# Researchers turn to museums to track down clues in mysterious amphibian declines

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*Pseudoeurycea rex*, a high elevation salamander that has declined severely at many sites in Guatemala. Credit: Sean M. Rovito

There's a crisis among the world's amphibians -- about 40 percent of amphibian species have dwindled in numbers in just three decades. Now, museum jars stuffed full of amphibians may help scientists decide whether this wave of extinctions was caused by a fungal infection.

DNA swabbed from the preservative-soaked skins of salamanders, frogs and toads—collected from some of Central America's best-known extinction hotspots— revealed a startling but clear pattern. Salamanders in parts of Mexico and Guatemala, and frogs and salamanders in Costa Rica's Monteverde cloud forests began to disappear at the same time that the deadly *Batrachochytrium dendrobatidis* or *Bd* fungus first appeared in these areas.

The museum specimens helped SF State biology graduate student Tina Cheng and her colleagues track a *Bd* epidemic wave that began in southern Mexico in the early 1970s, spread southward to western Guatemala in the 1980s and 1990s, and to Costa Rica by 1987. They report their findings in the latest issue of the journal *Proceedings of the National Academy of Sciences.*

The disappearance of Monteverde's famed golden toad, which vanished within three years, brought widespread attention to the amphibian crisis. The extinctions have happened so rapidly and over such a wide swath of the globe that scientists have been scrambling to find their cause. The proposed culprits include *Bd* infection, global climate change, drought, among other explanations.

"One of the things that's so cool about Tina's research is that she's solving a mystery that's been hanging over science since 1989," said study co-author Vance Vredenburg, an assistant biology professor at SF State.and a research associate at Museum of Vertebrate Zoology at the University of California at Berkeley.

If you're solving a murder mystery, however, you're going to need a body. By the time most researchers realized the extent of the decline in amphibian populations, the amphibians themselves had disappeared. Cheng and Vredenburg, along with Berkeley researchers Sean Rovito and David Wake, decided to find their "bodies" in Berkeley's Museum of Vertebrate Zoology.

Since *Bd* is a skin infection, they reasoned, it might be possible to collect DNA from the museum specimens from the past 40 years and analyze it for traces of the fungus. But they were skeptical at first whether they could even recover DNA from the skins, Cheng said, "since they're basically pickled in formalin, which is known to degrade DNA over time."

Cheng said it was "quite a task to fish through all these jars," sometimes holding 400-500 salamanders at a time, "to find the ones we wanted. But the museum has done a great job of preserving these animals, and we didn't really encounter any in poor shape."

*Bolitoglossa lincolni*, one of the salamander species from San Marcos, Guatemala found to harbor the pathogenic chytrid fungus during recent surveys. Credit: Sean M. Rovito

The researchers used swabs to gently swipe the amphibian skins and collect the short DNA sequence needed to confirm *Bd*'s presence. The earliest confirmed data of *Bd* infections among the specimens was 1972.

But the researchers didn't stop with "dead things," Vredenburg noted. The study team also brought living amphibians into the lab and infected them with *Bd* to get a better handle on how the fungus might have felled so many of their comrades. The fungus doesn't kill indiscriminately, they confirmed. Some species seem to be more susceptible to infection, and the likelihood of death depends on the level of fungal spores. "We now know that the pathogen isn't going to drive every amphibian to extinction," Vredenburg said, "but we want to know why."

Funded by the National Science Foundation, the study suggests that this fungal infection may be the prime suspect in amphibian declines, but Vredenburg says it also offers a way to test whether climate change may have played an indirect role. "Now that we can say, 'here's the actual factor that's killing these amphibians,' we can go to 100 other sites and document when it showed up. Then you can go to the climate data, and find out what happened with the climate at these sites in the years when this pathogen showed up."

Vredenburg is excited about the possibilities of testing museum specimens from other collections around the world, hoping to find out why and how these extinctions are happening.

"Amphibians are long-term survivors," he said. "They've been on Earth for 360 million years, and have made it through four mass extinctions. They were doing just fine until now, but something unprecedented and worrisome has happened in the last 40 years."

"Coincident mass extirpation of neotropical amphibians with the emergence of the infectious fungal pathogen Batrachochytrium dendrobatidis" was published in the May 2, 2011, Early Edition of *Proceedings of the National Academy of Sciences*.

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