

## Fungal in the jungle: how researchers are trying to safeguard amphibians in Ecuador's rainforests—and beyond

According to the U.S. Geological Survey, amphibians are the most threatened groups of animals on the planet<sup>[ii]</sup>, with experts predicting that half of all amphibian species are currently at risk of extinction.<sup>[iii]</sup>

A key suspect in this crime against biodiversity is a fungal pathogen. But how, exactly, does this fungus kill—and how does it spread?

Much like highly trained crime detectives, Dr. David Rodriguez, his students and his research partners are trying to uncover answers to these questions. And they're doing it from deep in the Ecuadorian rainforest, not in a sterile, fully equipped lab thousands of miles from Ecuador.

Their goal: uncovering the host-pathogen dynamics of the amphibian-killing chytrid fungus, *Batrachochytrium dendrobatidis*, known to be responsible for the deaths of susceptible amphibians not only in Ecuador but around the world.

A faculty member at Texas State University, where he teaches Mycology and Applied Bioinformatics, Dr. Rodriguez investigates host-pathogen dynamics using state-of-the-art genetic, genomic and bioinformatic tools. His arsenal includes a portable genetics lab that's used to perform—and help teach his students about—genetic analyses in the field.



Dr. David Rodriguez (left), his student Toriann Molis (middle), and Ryan Lynch, Executive Director at TMA (right) prepare samples for analysis using the EMnetik PCR Cleanup System.

Together with the **Dr. Shawn McCracken** from the McCracken Lab at Texas A&M University Corpus Christi, Dr. Rodriguez' lab has partnered with **Third Millennium Alliance (TMA)** to carry out ongoing research and teaching activities in Ecuador. TMA is an NGO focused on purchasing land for conservation and restoration, and to promoting sustainable agroforestry practices.

Founded in 2007, TMA created what is now known as the Jama-Coaque Reserve (JCR) in the heart of Ecuador's Pacific Forest, which is now a research site for the teams led by Drs. Rodriguez and McCracken.

"Our (research) efforts include sampling the canopy habitats of these tropical forests, which are difficult to access," Rodriguez says. "A lot of research on this pathogen has focused on the forest floor, but we would like to more closely investigate the relatively unexplored canopy habitats of both low- and high-elevation forests."

## Solving a mystery while teaching tomorrow's "detectives"

While Rodriguez, McCracken and their TMA partners work tirelessly to gather unique clues about the amphibian-killing fungus, they also devote a lot of time to passing along their years of knowledge and expertise to students who are eager to learn how to solve mysteries about biodiversity in tropical environs.

"In addition to the science," Rodriguez says, "we are also very passionate about teaching and training the next generation of scientists—both from the U.S. and Ecuador."

And despite the inherent challenges, Rodriguez, McCracken and their student teams genuinely enjoy doing intense fieldwork in remote places like the Jama-Coaque Reserve.

"Because our methods involve performing genetic analyses in the field, and due to the dynamic nature of each site we visit, we certainly face many technical challenges," Rodriguez says.

Not only do they have to maintain the appropriate temperature for reagents, but their teams must create effective, efficient research protocols using portable lab instruments. To help address that specific challenge as it relates to PCR cleanups, the team relies on the small, lightweight EMnetik System from Beckman Coulter Life Sciences.

"The EMnetik system, with its highly responsive (superSPRI) beads, has helped to streamline our DNA extraction," Rodriguez explains.



Fungal pathogen *Batrachochytrium dendrobatidis* can kill amphibians such as the gliding leaf frog (*Agalychnis spurrelli*). Photo courtesy of Ryan Lynch and Third Millennium Alliance.

"We use EMnetik (superSPRI) beads to isolate and purify DNA from digested lysates, to then use in PCRs. The resulting amplicons are then sequenced using Nanopore technology—all while in the field. The EMnetik provides easy-to-follow protocols that students can master quickly to generate (clean) DNA...even in suboptimal non-laboratory conditions."



Student researchers use the EMnetik PCR Cleanup System to help uncover clues to amphibian deaths in the tropical forests of Ecuador.

Dr. Rodriguez and his colleagues are convinced that the advent of field-based genetics can have profound implications for biodiversity research—and hopefully for preventing the extinction of some amphibian species—because it enables researchers to generate valuable data quickly and *in situ*.

Moreover, students and scientists in under resourced countries will be able to leverage these portable technologies to drive a myriad of research interests, which can rapidly expand scientific knowledge about understudied ecosystems around the world.

## How are the clues adding up in Ecuador?

Dr. Rodriguez and his team have learned more about how *B. dendrobatidis* spreads, and how it acts on amphibian species in Ecuador's Jama-Coaque Reserve. They plan to present some of their preliminary data at the Society for Integrative and Comparative Biology (SICB) in Austin, Texas in 2023.

"We hope to help figure out all of the species that are driving the spread of chytrid in tropical amphibians," he says. "With this information, (forestry) managers can better implement mitigation strategies to help conserve amphibian biodiversity in this region of the world.

"And of course we hope our research benefits scientists investigating the same deadly pathogen in other parts of the world."

Beckman Coulter Life Sciences was proud to provide this field-based research team with the reagents and materials they needed to perform initial trials of EMnetik System protocols.

Our grant and collaboration programs help empower researchers like Drs. Rodriguez and McCracken, who seek answers to life's important scientific and healthcare questions.

Learn more about the Third Millennium Alliance at www.tma.earth/our-story/

Learn more about Beckman Coulter Life Sciences Genomic Reagents at www.beckman.com/ reagents/genomic

a. <sup>I</sup>USGS. Diseases of Aquatic Organisms. Available from: URL: www.usgs.gov/diseases-of-aquatic-organisms/amphibian-diseases.

b. "Bittel J. Half of all amphibian species at risk of extinction. Available from: URL: www.nationalgeographic.com/animals/article/moreamphibians-at-extinction-risk-than-thought.

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