



ASIAN UNIVERSITY FOR WOMEN

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SEARCH TO DEFY DEATH



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Abstract:

Turritopsis dohrnii has been identified to be an “immortal” species. It has been observed that *T. dohrnii* have the ability to revert back to its juvenile polyp state once exposed to stress or threat. Such capability of reverting into its polyp stage could be due to some special genetic make-up present in the jellyfish or perhaps due to the environment, the blue zone area, where the jellyfishes are usually present.



Image: Turritopsis dohrnii. The Creature That Defies Death immortal-jellyfish.com. Web. 26 November 2018.

The research aims to predict and understand whether environment or the presence of a particular gene is responsible for the unique trait of *Turritopsis dohrnii*. Therefore, in order to determine the “immortal” gene, the closely related species of the jellyfish was first studied and both pairwise and multiple alignment using Molecular Evolutionary Genetics Analysis (MEGA) and ClustalW was performed. The research used bioinformatic tool, MEGA to construct a phylogenetic tree using 16S ribosomal RNA sequence for certain species of the jellyfish belonging to the medusozoa clade, taken from various geographical locations. Once the research establishes that the environment is the not the major reason for the arise of such unique trait, it

can then proceed towards determining the sequence of interest and try to understand its expression followed by protein modelling.

Introduction:

Turritopsis dohrnii, the immortal jellyfish, is a species of small, biologically immortal jellyfish, mostly found in the Mediterranean Sea and in the waters of Japan. Also, in China, *Turritopsis* are widely distributed in the bays of Xiamen, however, their classification status and capability to reverse the life cycle have not been well characterized (Li 2018).

Turritopsis dohrnii is not immortal by definition. However, it does have an extraordinary ability to revert its cells back to their infancy stage as many times as they need, which is extremely unusual and quite impressive. Thus, the only known way they can die is if they get consumed by another fish or if a disease strikes the jelly. Although, the process of reverting from its adult-phase to a polyp was observed several times, it hasn't been observed yet in nature but only in laboratory environments (Stages of reverse development in *Turritopsis*; Kubota et.al; Kumar). In order to first understand and figure out the immortal gene it is first important to understand the life cycle of the jellyfish and to know which class this particular jellyfish belongs to. It is also essential to find out whether other species close to *Turritopsis dohrnii* portrays similar characteristics.

Turritopsis dohrnii belongs under the class Hydrozoa, in the clade of Medusozoa. This paper would comprise organisms from the clade Medusozoa only.

Medusozoa is a clade in the phylum Cnidaria, and is often considered a subphylum. It includes the classes Hydrozoa, Scyphozoa, Staurozoa and Cubozoa, and possibly the parasitic

Polypodiozoa. Medusozoa are distinguished by having a medusa stage in their often complex life cycle, a medusa typically being an umbrella-shaped body with stinging tentacles around the edge. With the exception of some Hydrozoa (and Polypodiozoa), all are called jellyfish in their free-swimming medusa phase.

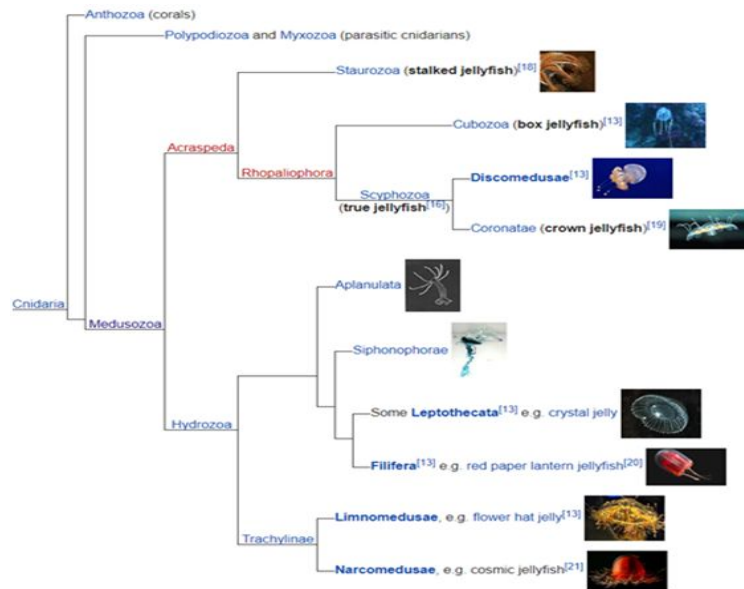


Figure: *The clades, classes of jellyfish* (Jellyfish Taxonomy)

