

Introduction

There are thousands of shrimp species around the world. Over 2,000 different shrimp species spread out all over the world in every known water bodies, from the tropics to the Antarctic Ocean. In Suzhou, there are many bodies of water within and surrounding the city; Suzhou is known as the "Venice of Asia." Growing up in this area has given me many opportunities to see the numerous types of shrimps being sold in markets and seafood restaurants. The rearing and selling of aquaculture products like shrimps are an important part of fishing agriculture and to the economy of this area. Some shrimp are small and pink, one kind has black zebra-like stripes, some have a big head and another species has a flat head, and some of them have large chelicera. This has led me think about the question, what is the evolutionary relationship between the different species of shrimps found in the region?

The DNA barcode consists of a standard short sequence of DNA that can be easily isolated and characterized for all the species on the earth, it allows users to quickly and accurately recognize known species and gives information about them. The concept of a universally recoverable segments of DNA that can be used as identification markers across species was initially applied to animals with the Cytochrome C oxidase gene region. I used the analyzed the evolutionary relatedness of 10 seemingly different shrimp using the CO1 gene.

Materials & Methods

Collect samples

1.Samples(QYT001,002,003a,004,005,006,007a,008a)were collected from Nanmen market. and samples from local restaurant(QYT003b,007b,008b,009,010) **DNA** extraction method

a). Use silica method to extracted DNA.(QYT001,002,003a,004,005,006,007a,008a).

b).And use Qiagen Dneasy kit to extract DNA from samples(003a,005,006,007a,008a).

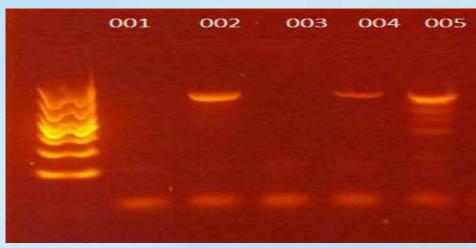
2. Analyze PCR products by gel electrophoresis at 130V 20minutes. View the gel using UV transillumination. 3.Sequencing

The DNA samples are sequenced wih Genewiz if there are visible bands around 600bp on the gel. DNASubway was used to analyze the DNA sequences. Q4-3(BRT003) and Q4-4(BRT001) sequences are collected from previous project "Exploring shrimps and crabs" of Lanxi. Y and Xuelin. W.

Discussion

I obtained 10 results from 11 samples. The QYT001, OYT002, QYT008, QYT011 all have a good quality. The quality of other samples is not as good. When I generated the tree I found that there are many amazing results. They are mainly divided into two groups. The first group also can be split into two parts. The QYT001, 002, 005, Q4-4 group and the QYT011, Q4-3 group. I found that QYT001 shares the same genus *Macrobrachium* with Q4-4 sample.





