The relationship of shellfish from Suzhou and Shanghai

—— DNA Barcoding Programme

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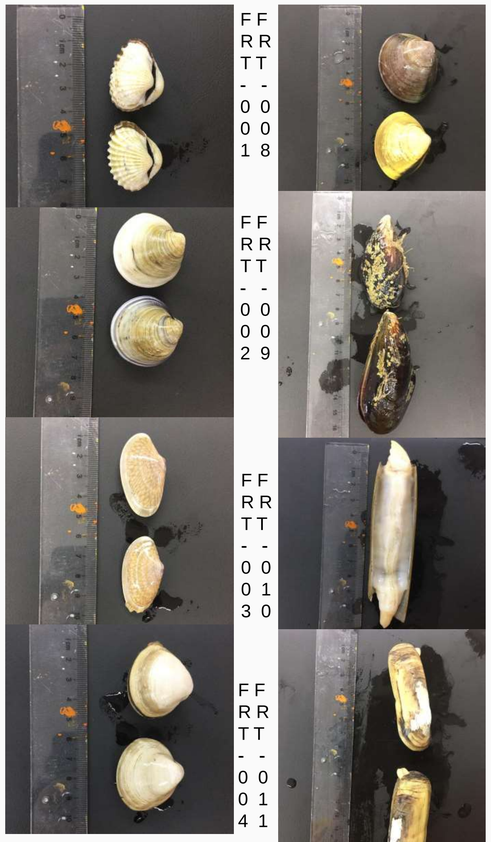

FRT-001：血蛤 FRT-002：蛤蜊 FRT-003：油蛤
FRT-004：白蛤 FRT-005：毛蛤 FRT-006：黄蚬
FRT-007：花甲 FRT-008：文蛤 FRT-009：贻贝
FRT-010：竹蛏 FRT-011：蛏子 FRT-012：黄金贝
FRT-013：石蚬 FRT-014：七彩贝

Abstract

This research focuses on shellfish, including the DNA sequencing and the building of phylogenetic trees. The DNA extraction is based on a combination of Silica method and DNeasy Blood & Tissue Kit. In the end, only 6 samples are succeeded in gaining the DNA sequences.
A comparison between the new phylogenetic tree and the traditional classification is carried out.

Introduction

The shellfish refers to a type of invertebrate animal with a shell, which belongs to the phylum *Mollusca*. *Mollusca* contains a large number of different species but with a relatively similar appearance.

Moreover, the environmental influence on their appearance is quite prominent . Therefore, setting up DNA barcoding information for them provides a remarkable scientific significance to further research and classification.

