The art of getting DNA out of decades-old pickled snakes

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Part of the Field Museum's underground "bunker" of reptile specimens. Credit: Field Museum

Two levels underground, Chicago's Field Museum has a secret bunker. The sub-basement Collections Resource Center houses millions of biological specimens for scientists around the world to use in their research, including countless bottles and jars containing pickled fish, lizards, and snakes, arranged like a library. Many of these specimens are decades or even centuries old, near-perfectly preserved by a combination of formalin and alcohol. But the process that preserves tissues often destroys or at least makes acquiring DNA for modern studies very difficult, which is bad news for scientists who study genetic relationships between organisms. A new study in *Frontiers in Ecology and Evolution*, however, reveals new approaches for getting and maximizing usable DNA from decades-old pickled specimens, and uses these techniques to solve a long-standing mystery about a small snake from the island of Borneo.

"As a true crime aficionado, it reminds me of how people didn't take DNA samples when a crime was committed in the 1960s, because nobody could have predicted that one day DNA samples would let you pinpoint who committed a crime," says Sara Ruane, assistant curator of herpetology at the Field Museum and the study's senior author. "These older museum specimens are sometimes the only available examples of a species, but they weren't preserved with DNA in mind— this paper is about how we can squeeze every bit of information possible out of them."

The project was born of the dissertation research of Justin Bernstein when he was Ruane's student at Rutgers University-Newark. "My primary study is on a group of snakes, called homalopsids or mud snakes, that live in South and Southeast Asia, New Guinea, and Australia," says Bernstein, the paper's lead author. "They're really fascinating; they live in muddy, aquatic environments, and there are 56 species of them. We use DNA to study their evolutionary history, to try to describe new species, and to learn what happened to these groups over tens of millions of years that led to the diversity we see today."

Alcohol-preserved specimens of Hydrablabes periops in the Field Museum's collections. Credit: Josh Mata, Field Museum

For this paper, there was one animal in particular that Bernstein and Ruane were trying to place into a family group, a two-foot-long greenish-brown snake called *Hydrablabes periops*, aka the olive small-eyed snake. It's found on Borneo, a large island east of mainland Malaysia and northwest from Australia that contains parts of Malaysia and Indonesia and the entire nation of Brunei. Based on its appearance, scientists had suggested two different families that it might be a part of. Analyzing its DNA could reveal its closest relatives and thus, its family, but that was easier said than done. "About half of mud snake diversity and all *Hydrablabes* vouchers are known from older specimens that were chemically treated, and this chemical treatment breaks down the DNA," says Bernstein.

Part of the process of preserving an animal in alcohol is "fixing" it with a substance called formalin, a solution of formaldehyde gas and water, that makes its tissues rubbery and rigid. Unfortunately, the DNA in its cells gets altered as well. "It does something called crosslinking, which binds up the DNA," says Ruane. "If you want to study its DNA, you need to undo or try to force the DNA out from those crosslinks."

Studying the olive small-eyed snake meant taking small samples of liver tissue from some of the few specimens in the United States, both from the Field Museum's collections. One from 1964, and the other from a 1993 collecting trip by the Field's then-curator Robert Inger and his wife Tan Fui Lian.

Lead author Justin Bernstein visiting the Field Museum's reptile collections. Credit: Sara Ruane, Field Museum

Such old specimens required new lab techniques. Normally, getting DNA out of a tissue sample involves adding digestive enzymes that break apart the tissue, leaving the DNA behind, and heating it to 130 °F for several hours. "We had to modify the way we got the DNA out by making it hotter for longer and using more of these digestive enzymes," says Ruane. These more extreme preparation methods have been effective for other snakes in previous studies, but the resulting genetic analysis still contained lots of gaps for Borneo's *Hydrablabes* snake specimen.

Lead author Justin Bernstein working with snake specimens at University of Kansas Biodiversity Institute & Natural History Museum, taking morphological data to examine homalopsid snake diversity. Credit: Justin Bernstein

"The chemicals used to preserve the snakes sheared their DNA into shorter pieces of code, which made them hard to compare with longer, more complete genes from other specimens," says Bernstein. "The first software that I used made it hard to understand how much fragmented DNA there was across the study specimens, but switching to a different software that visualized the pieces of genetic code made it easier to see where there were problems." And even the smaller, more fragmented pieces of code could be added to larger, published datasets to help build an evolutionary tree.

An important aspect of this paper for Bernstein was being transparent about the difficulties of using older specimens and the troubleshooting required to study them. "I wanted to show scientists that you can still do work with these specimens, it just requires a bit of finagling," he says. "On a broader level, the study is really showing how to leverage the data you do obtain and how you can combine it with previously published datasets to investigate some really cool hypotheses."


FMNH
RF1
FAM. S-9
REPTILIA
SPEC. 1
Field Museum of Natural History
CAT. NO. 251051
SPECIES Hydrablabes periops
LOCALITY Malaysia: Sabah
COLL. R.F. Inger
DATE 1993

One of the decades-old snake specimens from the Field Museum used in this study. Credit: Sara Ruane, Field Museum

As for the Bornean snake at the heart of the study, the researchers were able to determine that it's a member of the family Natricidae, which contains distant relatives such as the North American garter snakes. Which might not seem like a big deal, but "knowing that a particular species is part of a certain group can tell us a lot about biogeography and about how life on Earth has changed over time," says Ruane.

And beyond the study of snakes, she notes that overall, "this project underscores the importance of museum collections, because you never know what you'll be able to learn from specimens in the future."

More information: Maximizing molecular data from low-quality fluid-preserved specimens in natural history collections, *Frontiers in Ecology and Evolution* (2022). DOI: 10.3389/fevo.2022.893088.

Provided by Field Museum

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