Determining the Evolutionary Relationship of Tropical Fish Using DNA Barcoding

Authors: Ge Ge, Amy Morton, John Mark Olson

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-001 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 saltwater 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 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 more fish which are in specimens 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. Mix the supernatant away. Add wash buffer, vortex and centrifuge. Repeat the steps above to the supernatant, add the wash buffer, vortex and centrifuge, remove the supernatant, add silica 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.

Lot method
2. Add buffer AL mixed by vortexing. Add ethanol, mixed by vortexing. Pipette 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.
4. Elute the DNA by adding buffer AE to the center of the spin column membrane, incubate and centrifuge. Discard the flow through and collection tube.

Results
1. The silica method worked a lot in the experiment, the first group DNA extracting on the day 1. I can see that 4 samples are strong and 2 samples are weak and some samples on the gel in fig1, after send 6 samples to sequencing, their results looks well in DNA subarray.
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 to 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, I got 2 strong samples and 5 weak samples out of 10 samples, although 2 samples did not work well in the sequencing process, I still save some necessary samples.

Discussion
Questions and results
The ART-001 and ART-017 are called Gymnocorymbus Tene, 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 relation was depending on the artificial selection. Compared with Gymnocorymbus Tene, ART-006 Parachirodon innesi and ART-003 Moenkhausia Castor, they have similar characteristic and come from characidae family. ART-012, Maylandia Zebr, 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 Xiphophorus Helleri, and ART-009, the Xiphophorus Variato, the research says although they look similar but have almost disparate habitus. The Xiphophorus is a type of tropical fish after the artificial selection. They are in Poeciliidae, but Xiphophorus Helleri has strong adaptability and not aggressive to other tropical fish, Xiphophorus Variato is very aggressive 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 found 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 be easily spoiled outside because I could not control the force well when pipetting in and out. The biggest problem that the mixture could not be mixed cloudy. There was another problem when I did the vortex by machine, I did not cover the test tube well, so some solution overflowed 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 the samples are fresh.
4. And I got a practice dye which is blue to practice how to spot 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 will make some of the results.
7. I used the DNA subarray to analyse those samples. When doing the BLAST, select data and output file part, it should be really careful.
8. The sound of the centrifuge was loud sometimes 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
https://baike.sogou.com/mFull?emId=705740&req=29909kw2vswf
https://m.baidu.com/s?from=69696969&input=50PM0D0DwYwVb
https://m.baidu.com/s?from=69696969&input=50PM0D0DwYwVb
https://m.baidu.com/s?from=69696969&input=50PM0D0DwYwVb
https://m.baidu.com/s?from=69696969&input=50PM0D0DwYwVb
https://m.baidu.com/s?from=69696969&input=50PM0D0DwYwVb
https://m.baidu.com/s?from=69696969&input=50PM0D0DwYwVb
https://mp.weixin.qq.com/s?src=1&clienttimeamp=1573614078&ver=2104q&signature
https://mp.weixin.qq.com/s?src=1&clienttimeamp=1573614078&ver=2104q&signature
https://mp.weixin.qq.com/s?src=1&clienttimeamp=1573614078&ver=2104q&signature
https://mp.weixin.qq.com/s?src=1&clienttimeamp=1573614078&ver=2104q&signature

Acknowledgements
Thanks for John’s teaching, thanks for Cold Spring Harbor DNA Learning Centre in Asia assisted me in doing the research.