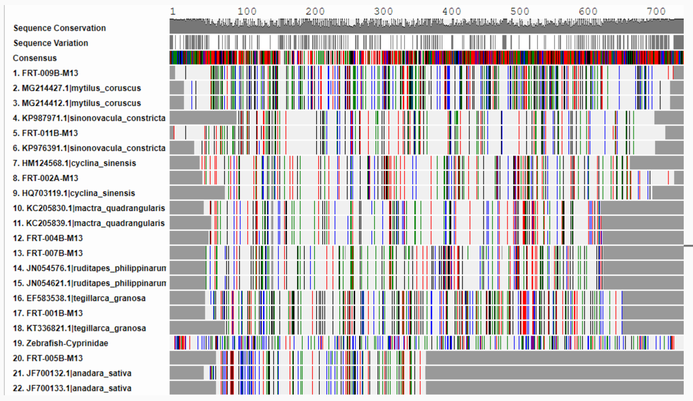

Figure 1, DNA barcode

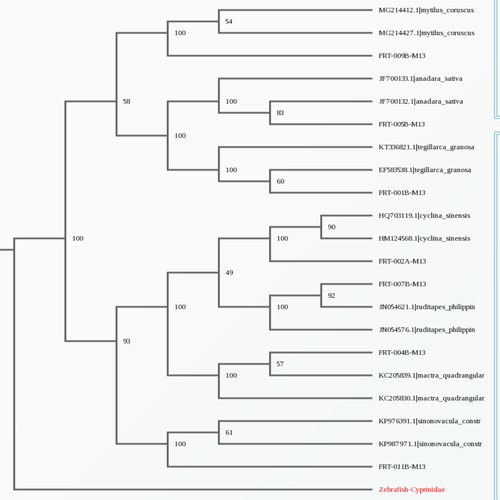

Figure 2, NJ phylogenetic tree

Materials and Methods

In all, 14 different shellfishes with similar appearance had been collected from two markets in Suzhou and Shanghai. They are *Tegillarca granosa*, *Paphia undulate*, *Mactra veneriformis Reeve*, *Scapharca subcrenata*, *Mactra Chinensis*, *Ruditapes philippinarum*, *Meretrix lusoria*, *Mytilus edulis*, *Solen strictus, Sinonovacula constricta*, and 4 other samples with common names in Chinese only (蛤蜊 , 黄金贝 , 石蚬 七彩贝).
(All scientific names come from Internet research based on samples' Chinese common names.)

The samples were collected on 1st August and 2nd August in 2020, and the experiment was carried in the week after that, from 3rd August to 5th August. All samples are stored in the freezer before and after obtaining the tissue samples for the DNA extraction.

All samples firstly completed the DNA extraction process by using the Silica method. Later, six samples that failed in the Silica method (FRT 008, FRT 010, FRT 011, FRT 012, FRT 013, and FRT 014) tried with DNeasy Blood & Tissue Kit.The extracted DNA samples were amplified by PCR with invertebrate COI primer(LCO1490/HC02198). PCR products were analyzed by 2% agarose gel electrophoresis.
The PCR products with positive gel electrophoresis were sent to GENEWIZ for DNA sequencing. In the DNA Subway, sequences sending back were compared with the existing sequences. Each sample only chose the most complete sequence for the phylogenetic tree building. In the end, both NJ and ML phylogenetic trees were built.

Result

Unfortunately,DNA exaction of FRT 003, FRT 006, FRT 008, FRT 010, FRT 012, FRT 013, and FRT 014 were failed. And they will not be discussed in the result part.
According to the matching results in the DNA Subway, some scientific names getting from the Internet research are inaccurate. Figure below shows the NJ phylogenetic tree of the seven samples of shellfishes in this research. As the tree is shown, FRT 004, FRT 007, FRT 002, and FRT 011 converge to one same group while FRT 005, FRT 001, and FRT 009 belong to another group.
Among them, FRT 001 and FRT 007 are the only two samples that can identify as the same species as its information provided by the Internet. In contrast, FRT 002 failed to determine as *Cyclina sinensis*, and FRT 011 failed to determine as *Sinonovacula constr*. Moreover, FRT 004 identify as *Mactra quadrangular*; FRT 005 identify as *Anadara Sativa*, and FRT 009 shows a close relationship with the species *Mytilus coruscus*.

Discussion

The result of the research cannot show the difference between the classifications of shellfishes based on DNA barcodes and traditional methods, since only seven shellfish samples are in the phylogenetic tree building. What can be seen is that the naming system of the shellfish group is not yet complete. To be more specific, the species names cannot be one to one correspondence among different languages.

The classification based on DNA barcodes contains shortcomings that make it unable to replace the traditional classification. Since there is no clear definition standard for the variation inside and between species, the DNA barcoding result cannot directly apply to the species authentication. Besides, the design of primer sets for DNA barcoding aims to be universal, that will miss considerate some specific species. In general, many obstacles in the process of species authenticating are required to break up

In this case, FRT 008, FRT 010, FRT 012, FRT 012, FRT 013, and FRT 014 were failed in DNA extraction with both methods, while FRT 003 and FRT 006 only tried the Silica method and failed. As mentioned above, unsuitable primer sets can cause failed DNA extractions. Contaminated or damaged tissue samples used and nonstandard operations during the process of DNA extraction can also cause an error .
Therefore, primer sets other than CO1(LCO1490/HC02198) are worth using in the DNA extraction of the shellfish . In the process of this research, researchers had difficulties in pairing the scientific names on the Internet with common names used in markets. Several websites provide primary information on some species but with incomplete naming system.

References:
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