Determining the Evolutionary Relationship of Tropical Fish Using DNA Barcoding

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Abstract

The purpose of DNA barcoding is to identify and classify tropical fish.I collected and analysed sequence data from tropical fish, used DNA sequence to identify species, then explored relationships between tropical fish species. I found ART-007 and ART-017 are the same species, ART-003 and ART-006 have some similarities too. ART-002, ART-009 and ART-008 are closely related. ART-011, ART-013 and ART-010 also has close relationships. ART-012 has close relationship to the common swordfish. I also learned how to improve my experimental techniques and how to analyse data.

Introduction

Tropical fish are generally those fish found in aquatic tropical environments around the world, including both freshwater and salt water species. Fishkeepers often keep tropical fish in freshwater and saltwater aquariums. Tropical fish come in different colors and size. There are different kinds of tropical fish we can see in our daily life, it becomes more and more popular nowadays, so a lot of people keep them as pets, some of them look similar, some of them look extremely different, but all of them look beautiful. Whether they have close relationship or not, whether some of tropical fish are related to each other because they have been hybridized by people. Tropical fish do not live in the same habitats, so I suggested that although some tropical fish look different but they are same species, and some fish looks similar and has similar common names but they are not closely related. It is interesting to turn out the amazing and complicated relationship between these tropical fish. I would like to achieve this by having an experiment on some typical tropical fish and barcoding them, by doing this I can find the relationships between some tropical fish species.

I can learn more about tropical fish in this research, after that maybe I can reproduce another tropical fish with better genes.

Materials and methods

I collected dead fish for my specimens from the market, I asked assistants to give me some dead fish for the specimen collection. Document the specimen and identify them first, then record them into the database.

Silica method

1.Cut muscle pieces of different fish as tissue samples.

2.Add lysis solution, grind samples, vortex and incubate. Then centrifuge and transfer the supernatant.

3.Add silica resin, mixed by pipetting in and out, vortex, incubate and centrifuge. Move the supernatant away. Add wash buffer, vortex and centrifuge. Repeat the steeps remove the supernatant, added the wash buffer, vortex and centrifuge, remove the supernantant, centrifuge again and remove the remaining supernatant.

4.Add dH2O and mixed by pipetting in and out, vortex, incubate and centrifuge. Transfer the supernatant to the fresh test tubes and store it.

Not all the samples successful in extracting DNA by using silica method, so Dneasy blood&tissue kit method can be done for those samples which failed in DNA extracting.

Kit method

1.Cut some tissue into a tube. Add buffer ATL and proteinase K, mixed by vortexing, and incubate, vortex occasionally during incubation. Vortex before proceeding to next step.

2.Add buffer AL, mixed by vortexing. Add ethanol, mixed by vortexing. Pipet the mixture into a DNeasy Mini spin column placed in a tube and centrifuge. Discard the flow-through and collection tube.

3.Place the spin column in a new tube. Add buffer AW1 and centrifuge. Discard the flow-through and collection tube. Place the spin column in a new tube, add buffer AW2, and centrifuge. Discard the flow-through and collection tube. Transfer the spin column to a new tube.

4.Elute the DNA by adding buffer AE to the center of the spin column membrane, then incubate and centrifuge.

After extracting the DNA,I did :

PCR (polymerase chain reaction)

Gel electrophoresis

Send samples for sequencing

Analyse

Tables & Figures

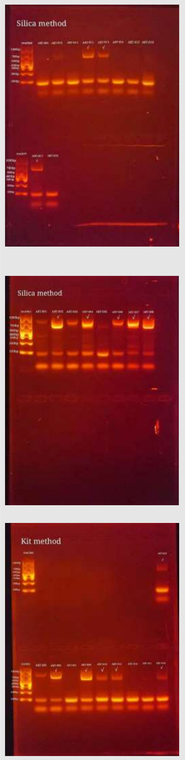

Figure2:The photo of the gel.
The appearance of PCR products of silica and kit method after running the gel.

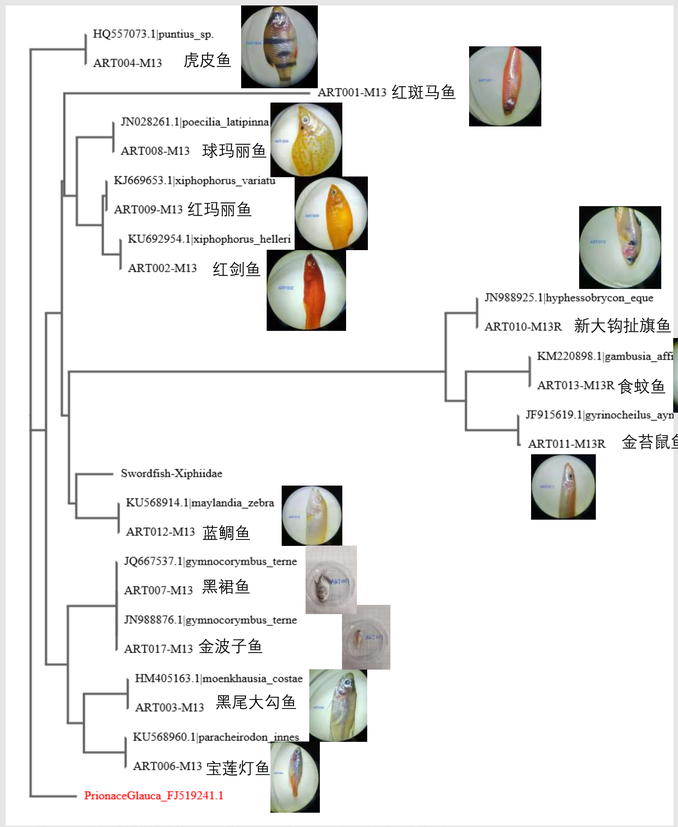

Figure1:The PHYLIP ML tree. The tree shows the relationships between tropical fish and photos of the fish.

figure 3:the MUSCLE of samples ART-007 and ART-017 are same. They are same species.