Jellyfish or sea jellies are the informal common names given to the medusa-phase of certain gelatinous members of the subphylum Medusozoa, a major part of the phylum Cnidaria. Jellyfish are mainly free-swimming marine animals with umbrella-shaped bells and trailing tentacles, although a few are not mobile, being anchored to the seabed by stalks. The bell can pulsate to provide propulsion and highly efficient locomotion (Brooks). The tentacles are armed with stinging cells and may be used to capture prey and defend against predators. Jellyfish have a complex life cycle; the medusa is normally the sexual phase, the planula larva can disperse widely and is followed by a sedentary polyp phase.

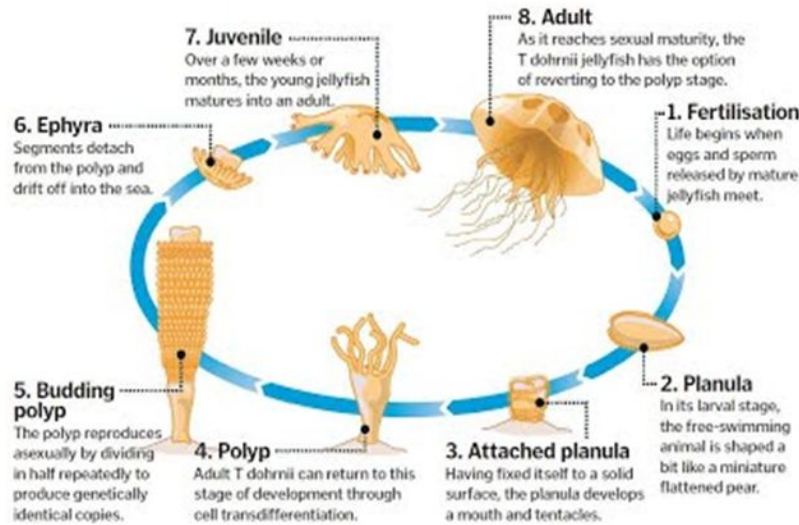


Figure: *The life-cycle of Turritopsis dohrnii.* (How it works)

Similar to *Turritopsis Dohrnii*, the other two organisms of the *Turritopsis* genus have been found to be quite closely related. In fact, there has been a lot of confusion even inside the scientific community between the three types of turritopsis jellyfish: the *dohrnii*, the *nutricula* and the *rubra* (The Immortal Jellyfish; Rogaev, 2017; Piraino, et.al). Since the *Turritopsis* genus is common in many parts of the world, it was not an easy task to differentiate between these tiny jellyfishes. *T. nutricula* is found to be more genetically similar to *T. dohrnii* than *T.rubra*, residing in the Caribbean and North America and the cycle reversal was not in fact observed on the *nutricula* (Carla; et.al; The Immortal Jellyfish; Piraino, et.al; Lisenkova et.al, 2017).

However, that does not refer to the fact that *T. nutricula* is biologically mortal but rather it has not been observed and proven yet. The research uses MEGA to construct a phylogenetic tree for the species taken from various location and regions. MEGA is the abbreviation of Molecular Evolutionary Genetics Analysis. It is a computer software for conducting statistical evolution for conducting statistical analysis of molecular evolution and for constructing phylogenetic tree. In

an article named “MEGA: Molecular Evolutionary Genetics Analysis (MEGA) software version 10.0”, the author Koichiro Tamura et.al, mentioned that “Since the early 1990s, MEGA software functionality has evolved to include the creation and exploration of sequence alignments, the estimation of sequence divergence, the reconstruction and visualization of phylogenetic trees, and the testing of molecular evolutionary hypotheses” (Kumar, Tamura, Nei, 2007). Hence, the project would compare similarities of sequences collected from National Center of Biotechnology Information (NCBI) and construct the tree.

