Graduate Research Plan Statement

Background: The development of the amniote egg was a key factor in the evolution of vertebrates, since it decoupled their life cycle from water and allowed them to invade land[1]. After this invasion, eggs took a variety of shapes, sizes, and structure characteristics, with extant taxa being living evidence of such diversity[2]. The evolution of archosaur eggshells has been viewed primarily through the lenses of taxonomical classification, phylogeny, and nesting habits[3,4]. However, studies of extant taxa, mainly birds, suggest structural aspects of the eggshell (thickness and porosity) may have been the results of environmental evolutionary adaptations of theropods unrelated to nesting[5]. In addition to this, several studies have investigated the water vapor conductance of archosaur eggshell[6,7], which is a useful trait of eggshell that relates to thickness and porosity. These projects are promising because they provide a framework from which to delve into the environmental controls on the evolution of archosaur eggshells.

Objectives: Due to the lack of study of the controlling factors on the evolution of structural characteristics in archosaur eggshell, I propose to **study the effects of humidity over archosaur eggshell thickness and porosity**, to observe if these traits **respond to the surroundings of the egg-laying site**. With this, we can directly observe if the eggshell formed part of the suite of adaptive measures archosaurs had to survive their environment and proliferate, and if thickness and porosity are traits in which changes can be tracked in archosaur lineages.

Humidity has been found to influence both thickness and the pores in the eggshell of modern avian specimens[5]. Such study hypothesized that an inverse relationship exists between humidity and thickness (see Figure 1), and that a direct relationship exists between humidity and pore area. This was proposed because in drier climates the egg would need to limit its water vapor conductance (diffusive capacity) with its surroundings to retain water, and therefore increase shell thickness and decrease pore area. The opposite would happen if the climate is humid. Both eggshell thickness and pore area have been measured in fossilized archosaur eggshell[4], and therefore are assessable features useful for this study. In the case of humidity, it can be assessed using $\delta^{18}O$, since these isotopes in eggshell mainly reflect the archosaur's ingested water[8]. This ingested water can tell us more about the humidity of the surroundings of the egg-laying site, since the distribution of values in $\delta^{18}O$ is controlled mainly by the location of the water in a landmass, the latitude, and the amount of evaporation at the site.

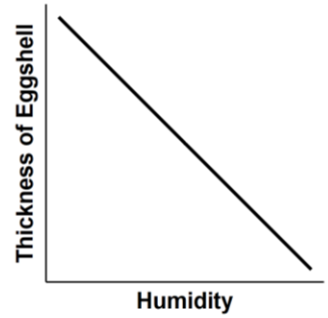

Figure 1. Hypothesized relationship between eggshell thickness and humidity.

Research Plan: The project will consist of various phases: Specimen selection (from the [repository]) and eggshell collection (fieldwork), Microstructural analysis of the eggshell for classification, Eggshell thickness and porosity measurements, $\delta^{18}O$ analyses of archosaur eggshells, and Analysis of eggshell structure versus $\delta^{18}O$. The purpose of the first two phases will be to first establish the framework of specimens available, their origin, their egg taxonomy (microstructure), age, state of preservation, and environmental context to establish their suitability for the proposed analyses of structure and $\delta^{18}O$. After selecting and/or collecting the necessary specimens, eggshell thickness data can be collected using a digital micrometer. Thickness will be the main trait studied since it is the most accessible in eggshell, and porosity will be assessed in specimens that allow for tangential thin sections to be made. In the thin sections, total pore area (A_p) will be calculated by multiplying the average pore area (A), times the pore density (D; how many pores per area), times the eggshell surface area (A_s). With this, porosity can be calculated as total pore area divided by pore length ($A_p \cdot L_s^{-1}$), which is determined by the eggshell thickness[4]. Finally, $\delta^{18}O$ analyses would be conducted on the eggshell carbonate, to assess the drinking water consumed by the egg-laying organism. The data analysis will consist of first grouping eggshells with similar thickness using a *k*-factor cluster analysis, which will allow me to observe the distribution of $\delta^{18}O$ amongst each cluster. The $\delta^{18}O$ distribution will be analyzed based on its mean and its skewness. Its mean will be correlated to the predominant location of the samples in the distribution, since $\delta^{18}O$ can be controlled by latitude and continental location (closer or further away from oceans). The skewness of the distribution will be associated with the humidity of the environment, since normally distributed samples would be closer to the slope of the meteoric water line and left-skewness in the distributed samples would point towards environments enriched in ^{18}O, due to local prevalent evaporation[9]. Normal distributions would point towards more humid environments and left-skewed distributions would point towards more arid environments. By observing the distribution of $\delta^{18}O$ within each thickness cluster, I will be able to assess if thicker eggshells are usually present in arid environments and thinner eggshells in more humid ones. The cluster analysis will be similarly applied to porosity to correlate to the characteristics of the $\delta^{18}O$ distributions associated with each cluster. With the paired humidity/thickness constraints, I will test whether older eggshells had a great variance in thickness that allowed archosaurs to adapt to drier environments, producing a correlation like in Fig. 1, or if early eggshells were predominantly thin, and later became thicker as a response to archosaur expansion into less humid environments, ultimately filling out a correlation like in Fig. 1.

Resources: My connection to the University of Colorado Boulder (CU Boulder), would allow for direct access to the [repository], which contains about 3,000 specimens of eggshells from as far back as the Jurassic. Also, some specimens include useful notes, thin sections, and electron micrographs that will provide important context for this study. Additionally, CU Boulder has the mass spectrometry capabilities and equipment to analyze the $\delta^{18}O$ composition of the eggshells at the CUBES-SIL laboratory.

Intellectual Merit: This work will extend our knowledge of the optimization of the amniote egg through time. Since this field has been understudied, in comparison to other archosaur fossils, any constraints on the controls on eggshell structure will be beneficial for future studies of oviparous tetrapod evolution, environmental reconstruction, and the mechanical functionality of the egg. This is even true if the primary hypothesis in this research plan were falsified (if humidity were completely uncorrelated to eggshell thickness), as the geochemical/structural record built in this study could be utilized to assess gas conductance and nesting behaviors.

Broader Impacts: This research could bridge the gap between paleontology and geochemistry and show the applicability of stable isotopes to archosaur research. Also, given many archosaurs in the study will likely be dinosaurs, which are an eye-catching topic within paleontology, I could exploit this fact to attract attention to this project. Using the novel approaches of my research, I plan to attract new students into geology, offer them research mentoring through internships like the SMART program (see Personal Statement), and recruit individuals as assistants for fieldwork. This direct one-on-one exposure could allow me to showcase the broad range of subjects in geology and connect people to future research experiences. Finally, I will use my connection to [Uni] to attract minorities to the geosciences, through webinars and seminars about my project at Puerto Rico, and orientations on how to get research opportunities abroad.

1: Stein et al., 2019. Scientific Reports, 9(1):4424. **2:** Deeming & Ruta, 2014. R. Soc. Open Sci., 1(3). **3:** Mikhailov, 1997. Special Papers in Paleontology, No. 56. **4:** Tanaka et al., 2015. PLoS ONE, 10(11):e0142829. **5:** Stein & Badyaev, 2011. Funct. Ecol., 25. **6:** Hechenleitner et al., 2016**.** J. Royal Soc. Interface, 13(116). **7:** Portugal et al., 2014. J. Exp. Biol. 217(18). **8:** Montanari, 2018. R. Soc. Open Sci., 5(6):180006. **9:** Alley & Cuffey, 2001. Rev. Mineral. Geochem., 43(1).