SELECTING EDIBLE MEDUSAE BY 18S RDNA SEQUENCING

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Introduction

The Food and Drug Administration Agency of United States of America (FDA) has created, in the last seven years, a DNA Barcoding database with more than 3 million sequences of DNA. The project global name is BOLD, Barcode of Life Data Systems, which aims to contribute for the public health, as well as to the information on species distributions and taxonomy.

In relation to fungi, the BOLD system has adopted sequences from the Internal Transcribed Spacer Region, ITS. The respective validation is achieved with the BLAST algorithm. When considering plants, it was adopted the sequences for rbcL (Ribulose-bisphosphate carboxylase) and matK (Maturase K) genes. Regarding animals, the BOLD Identification System (IDS) has adopted COI sequences from the 5' region of the mitochondrial Cytochrome c oxidase subunit I gene.

Considering cnidarians, other DNA sequences may be used as biomarkers, in addition to FDA's selected one. For example, in Europe, an Italian research group has proposed the use of the cytochrome b gene (*cytb*) to discriminate close medusa species (Armani *et al*, 2013) while a German group has used the ribosome small subunit, 18S rDNA, to compare Scyphozoan exemplars (Holst & Laakmann, 2014) - once it is one of the most frequently used genes in phylogenetic studies.

In the current study, we present the result of a comparison between edible and non-edible cnidarians which were successfully separated by a cladogram based on 18S rDNA sequencing. The experimental assays were made with *Catostylus tagi*, a native Scyphozoa from Tagus and Sado estuaries. Other cnidarian sequences, namely *Catostylus mosaicus*, *Cyanea capillata*, *Hydra magnipapillata*, *Lychonorhiza lucerna* and *Rhopilema esculentum* were obtained from NCBI database.

Laboratorial Procedure

Gonads were taken out from jellyfish and processed using two dialysis membranes , one with less then 1kDa and another with less then 15 kDa (Membrane filtration products inc,). The process ran on 2 liters water column, for 5 days, the water was periodically swapped. The sample was then lyophilized to concentrate. The DNA extraction was performed with E.Z.N.A Mollusc DNA kit and Omega Bio-TEK procedures. The primers for DNA sequencing were taken out from Bayha *et al* (2010), forward : 18 Sa 5´-AACCTGGTTGATCCTGCCAGT-3´; and reverse 5´-GATCCTTCTGCAGGTTCACCTAC-3´. Temperature program for PCR is presented in table 1. DNA sequencing equipment was a 3730xl DNA Analyzer (Applied Biosystems) with BigDye® kit Terminator v3.1.

Table 1 – PCR Program

1x	95ºC for 15 min
35 cicles	94ºC for 30s
	60ºC for 15s
	70ºC for 30s
	72ºC for 6 min
Ends at 4ºC	

Results

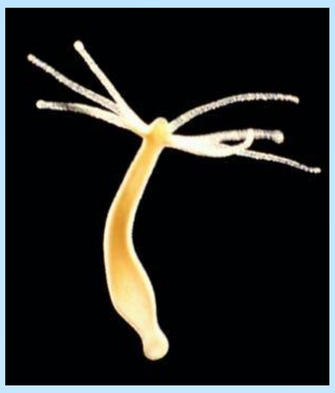
Catostylus Tagi
http://www.perseus-net.eu/en/species_of_jellyfish/index.html

***Lychnorhiza lucerna* (Haeckel, 1880)**
http://www.cenemar.org.br/foto_do_dia/foto_25.htm

Catostylus mosaicus
http://www.aqua.org/explore/animals/jelly fish-blue-blubber-jelly

Rhopilema esculentum
http://www.typesofeverything.com/types-of-jellyfish/

Cyanea capillata
http://www.habitas.org.uk/marinelife/species.asp?item=D760



Hydra magnipapillata
http://www.coolweirdo.com/freshwaterpolyp-lifespeciesthat-livesforever.html

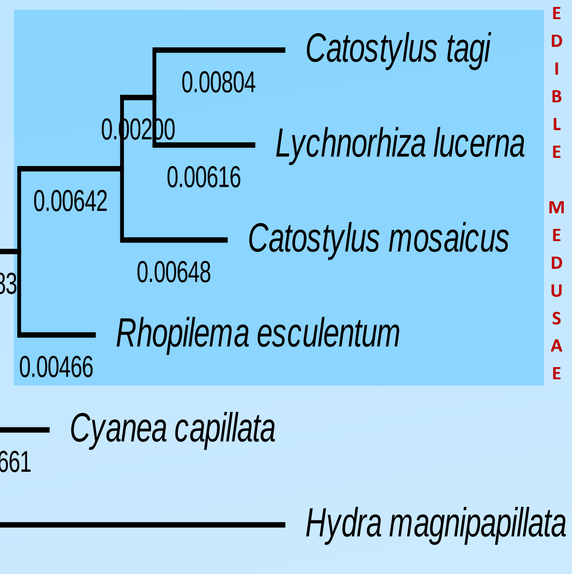


Fig. 1- Cladogram based on 18S rDNA

Conclusion

The cladogram based on 18S rDNA established a separation between the edible and non-edible cnidarians tested. One group was composed by *Catostylus tagi*; *Catostylus mosaicus*; *Lychnorhiza lucerna and Rhopilema esculentum,* which forms the edible group, while *Hydra magnipapillata and cyanea capillata* belong to the non-edible group (Fig. 1).

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