Skinks in Vietnam: Determining If There Are New Species in Vietnam Through DNA Barcoding

Authors: Ringpue Chan,[1] James Gallagher[2]; Mentor: David Kizirian[3]

[1] Brooklyn Technical High School, [2] Regis High School, [3]American Museum of Natural History

Funded by:

The Pinkerton Foundation

SCIENCE Simons Foundation SANDBOX

Abstract

Scincid lizards have a nearly global distribution and include approximately 1658 species. Our experiment revolves around barcoding tissue samples collected from poorly known regions in Vietnam in order to determine if a new species of skinks can be found. To do this, we amplified segments of the DNA through PCR and isolated DNA fragments through gel electrophoresis. After doing so, we sent our DNA to be sequenced and then we compared our DNA to other DNA of similar species. We discovered a candidate new species of the *Eutropis multifasciata* complex.

Introduction

Many species of skinks can be found throughout the world. Each skink has its own distinctive characteristics because of the various environment they live in. With so many different characteristics, it is hard to identify the species of a skink just by looking at it. Vietnam is a known area for inhabiting many skinks. However, many places have not been studied since they are not as well known as others. Tissue samples of unidentified skinks have been collected in poorly known regions in Vietnam. Our experiment was to barcode the DNA found in these tissue samples to identify the species of these unknown skinks and most importantly, see if any of the skinks can be considered as an undescribed species.

AMCC 193164 (E5)

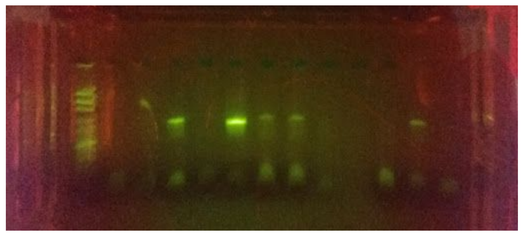
AMCC 193164 (E7)

Methods and Materials

For this project, we followed a very standard procedure for extracting and utilizing the DNA in the skinks. We started by isolating the DNA from the small tissue samples that were given to us by first repeatedly grinding the tissue with a lysis solution. After incubating the sample for about ten minutes, we were able to separate the DNA from the tissue sample found in the supernatant and added silica resin to it to bind the DNA. After washing the mixture of supernatant and silica resin for about three times, we incubated the mixture one last time before taking the supernatant out and storing it at 4°C overnight. The next day, we performed PCR in order to amplify the DNA and finally performed gel electrophoresis to separate the DNA. After seeing which extract we correctly barcoded, we sent the corresponding tissue sample to the Cold Spring Harbor Laboratory to be barcoded and analyzed the results through DNA Subway. We used 12 samples of skinks taken from roughly the same geographic area in Central Vietnam. These were tested over the course of two weeks, and we received the samples from a trip the David took to Vietnam a few years earlier.

Results

E3 and E7, represented by 5 and 6 on the table, appear to be very closely related to each other. They share a 99.36% similarity, so we can surmise that they are from the same species of skink. Furthermore, this species can be identified as *"Eutropis multifasciata"* based on the 98.31% similarity with E3 and the 98.09% similarity with E7. Phylogenetic analysis of *E. multifasciata*, including GenBank samples, suggests that this widespread taxon is a complex of species, with the central Vietnam lineage nested within it. Hence, the new material might represent a new species within the *E. multifasciata* complex. E12 exhibited no clear matches to any species of skink and may represent contaminant. In conclusion, E3 and E7 may represent an undescribed species of the *Eutropis multifasciata* complex and E12 sample may have been contaminated.