Results

1.The silica method worked a lot in the experiment for the group 1 DNA extracting on the first day. I can see that 4samples are strong and 2samples are weak among 8 samples on the gel in fig2, after send 6 samples to sequencing, their results looks well in DNA subway.

2.I did the second group of DNA extracting on the second day, the results of running the gel is much worse than the first group in fig2, only 3 of the DNA samples worked well out of 10 DNA samples, so I just sent 3 samples to sequencing and they sequenced well.

3.I tried another method called DNeasy blood & tissue Kit try to extract DNA from the samples which I failed to extract before. This method worked better than the silica one, for I looked through the appearance of the gel in fig 2, it got 2 strong samples and 5 weak samples out of 10 samples, although 2 samples did not worked well in the sequencing process, I still save some necessary samples.

Sequencing analyse

After getting the sequences, I used DNA subway to analyse it. I focus on the results on fig1.

On the graph, the ART-002 is the *Xiphophuros Helleri*, it is similar to ART-009, the *Xiphophuros Variatu* species. ART-008, the *Poecilia Latipinna*, also has some similarity with ART-009 and ART-002.

The ART-007 called *Gymnocorymbus Terne* and ART-017 also called *Gymnocorymbus Terne*, the tree shows they are the same species, it is a surprised discover because they looks different, but they still have some similar part when I took a close look on them. They are also closely related to ART-003*, Moenkhausia Costae* and ART-006, *Paracheirodon Innes*.

ART-012, *Maylandia Zebra*, is closely related to a common fish called *Swordfish Xiphiidae.*

ART-013, *Gambusia Affi*, ART-011, *Gyrinocheilus Ayn*, and ART-010 *Hyphessobrycon Eque* are closely related too. On the tree, it shows they developed more faster than other fishes.

ART-001 is a really special tropical fish and I can not found what it is, and it is different from other fishes. ART-004 *Puntius Sp* also has a big difference compared with each other.

Discussion

Questions and results

The ART-007 and ART-017 are called *Gymnocorymbus Terne*, I got some information on the internet, the environments they required is similar, they are alike in the gentle character, the source of their ancestor comes from the same place too. So maybe their evolution was depending on the artificial selection. Compared with *Gymnocorymbus Terne*, ART-006 *Paracheirodon Innes* and ART-003 *Moenkhausia Costae*, they have similar characteristic and come from characidae family.

ART-012, *Maylandia Zebra*, has so aggressive action like *swordfish*, for they contain a lot of same features, they all can live in the warm sea water, although they look different, they are closely related.

ART-002, the *Xiphophuros Helleri*, and ART-009, the *Xiphophuros Variatu*, the research says although they look similar but have almost disparate habits. The *Xiphophuros* is a type of tropical fish after the artifical selection. They are in the *Poeciliidae*, but *Xiphophuros Helleri* has strong adaptability and not aggressive to other tropical fish, *Xiphophuros Variatu* is very aggresive and needs special requirements for it's living environment.

Difficulties and mistakes

1. When I took photos of the samples, the photo seemed dark,so I decided to put them under the microscope, it looked more brighter and clear under the microscope but was dark in the camera. It was difficult to take a bright photo.
2. I decided to get some muscle from the back of the fish to prepare for the DNA extracting. I find some of the fish had hard squama which make the extracting process takes a long time. Some samples have to obtain some bones of the fish or the squama on it for the fish is too small, I think this might be a reason for not enough DNA after extracting.
3. When doing the silica extracting of DNA, the mixture can easily be spilled out because I could not control the force well when pipetting in and out. The biggest problem was that the mixture could not be mixed cloudy. There was another problem when I did the vortex by machine, I did not cover of the test tube well, so some solution overflow out of the test tube. After the analysing , I suggested that less fresh the samples were, more DNA will be lysed. So it is important to make sure the samples are fresh.
4. And I got a practice dye which is blue to practice how to pipet the PCR, and I thought it was easy to confuse the blue practice dye with the marker.
5. I found the kit method is more difficult than silica method, but it works better than the silica one at last.
6. Before running the PCR, I forgot to centrifuge for the first day samples, so some PCR product may on the wall of the test tube, which can trouble some of the results.
7. I used the DNA subway to analyse those samples. When doing the BLAST, select data and outgroup selection part, it should be really careful.
8. The sound of the centrifuge was loud sometimes especially during the kit box experiment, avoided the accident happened, I added a suitable force on the cover of the centrifuge machine to make sure it could work properly.

References

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