

Spotlight on mud snakes: Study unveils evolutionary secrets of enigmatic snake family from Southeastern Asia

September 20 2023



A species of Mud Snake, *Enhydris enhydris*, from Southeast Asia. Credit: Kenneth Chin

A new University of Kansas genomic investigation of a group of unheralded but unique "mud snakes" from Southeast Asia is rewriting the evolutionary history of this family, named the Homalopsidae. The results just were [published](#) in the *Bulletin of the Society of Systematic Biologists*.

Species of mud [snake](#) can inhabit fresh, brackish or saltwater coastal and inland areas, mostly sleeping by day and munching on fish and crustaceans by night. Now, they've also fascinated two generations of KU researchers.

The new DNA-driven investigation by lead author Justin Bernstein, a KU Center for Genomics postdoctoral researcher, is an example of systematics: the study of how species evolve and their relationships through time.

It builds on a "monumental" study of mud snakes conducted without benefit of DNA analysis in 1970 by Ko Ko Gyi. Gyi was a promising evolutionary biologist from Myanmar who conducted research in the Division of Herpetology of the KU Natural History Museum during a three-year fellowship from the United Nations Educational, Scientific and Cultural Organization.

"Our new paper looks at the evolutionary relationships and history of mud snakes, which are found in primarily South Asia, Southeast Asia, Australia and New Guinea—and they're very poorly studied," Bernstein said. "We don't know too much about them, despite decades of effort, including the efforts of Gyi, because they're kind of hard to find. They're aquatic snakes who prefer muddy environments. They're nocturnal. Very few people have studied their evolution."

Bernstein and his co-authors shed light on mud snake evolutionary history using genomics by combining genetic analyses of older museum

specimens' mitochondrial DNA with fresher genetic samples from recent field collections (which allow for much higher-resolution molecular analysis—they used data from 4,800 nuclear loci in each genome).

"If you have an animal that's been dead for days and extract DNA from that tissue, it's going to be degraded—it's not going to be high quality, which interferes with our results," Bernstein said.

"That means we either can't analyze it, or the results might be kind of weird and we can't trust them. But this idea of getting DNA from old specimens in natural history museums has really been on the rise in the last decade. While the DNA is degraded, there've been protocols in the last 10 years to get high-quality DNA out of old specimens that are over 100 years old from natural history collections."

The researchers used the mitochondrial data from those museum-specimen samples to fill in knowledge gaps for a more accurate biogeographic history of Homalopsidae. Combining that with DNA samples from recent specimens, they reconstructed the most comprehensive family tree of Homalopsidae relationships, showing which species branched from common ancestors and roughly when.

Bernstein's co-authors were Hugo de Souza and Kartik Shanker of the Indian Institute of Science; John Murphy, Harold Voris and Sara Ruane of the Field Museum in Chicago; Edward Myers of Clemson University and the California Academy of Sciences; Sean Harrington of the University of Wyoming and American Museum of Natural History; and Rafe Brown, curator-in-charge at the KU Biodiversity Institute and Natural History Museum and professor of ecology & evolutionary biology.

"Following up on Gyi's pioneering and foundational work, Justin has

been able to avail of a technological breakthrough, which we refer to now as 'museumomics'—the extraction and sequencing of degraded DNA, even from very old formalin-preserved specimens that were preserved dozens to hundreds of years ago," said Brown, who served as an "outside" member of Bernstein's doctoral committee at Rutgers.

"Obviously, the quality of that highly degraded DNA is variable, but new genomic sequencing technologies, bioinformatic tools, and diligent studies on the part of museum biologists have developed best practices for reconstructing highly fragmented genomes of specimens that were preserved upwards of a century ago. Justin successfully harnessed these new state-of-the-art tools, but also brought his own research 'full circle', historically following up on Gyi's work."

The new paper shows one long-held concept regarding mud snakes was incorrect. For years, scientists (including Bernstein) thought ice age fluctuations in [sea level](#) might have acted as a "species pump" that drove diversification of the mud snakes. Indeed, much regional biodiversity can be traced to sea level rise and fall. But the new paper shows, "Pleistocene sea level changes and habitat specificity did not primarily lead to the extant species richness of Homalopsidae."

Instead, Bernstein and his colleagues believe a more likely driver of mud snake species richness happened around 20 million years earlier, during the Oligocene, when sliding tectonic plates and shifting rivers in Southeast Asia might have severed and reestablished gene flow repeatedly between mud snake groups, driving diversification.

"Although we can never prove what did happen, exactly, to trigger diversification in mud snakes, we can rule out, or reject, some previously articulated hypotheses," Bernstein said. "Because we found strong statistical support for diversification pre-dating Pleistocene sea level fluctuations by 15-20 million years, we need to look to alternative

explanations, or novel ways of interpreting the production of biodiversity."

Bernstein and Brown are quick to point to the pioneering work on mud snakes performed 50 years ago by Gyi, whose work not only figured into the new paper but had several ideas confirmed by later DNA testing.

"Gyi completed a monumental work on this group of snakes, more than 50 years ago, and without the benefit of molecular analysis," Brown said.

"That, in and of itself, is quite an accomplishment—his work was thoroughly comprehensive for the time, he really made the most of the available technologies, such as detailed X-rays of the skulls of all the species in this mysterious group of snakes, and he totally took the study of the evolutionary relationships of mud snakes 'from zero to 60' in his 1970 monograph."

"I know his adviser, the late Bill Duellman, was heartbroken to find out that Gyi passed away unexpectedly back in Myanmar, shortly after he left KU. But, here at KU, we still honor revisit and celebrate Ko Ko Gyi's unique contributions, even to this day, and his work is continued on by Field Museum of Natural History researchers John Murphy and Harold K. Voris—and now by Justin Bernstein."

More information: Justin M. Bernstein et al, Phylogenomics of Fresh and Formalin Specimens Resolves the Systematics of Old World Mud Snakes (Serpentes: Homalopsidae) and Expands Biogeographic Inference, *Bulletin of the Society of Systematic Biologists* (2023). [DOI: 10.18061/bssb.v2i1.9393](https://doi.org/10.18061/bssb.v2i1.9393)

Provided by University of Kansas

Citation: Spotlight on mud snakes: Study unveils evolutionary secrets of enigmatic snake family from Southeastern Asia (2023, September 20) retrieved 2 May 2024 from <https://phys.org/news/2023-09-spotlight-mud-snakes-unveils-evolutionary.html>

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