Materials and Methods:

The research aimed to observe the similarity between the mortal and immortal jellyfishes from different geographical locations but keeping the type of sequence taken for comparison constant. In this project, few steps were performed using various databases and different bioinformatics tools. The sequences used for comparison were taken from 16S ribosomal RNA gene (mitochondrial partial sequence) but from different organisms (28 organisms in total) who are present in different geographical locations. The initial step was to get the DNA sequences from both mortal and immortal jellyfishes and this was done using MEGA 10.1 which is a specialized software to work on Molecular and Evolutionary Genetic Analysis. The NCBI database which contained all the required sequences was accessed through MEGA 10.1 in order to view ‘Add to Alignment’ bar so that similarity between all observed sequences could be identified. Using “Add to Alignment” the 16S ribosomal RNA sequences were added to MEGA 10.1 from Basic Local Alignment Search Tool (BLAST) search tool. This step was repeated depending on the number of both same and different species taken for alignment. After adding it to alignment

successfully, the sequences were renamed based on the scientific name or the general name of the species. The sequences were then used for sequence alignment (both pairwise and multiple alignment) using Clustal W through MEGA 10.1 that showed the similarity between the sequences of different organisms with a colorful notation for each nucleotide, in order to facilitate the interpretation of sequence similarity. Next, using the result of alignment, the MEGA software was used to construct a phylogenetic tree that showed evolutionary relationship between the various organisms which says how closely or distantly they were related. Therefore, the bioinformatic tools we used were MEGA 10.1 and Clustal W, to construct our Phylogenetic tree and do both pairwise and multiple alignment. Then, in order to assume structural similarity at the sequence level, protein modeling of most similar sequences among different species would be performed. In this case, we did protein modeling of 16S rRNA gene of *Turritopsis dohrnii* and *Turritopsis nutricula* since it was observed that *Turritopsis dohrnii* and *Turritopsis nutricula* were the organisms that are closely related and they might have arisen from most recent ancestors.

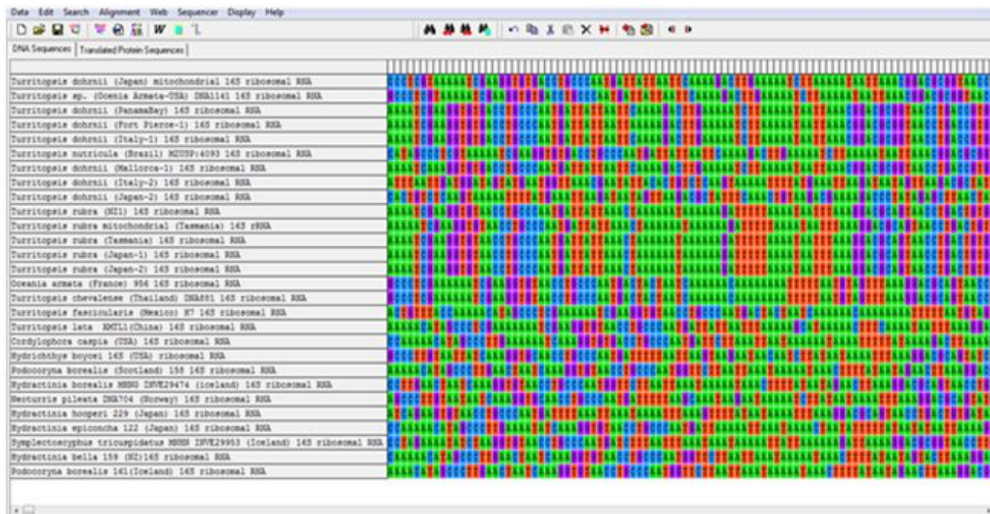


Figure: Pairwise alignment using MEGA

Results and Discussion:

The research used 16S ribosomal RNA of *T. dohrnii*, taken from several geographical regions, which demonstrated that *T. dohrnii* does in fact consists a certain gene which results its immortal trait and not mainly because of the region. After observing the phylogenetic tree constructed by MEGA, it was observed that *Turritopsis dohrnii* and *Turritopsis nutricula* were the organisms that are closely related and they might have arisen from most recent ancestors despite the fact that they share different geography. Therefore, this indicates that years ago, they might have been the same species but evolution has caused them to result into two different species. Thus, there is a high probability that *T. nutricula* might also have the “immortal” trait like *T. dohrnii* due to their closeness in the phylogenetic tree.