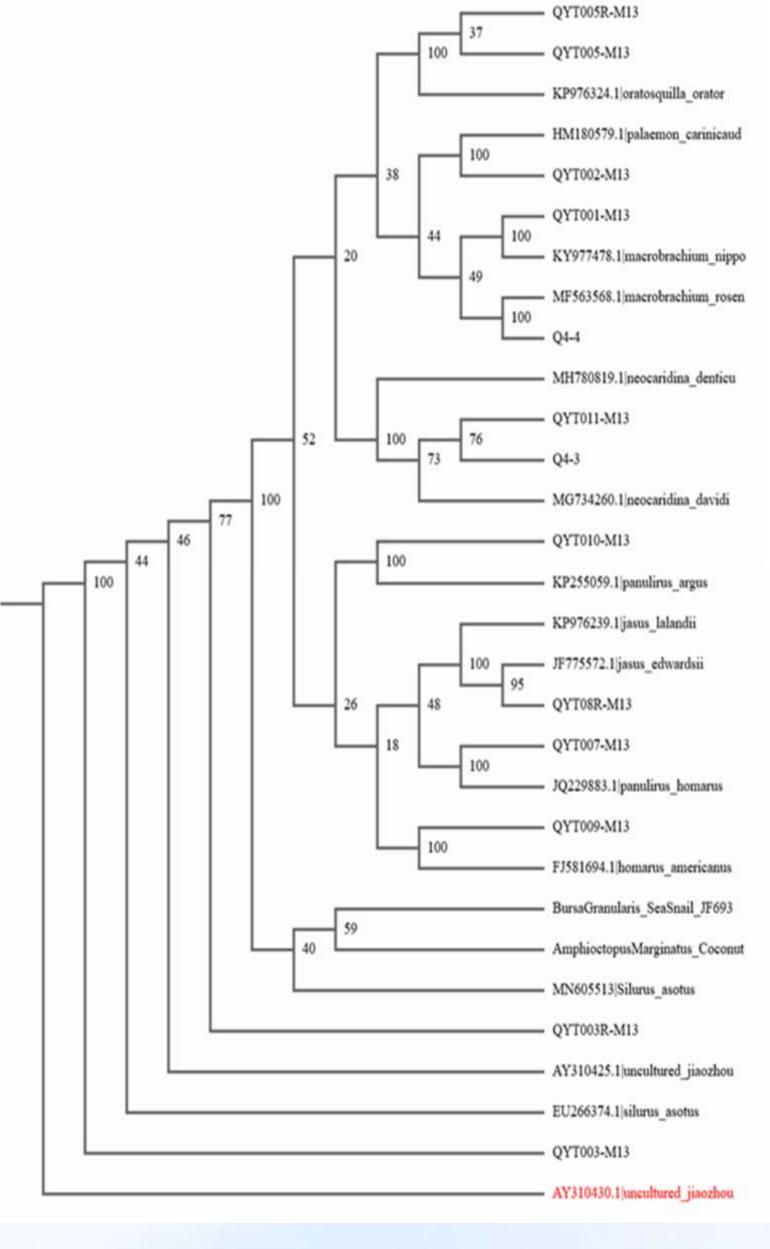


Fig.6 The evolutionary relationship



The evolutionary relationship of shrimps

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Facts and Figures

Fig.1 The 11 shrimp samples collected at different areas of Suzhou, QYT001-006and QYT011 are collected from local market and QYT007-010 are collected from local restaurant.

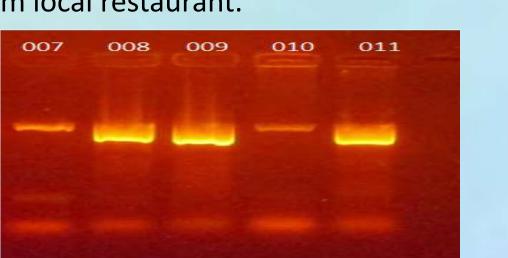
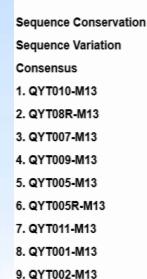
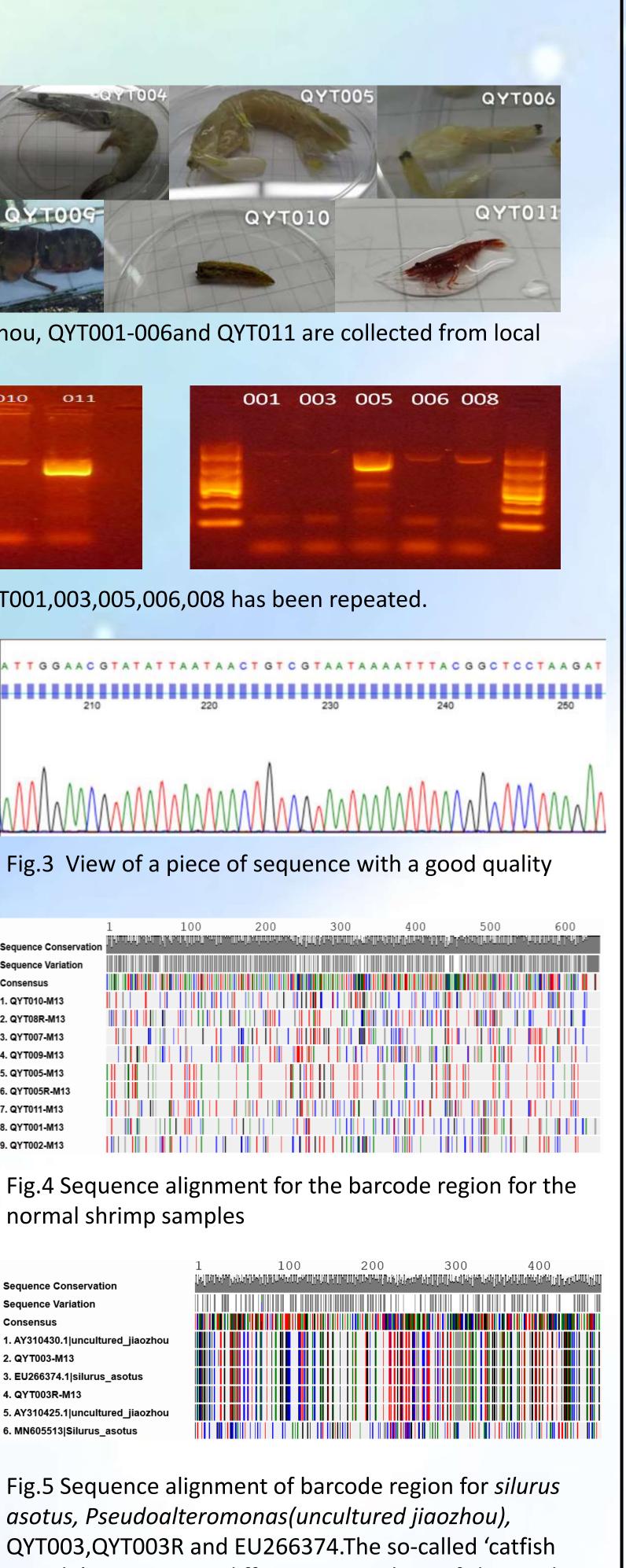