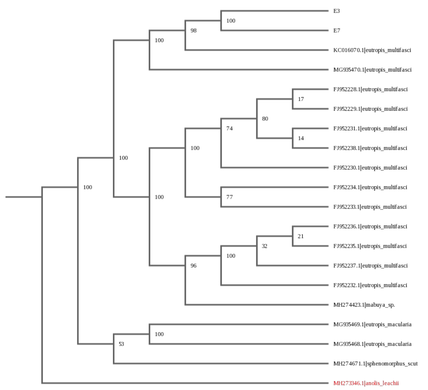
Alignment Viewer

ATCG | TRIM ALIGNMENT | SEQUENCE SIMILARITY

	C	1	2	3	4	5	6	7	8	9	10	11	12	13
Sequence Conservation														
Sequence Variation														
Consensus														
1. MG935469.1|eutropis_macularia	C	-	86.00	91.06	95.34	94.92	95.35	94.30	82.42	83.11	81.98	84.84	83.77	81.14	84.31
2. MH274423.1|mabuya_sp.	1	86.00	-	83.30	83.73	83.51	83.94	83.20	80.88	78.95	79.34	83.08	79.61	80.48	80.26
3. MG386477.1|leptobrachium_hendrick	2	91.06	83.30	-	92.13	91.06	91.91	91.28	78.92	78.48	78.25	81.61	79.60	80.27	80.49
4. KC016070.1|eutropis_multifasciata	3	95.34	83.73	92.13	-	98.31	98.31	98.09	79.37	79.15	78.25	81.61	79.82	78.25	80.27
5. E3	4	94.92	83.51	91.06	98.31	-	98.31	98.09	79.82	80.04	77.80	81.17	80.27	78.25	80.49
6. E7	5	95.35	83.94	91.91	98.31	98.31	-	99.36	80.27	79.82	77.13	81.39	80.27	78.48	80.72
7. JF458855.1|proechimys_gularis	6	94.30	83.20	91.28	98.09	98.09	99.36	-	80.00	79.17	77.14	80.66	79.61	77.63	79.82
8. JX962208.1|apodemus_peninsulae	7	82.42	80.88	78.92	79.37	79.82	80.27	80.00	-	85.05	81.32	80.22	78.90	79.12	83.52
9. KP149252.1|rulyrana_flavopunctata	8	83.11	78.95	78.48	79.15	80.04	79.82	79.17	85.05	-	80.00	80.22	78.07	79.17	83.33
10. MH140019.1|anolis_lemurinus	9	81.98	79.34	78.25	78.25	77.80	77.13	77.14	81.32	80.00	-	80.00	78.90	79.34	83.30
11. JN700854.1|paramesotriton_ermizha	10	84.84	83.08	81.61	81.61	81.17	81.39	80.66	80.22	80.22	80.00	-	80.22	79.34	83.96
12. MH237972.1|sphenomorphus_yersir	11	83.77	79.61	79.60	79.82	80.27	80.27	79.61	78.90	78.07	78.90	80.22	-	82.24	83.55
13. E12	12	81.14	80.48	80.27	78.25	78.25	78.48	77.63	79.12	79.17	79.34	79.34	82.24	-	83.77

Discussion

This project set out to possibly identify possible new species of skinks in Vietnam. We successfully barcode two samples from different skinks and identify them as candidate new species of the *Eutropis multifasciata* complex. Yet, we would still like to discuss E12, the obvious and odd outlier of the group deserves recognition. One thing to note is that E12 had a distinct lack of consensus throughout the strand. E12 had roughly the same range for all of the samples: 80-84 with nothing exceeding 84.5. This is an odd phenomenon, but is probably explained by the lack of consensus within the sample. In addition to the lack of consensus in the sample, we sent in two more samples to be sequenced but these two returned with only one strand intact; that is, they had a forward or a back but not both.



References

Mausfeld and Schmitz (2003). Molecular phylogeography, intraspecific variation and speciation of the Asian scincid lizard genus Eutropis, 1843 (Squamata:Reptilia: Scincidae): taxonomic and biogeographic implications. Organisms Diversity & Evolution 3: 161–171.

Nguyen, Ho, and Nguyen (2009). Herpetofauna of Vietnam. Chimaira, Frankfurt, 768 pp.

Acknowledgements

Thank you to Prof. David Kizirian for helping, instructing, and supporting us through the whole project. We could have never done it without you!
Thank you also to the Cold Spring Harbor Laboratory for providing all the necessary material needed and the UBRP foundation for funding this whole ex[eriement.