopsis bachei* and *Amphinema dinema*) belongs to same suborder *Folifera*, while the rest two (*Eucheilota menoni and Eutima levuka*) belongs to same order *Leptothecata*. Precisely, these all species including *T. nutricula* are grouped under a common order *Leptothecata* **[26].** Limited studies have been done on those animals and none of these organisms is so far reported to be immortal similar to *T. nutricula*. There is high probability that these animals may have features of immortality. Detail physiology and molecular studies of these animals along with *T. nutricula* will shed light on aging biology and therefore the knowledge may be translated and would be applied to biomedical sciences especially in proliferative, degenerative, and aging related disorders.

Acknowledgement:

The authors thank DST-SERB for financial support in young scientist award scheme and Vasco Azevedo is sponsored by CNPq and UFMG

Conflict of Interest:

The authors are no conflict of interest of this article

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Edited by P Kangueane
Citation: **Devarapalli *et al.*** Bioinformation 10(9): 586-591 (2014)

Supplementary material:

Table 1: *Turritopsis nutricula* with its most related individuals *T. Dohnrnii, T. Rubra, Hydractinia sp., Podocoryna borealis, Symplectoscyphus tricuspidatus, Halecium delicatulum, Eucheilota menoni, Bimeria vestita* and *Salacia desmoides* arranged according to their E-Value and Max Ident based on gene relative study.

Relative Species	**Max score**	**Total score**	**Query coverage**	**E value**	**Max Ident**
Turritopsis nutricula isolate WHOI_1 16S ribosomal RNA gene, partial sequence; mitochondrial	1081	1081	100%	0	100%
Turritopsis sp. 1 MPM-2008 isolate Bocas_2 16S ribosomal RNA gene, partial sequence; mitochondrial	1037	1037	100%	0	99%
Turritopsis dohrnii isolate Fort Pierce_2 16S ribosomal RNA gene, partial sequence; mitochondrial	961	961	100%	0	96%
Turritopsis rubra isolate Japan_1 16S ribosomal RNA gene, partial sequence; mitochondrial	778	778	100%	0	91%
Hydractinia sp. 1 MPM-2009 isolate 078 16S ribosomal RNA gene, partial sequence; mitochondrial	614	614	99%	3.00E-172	86%
Podocoryna borealis isolate 152 16S ribosomal RNA gene, partial sequence; mitochondrial	601	601	99%	2.00E-168	85%
Symplectoscyphus tricuspidatus voucher MNHN INVE29953 16S ribosomal RNA gene, partial sequence; mitochondrial	599	599	99%	8.00E-168	85%
Halecium delicatulum mitochondrial partial 16S rRNA gene, isolated from Antarctica, Ross Sea	595	595	99%	1.00E-166	85%
Eucheilota menoni voucher MHNG INVE33457 16S ribosomal RNA gene, partial sequence; mitochondrial	590	590	100%	5.00E-165	85%
Bimeria vestita mitochondrial partial 16S rRNA gene	590	590	99%	5.00E-165	85%
Salacia desmoides 16S ribosomal RNA gene, partial sequence; mitochondrial	588	588	100%	2.00E-164	85%

Table 2: *Turritopsis nutricula* with its most related individuals *Clytia sp, Eirene ceylonensis, Nemopsis bachei, Clytia folleata, Clytia elsaeoswaldae, Obelia bidentata, Clytia gracilis, Eirene brevistylus* and *Turritopsis lata* arranged according to their E-Value and Max Indent based on 16s rRNA relative study.

Relative Species	**Max score**	**Total score**	**Query coverage**	**E value**	**Max ident**
Turritopsis nutricula isolate 1 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	1147	1147	100%	0	100%
Clytia sp. KC JRH-2012 isolate 1 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	625	625	98%	1.00E-175	85%
Eirene ceylonensis isolate 3 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	625	625	99%	1.00E-175	85%
Nemopsis bachei mitochondrion, partial genome	625	625	99%	1.00E-175	85%
Clytia folleata cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	619	619	99%	7.00E-174	85%
Clytia elsaeoswaldae cytochrome oxidase subunit I gene, partial cds; mitochondrial	619	619	97%	7.00E-174	85%
Obelia bidentata voucher 76NC cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	619	619	95%	7.00E-174	85%
Clytia gracilis voucher Brazil.5 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	617	617	98%	2.00E-173	85%
Eirene brevistylus isolate 4 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	614	614	99%	3.00E-172	85%
Turritopsis lata cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	593	593	100%	4.00E-166	84%

Table 3: *Turritopsis nutricula* with its most related individuals *Turritopsis lata, Didiscus sp., Tiaricodon coeruleus, Amphinema dinema, Laodicea undulata, Bougainvillia muscus, Clytia sp., Eirene ceylonensis, Eutima levuka, Eucheilota menoni* and *Helgicirrha malayensis* arranged according to their E-Value and Max Indent based on protein relative study.

Description	Max score	Total score	Query coverage	E value	Max ident
cytochrome oxidase subunit I, partial (mitochondrion) [Turritopsis nutricula]	407	407	100%	1.00E-142	100%
cytochrome oxidase subunit I, partial (mitochondrion) [Turritopsis lata]	362	362	100%	6.00E-125	97%
cytochrome oxidase subunit I [Didiscus sp. UCMPWC1040]	363	363	100%	6.00E-125	87%
cytochrome oxidase subunit I, partial (mitochondrion) [Tiaricodon coeruleus]	359	359	100%	9.00E-124	96%
cytochrome oxidase subunit I, partial (mitochondrion) [Amphinema dinema]	353	353	100%	3.00E-121	97%
cytochrome oxidase subunit I, partial (mitochondrion) [Laodicea undulata]	350	350	100%	4.00E-120	97%
cytochrome oxidase subunit I, partial (mitochondrion) [Bougainvillia muscus]	350	350	100%	5.00E-120	94%
cytochrome oxidase subunit I, partial (mitochondrion) [Clytia sp. KC JRH-2012]	349	349	100%	7.00E-120	97%
cytochrome oxidase subunit I, partial (mitochondrion) [Eirene ceylonensis]	349	349	100%	8.00E-120	97%
cytochrome oxidase subunit I, partial (mitochondrion) [Eutima levuka]	349	349	100%	9.00E-120	97%
cytochrome oxidase subunit I, partial (mitochondrion) [Eucheilota menoni]	349	349	100%	9.00E-120	97%
cytochrome oxidase subunit I, partial (mitochondrion) [Helgicirrha malayensis]	349	349	100%	1.00E-119	97%