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Project title: The Origin and Evolution of the acquisition of Guanidinium Alkaloids in Harlequin Frogs (*Atelopus*, Bufonidae) from Ecuador and Colombia

The Sierra Nevada de Santa Marta (SNSM) is the highest coastal mountain range on Earth, located on the Caribbean coast of the Magdalena Department in Colombia. Its intricate geological history and the unique climatic conditions of its towering peaks have resulted in an exceptional array of biodiversity. Species assemblages are so unique because the significant geographic separation from the Andes and the isolation provided by the surrounding lowlands allow the Sierra Nevada de Santa Marta to function like an island. Here, populations become isolated, undergo speciation, and adapt to specific climatic conditions, resulting in a phenomenon known as endemism.

The diversity of amphibians in the Sierra Nevada de Santa Marta is particularly striking, not only in the number of species but also in the abundance of individuals. Unfortunately, such large population sizes are becoming increasingly rare for most amphibian species worldwide. For example, many species of the highly endangered harlequin toads of the genus *Atelopus* in Central and South America have faced severe population declines over the last few decades, with some even categorized as extinct. However, this is not the case for *Atelopus* of the SNSM. Unlike the more than two-thirds of harlequin toads categorized as Critically Endangered or Possibly Extinct by the IUCN, *Atelopus* species from the SNSM exhibit stable, healthy, and abundant populations that have shown resilience over the years. What makes the *Atelopus* of the Sierra Nevada de Santa Marta so unique that they seem to have escaped the severe amphibian extinction crisis affecting other parts of the world?

To better address this question, it's crucial to understand that the most widely accepted hypothesis for the mass die-offs of amphibians is a fungal pandemic disease known as chytridiomycosis. This infection can be lethal to frogs as it affects their skin, disrupting normal gas exchange and interfering with respiration. Remarkably, *Atelopus* species in the Sierra Nevada de Santa Marta seem to coexist with this deadly chytrid fungus, although the mechanism for their resistance remains elusive. One potential key trait that might confer resistance to chytridiomycosis in some *Atelopus* species is the presence of potent neurotoxic compounds known as guanidinium alkaloids, including the enigmatic Tetrodotoxin (TTX). While these alkaloids are primarily thought to deter predators, some species might have also repurposed them to prevent infection from pathogens.

Several studies have reported TTX and unique TTX-analogs seen nowhere else in nature from 13 of the 99 formally described species of *Atelopus,* with 9 of these corresponding to Central American species. However, the vast diversity of harlequin toads occurs in South America, with 65 species occurring in Ecuador and Colombia alone. A comprehensive study of *Atelopus* toxicity will provide additional evidence for understanding the ecological role and the biological significance of guanidinium alkaloids, a more complete evolutionary history of the TTX-bearing phenotype in animals, as well as novel insights into one of the world's most imperiled amphibian groups.

The Tinker Field Research Grant, provided by the Center for Latin American and Caribbean Studies, enabled me to cover the airfare expenses to travel to the Sierra Nevada de Santa Marta in search

of three of the four endemic *Atelopus* species: the Santa Marta harlequin toad (*A. laetissimus*), the Guajira harlequin toad (*A. carrikeri*), and the San Lorenzo harlequin toad (*A. nahumae*). However, I decided not to pursue the search of the fourth species, the starry night harlequin toad (*A. arsyecue*) as it is considered a sacred animal by the Wayuu people, an indigenous community in the Sierra Nevada de Santa Marta. Access to Wayuu territory is highly restricted, and invasive sampling methods are prohibited. The CLACS grant provided me with the invaluable opportunity to conduct a preliminary visit to the region, helping me determine which species could be sampled. I deeply value this experience as it allowed me to show respect to indigenous communities and build trust with local researchers who guided me through the process of accessing the populations for which I had permits.

During my trip, I visited three localities across two different mountains. Two of these localities in San Lorenzo and San Pedro, situated at elevations of 2,500–2,600 meters, were home to *A. laetissimus* and *A. nahumae*. The third locality, Páramo de las Cebolletas at 3,500 meters above sea level, was the habitat of *A. carrikeri*. All trips began in Santa Marta, at sea level. From there, I traveled by car to a designated point, after which the remainder of the journey required hiking. The expeditions were both physically demanding and logistically challenging, particularly due to my sampling methodology. I needed to flash freeze and store swab samples and skin tissues in liquid nitrogen at -80ºC. Using a 17-liter liquid nitrogen tank, which weighs up to 30 kg when full, transporting this equipment up the mountains was unfeasible without assistance. To solve this issue, I rented mules from local people to help carry the tank and preserve my samples.

The final expedition to the Páramo was especially strenuous, involving an eight-hour hike up the mountain, where the mules were crucial for transporting the tank. Without the support of the Tinker grant, I would not have been able to cover the mule expenses and reach the localities to collect samples for the preliminary assessment of the species' toxicity. Thanks to the support from CLACS, I successfully obtained 18 skin samples and swabs from the three *Atelopus* species in Santa Marta and was able to identify which populations were most accessible and abundant for future in-depth collection and analysis of the chemical defenses and microbiome composition.

Additionally, this trip strengthened my collaboration with researchers from Universidad del Magdalena and biologists from Fundación Atelopus who are dedicated to studying and protecting these frogs. I learned from them about where to look for the frogs and important behavioral information on the species as well as about their research projects on uncovering the genetic diversity of the species, experiments on mate choice, diet preferences, and bioacoustics. From my side, I had the opportunity to share the methodology for biopsy collection with Luis Polo, an undergraduate student, and Aldair Barros, a Master's student. This methodology cannot only be useful for toxin detection, but it is also suitable for DNA and transcriptome analysis, as well as disease detection and experimental infection of samples.

Finally, I will send the samples for chemical analysis and toxin detection using liquid chromatography with fluorescent detection (LC-FLD) and high-resolution liquid chromatography-mass spectrometry (HR-LC-MS). The results from these analyses will be integrated with the data I have already obtained and analyzed from Ecuadorian Atelopus species. These analyses are crucial for completing the first chapter of my dissertation.