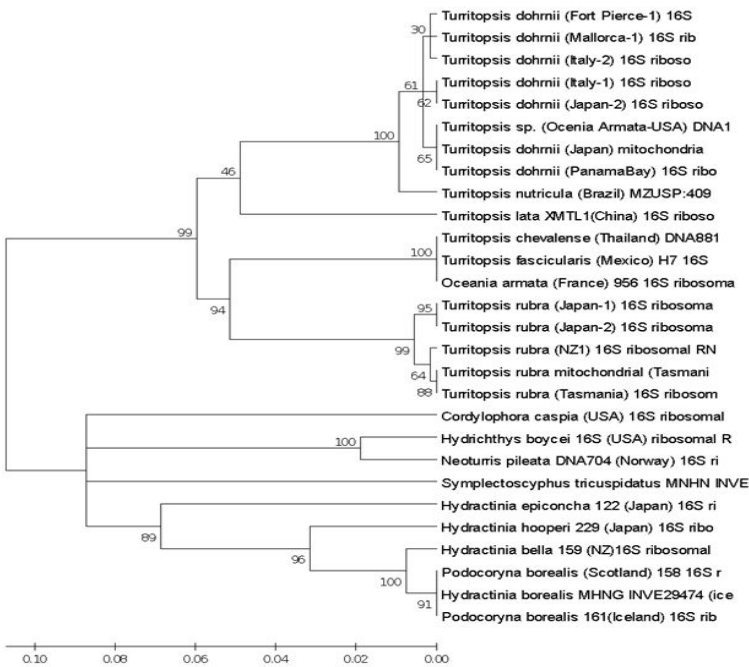


Figure: Phylogenetic Tree constructed using MEGA demonstrates the closely related species of *T. dohrnii* taken from different regions.

This result matched with the initial hypothesis and was not at all surprising. However, the initial study involved a third organism, *Turritopsis Rubra*, along with *T. dohrnii* and *T. nutricula* which surprisingly was seen to be ancestrally quite distantly related from them according to the result of this phylogenetic tree. Although we predicted *T. rubra* to be very closely related to these two, it is quite remote from them and so this might portray why *Turritopsis rubra* has not shown traits of “immortality”. Some other organisms who were also distant, yet relatively closely related from *T. dohrnii* were *Turritopsis lata*, *Turritopsis chevalense*, *Turritopsis fascicularis* and *Oceania Armata*. However, very less information has been yet known about these species of the *Turritopsis* genus unlike *T. nutricula* and *T. rubra*. Even though these organisms were somewhat closely related to *T. dohrnii*, they too didn’t show the immortal trait like *T. dohrnii*. Now, it can be stated that either the distantly related species of *T. dohrnii* either abstain from expressing the “immortal” gene due to nutrigenetics or other factors, or the related species do not contain the “immortal” gene within their genome. For now, since there is no evidence that these organisms are immortal, sequence comparison and analysis among *T. dohrnii* and the other *Turritopsis* species could ultimately show how they differ in order to find the gene that is unique to *T. dohrnii*.

Conclusion:

Turritopsis jellyfish potentially could be the key towards longer life span, if not immortality. Scientists are already trying to generate stem cells, studying this particular jellyfish. Besides, as regenerative medicine continues to grow in the future of medicine, it’s clear that this tiny jellyfish may hold the answers to not only addressing many age-related issues face, but also

owning mortality. Much of the advances in stem cell technology came from understanding stem cells itself. So, for transdifferentiation, hope still remains to eventually learn how creatures like *Turritopsis* skip the stem cell step and go directly from one cell type to another (Dimberu, 2011). This indicates that along with stem cell-based treatments, researchers can parallelly put their time and money into exploring transdifferentiation-based treatments as well for advancing in the field of regenerative medicines, where the phenomena of *Turritopsis dohrnii* stands to be potential. Essentially there are many future aspects towards this project such as studying nutrigenetics of the species. There is high probability that the nutrients the jellyfish feed on, effectively is responsible to activate the immortal gene in *Dohrnii*. Additionally, using bioinformatics tools such as LAST, DNASTAR, detect the differences in the gene sequence as present in the genetic sequence between mortal and immortal jellyfish. The next step, a continuation of the project, is to use LAST and determine the difference in sequences for the mortal and immortal species and predict the particular gene, which possibly is responsible for “immortality”. Some of online databases and tools to be used will be Ensemble server, ExPASy Translator Tools, PSIPRED, Swiss Model and I-TASSER. Then, once the predicted gene is identified, the gene can be inserted in *Scyphozoa* (true jellyfish under medusozoa) embryo or other closely related jellyfish species, such as *T.rubra*, to observe whether the organism behaves accordingly to *Dohrnii* once exposed towards stress.

With this project, thus, having many future aspects, the search to defy death can be continued and if not, may thrive to search ways in nature and integrate the findings within people to live a healthy, long life.

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Figure 4: Stages of reverse development in *Turritopsis* sp.5 from Xiamen, China. (n.d.).

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Image: *Turritopsis dohrnii*. *The Creature That Defies Death* immortal-jellyfish.com. Web. 26

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