Indochinese Big Tooth Snakes

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Abstract

This project focused on identification of newly collected unknown samples of Big Tooth Snakes (*Dinodon* clade of *Lycodon*) to samples of known species including *Lycodon flavozonatum, L. rufozonatum*, and *L. septentrionalis.* By using the information that was collected from seven snake DNA samples, the team would be able to find out if the snakes whose DNA was used in the experiment are of known species or possibly a new species. In order to complete this project, there were multiple materials needed for the results; DNA was extracted from seven snake tissue samples already prepared upon experimenting; these DNA extractions then underwent a polymerase chain reaction process; gel electrophoresis was then performed with the PCR products. However, the gel showed no bands for the snake DNA after five trials of undergoing PCR and gel electrophoresis.

Introduction

Colubroidea family of snakes is the most diverse living vertebrates on this earth. There have been numerous studies on these snakes, and more than 2500 species are known to exist. However, most of these studies do not go so far in depth in explaining the relationships among the different subfamilies in terms of phylogenetic research. For example, scientists have discovered thirty six species of the genus *Lycodon* but only three species were part of phylogenetic studies. Specifically, the *Dinodon* clade, which is nested within *Lycodon*, had not been focused upon in many research studies. In addition, new species are still being discovered in poorly known areas. Preliminary study of recently collected material from the Truong Son Range of Vietnam suggest that an undescribed species of *Lycodon* (*Dinodon*) may occur there.

In this experiment, we explored the DNA sequences of seven snakes by extracting the DNA, performing PCR (polymerase chain reaction), doing gel electrophoresis, and using the DNA sequencing data on DNASubway. Based on the results, we can try to determine the relationships between the snakes used in the experiment versus identified *Lycodon*, and see if there are any new/unidentified species within the seven snakes.

Figure 1 (left) and Figure 2 (right). Two specimens of uncertain taxonomic status from Vietnam investigated in this study.

Materials & Methods

In this experiment, seven snake tissue samples of Lycodon collected in poorly known areas of Vietnam during expeditions mounted by AMNH scientists during 1998-2011. The area of exploration and collection as well as the species of snake collected was chosen because there has not been much information on the specific genus and other related species of snake.

In order to perform the experiment, three processes were done: DNA extraction, polymerase chain reaction (PCR), and gel electrophoresis. First, the DNA was extracted from tissue samples collected at the time of the snake finding. The standard DNA extraction procedure was performed. The samples were stored at -20°C until the PCR process. A standard PCR reagent procedure was performed using vertebrate primers. For the PCR, the temperatures for initialization was 94°C, for denaturation, 55°C for annealing, 72°C for extension/elongation, and a 4°C hold. For the gel electrophoresis, an Invitrogen 2% Agarose E-Gel was used. In the first well, 15 µl of dH_2O and 5 µl of ladder was added. In wells 2-8, 15 µl of distilled water (dH_2O) and 5 µl of the PCR reagent was added. The gel ran for 30 minutes. Then the gels were examined for distinct bars and the PCR reagents were sent to GENEWIZ for sequencing.

This experiment was conducted five times because in each of the five attempts of getting clear bands, no bands or fuzzy, unclear bands appeared in the gels. In the first trial no bands appeared on the gel, so the PCR was redone and reconducted. However, the mistake that was made in the first trial was that we did not put 15uL DH_2O in the wells of the gel. In the second trial no bands appeared on the gel, and a third was conducted with a similar outcome. In the third, however, there was one fuzzy band across wells 2-8. After approximately 30 seconds under the UV light, the one fuzzy band separated and semi-individual fuzzy bands appeared in each well. The PCR was redone and reconducted a second time, and the same results as trial 3 occurred. For the fifth trial, the annealing temperature was changed to 53°C. The bands in the gel appeared to be fuzzy.

Figure 4 (left) and Figure 5 (right). E-Gel of a ladder and the seven DNA samples from *Lycodon.*

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Discussion

In all five trials, the ladder bands appeared in the first well, while fuzzy bands appeared in remaining wells. Sequencing of PCR product yielded no data. It is unclear why PCR did not result in sequenceable product.

References

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Acknowledgements

We would like to thank Dr.Kizirian for helping us with this experiment. Without his guidance, we would not have made it this far. We would also like to thank Christine Marizzi and Alison Cucco for giving us the materials we need in order to complete the project.

Figure 6. Truong Son Range of northern Vietnam where specimens of *Lycodon* were collected.