Fig.2 PCR products gel electrophoresis results for samples, QYT001,003,005,006,008 has been repeated.





normal shrimp samples

Sequence Conservation Sequence Variation 1. AY310430.1 uncultured jiaozho 2. QYT003-M13 3. EU266374.1|silurus asotus 4. QYT003R-M13 5. AY310425.1 uncultured_jiaozhou 6. MN605513ISilurus asotus



Fig.5 Sequence alignment of barcode region for *silurus* asotus, Pseudoalteromonas(uncultured jiaozhou), QYT003,QYT003R and EU266374.The so-called 'catfish sample' EU266374 is different to another cafish sample but more similar to those bacteria.

Description	
seudoalteromonas marina strain ECSMB14103 chromosome, complete genome	
seudoalteromonas sp. 13-15 chromosome 1, complete sequence	
ilurus asotus isolate CO1A6 cytochrome oxidase subunit I-like (COI) gene, partial sequence; mitochondrial	

zooplankton isolate MC39 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondi oplankton isolate MC27 cytochrome oxidase subunit I (COI) gene, partial cds: mitochond u Bay zooplankton isolate MC36 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondi ankton isolate MC29 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondi ou Bay zooplankton isolate MC26 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondri

Max Score	Total Score	Query Cover		Per. Ident	Accession
1140	1140	100%	0.0	99.06%	CP023558.1
1118	1118	100%	0.0	98.43%	CP019162.1
1059	1059	93%	0.0	98.66%	EU266374.1
1020	1020	89%	0.0	98.95%	AY310434.1
985	985	85%	0.0	99.45%	AY310430.1
968	968	85%	0.0	98.89%	AY310433.1
952	952	85%	0.0	98. <mark>34</mark> %	AY310431.1
939	939	81%	0.0	99.42%	AY310429.1
937	937	83%	0.0	98.67%	AY310436.1

Fig.7 Further blastn results for EU266374



They share 100% similarity with their blastn results. But they have different species. QYT001 is Macrobrachium nipponense which is a native shrimp species in China. While Q4-4 is Macrobrachium rosenbergii which comes from South East Asia. It has been introduced in China for food because it can grow bigger and faster. QYT002 has a close relationship to the *Macrobrachium,* which is *Palaemon carinicauda*. QYT005 is likely to be a Oratosquilla orator. the repeated experiment has the same results, though the first results has a bit of difference. Another split which is the QYT011 and Q4-3 are the most similar; they may even be the same species of cherry shrimp, Neocaradina denticulata. The second group are mostly the lobsters. QYT007 and QYT008 have the closest relationship compared with other samples. QYT007 is Panulirus homarus, because it shows 100% of similarity with blastn. QYT008 is known as 'Australian spiny lobster' in people but results actually shows that is the genus Jasus, it is more closely related to species Jasus edwardsii. The QYT009 is likely be a Homarus americanus because it shows a 100% similarity with blastn result. QYT010 is Panulirus argus, which shows the same genus with the QYT007. But QYT010 has the least similarity with the other shrimps. The blastn results for sample QYT003 firstly shows a catfish compared with EU266374. But other blastn results shows it is a kind of *Pseudoalteromonas*. From the blastn it is nearest to uncultured jiaozhou. The extraction and blastn has been repeated in QYT003R and they show the same results. So I add another sample for catfish MN605513 as a comparsion. To my surprise, two catfish samples MN605513 and EU266374 looked different in their sequences alignment. In further blastn in nbci website for the Silurus asotus EU266374. It has over 98% similarity with those uncultured jiaozhou *Pseudoalteromonas*. This means actually EU266374 contains gene from those bacteria too. The collector of it is come from S.Korea. I hope someone corrects the sample on the website. I think there must be something wrong when my samples collected or extraction process, also wrong with the blastn. And the CO1 primer has copy the gene from bacteria. From the internet, I know that CO1 gene come from the mitochondria DNA. And mitochondria has a theory to evolving from small bacteria living in other cells, so they might share the same pieces of gene to match the CO1 primer. *Pseudoalteromonas* is a kind of bacteria widely spread in the sea. It can live almost everywhere in water (including the surface of a marine organism, like shrimp). It may be attached on the shell of the shrimp and mix into the sample.

Sample QYT006 was found to have human DNA, and that may have been caused by skin cells falling from human body being mixed into the same tube during the extraction. Also, the skin cell might be mixed in during the manufacturing or even cooking because the container of shrimp had been kept in the fridge for at least one year.

Reference

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