Fossil Egg Shell aDNA revises elephant bird phylogeography and systematics

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The elephant birds of Madagascar (Aves: Aepyornithidae) were large, flightless ratites that became extinct around a millennium ago. Little is known about these birds, though they offer excellent models to study many aspects of evolution, including speciation and extinction. Within the two known genera, multiple species of elephant birds are recognised, but the relationships among them remain controversial due to the rarity of skeletal fossils. Furthermore, it has been challenging to recover ancient DNA (aDNA) from skeletal fossils due to the warm climate of Madagascar, which is not conducive to aDNA preservation. With approximately 3% endogenous aDNA retrievable from elephant bird eggshell, it is a promising substrate for recovering high-quality aDNA. Furthermore, the morphology and chemical makeup of avian eggshell can reveal information about the relationship between the bird and its environment[1]: for instance, eggshell porosity is a proxy the amount of gas exchange between the developing fetus and the environment and therefore provides clues about the metabolic demands of the embryo within[2], as well as the environment in which it was incubated; likewise**,** diet can be reflected in the carbon and oxygen isotope content of eggshell.

We provide the first description of elephant bird phylogeography based on molecular analysis of fossil eggshells. High-throughput sequencing of 20 near-complete mitochondrial genomes from across Madagascar reveals genetic variation that is correlated with eggshell macro- and micromorphology, isotope content, and geographic distribution. High levels of between-genera genetic variation suggest that Aepyornis and Mullerornis should be reclassified into two families instead of one. Low levels of within-genera genetic variation suggest that only one species within each genus existed in South-Southwest Madagascar during the Holocene; however, we describe a novel oospecies of Aepyornis from Madagascar's North that is phylogenetically and morphologically distinct. Furthermore, molecular dating suggests that changes in Madagascar's palaeo environment 20 Ma (million years ago) may have prompted the divergence between genera, while divergence within Aepyornis coincides with the drying climate of the Pleistocene, 2 Ma. We offer the first insight into elephant bird speciation and advocate for a revision of their taxonomy that integrates a molecular perspective. We foresee that elephant bird whole genome recovery is ultimately achievable, and will provide further insights into the evolution these birds.

References:

1. Burton, F. G. & Tullett, S. G. A comparison of the effects of eggshell porosity on the respiration and growth of domestic-fowl, duck and turkey embryos. Comparative Biochemistry and Physiology a-Physiology 75, 167-174 (1983).

2. Tullett, S. G. The porosity of avian eggshells. Comparative Biochemistry and Physiology a-Physiology 78, 5-13 (1984).

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3D Volume Rendering- Aepyronis Egg Shell-

https://cloudstor.aarnet.edu.au/plus/s/SOhzqs8XF7c0F3u/download

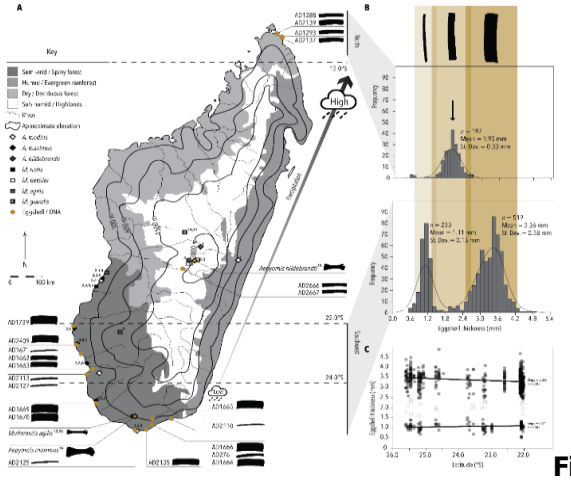

Figure 1 | A Map of Madagascar depicting the geographic location of eggshell samples collected and analysed (circles). Samples are represented by their ID# and the thickness of the sample is given in mm in the brackets to the right of the sample number. The location of fossil specimens of *Aepyornis* (diamonds) and *Mullerornis* (squares) are shown. Superscripts next to the symbol are primary and secondary references for those fossils. Specimens for which DNA data were available are coloured yellow. Simplified topography of the landscape is shown with approximate elevation represented by contour lines, rivers represented by fine lines, and biomes represented by shades of grey. **B** The distribution of eggshell thicknesses derived from the total number of eggshells collected across the range in the North, Southwest, and South of Madagascar.

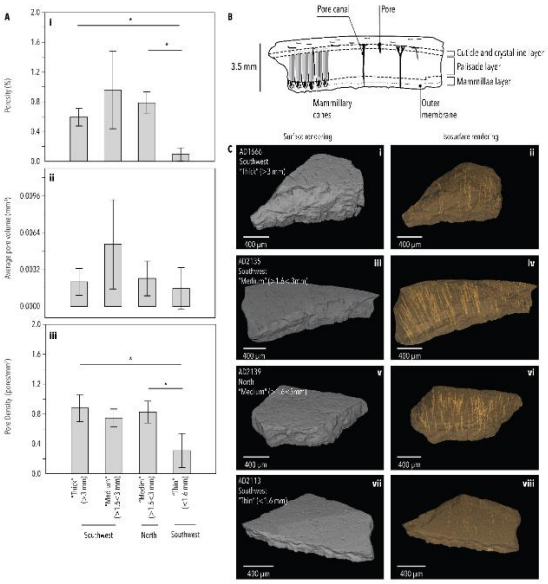

Figure 2 | **A** Bar chart comparing the mean porosity, mean 'average volume per pore', and mean pore density within the ROI examined of each eggshell morphotype. Significant differences (p<0.013) are indicated by an asterisk. **B** A cross-sectional schematic diagram of elephant bird eggshell macromolecular structure depicting the main morphological features, including both simple (unchbranced) and multifurcate (braching) longitudinal pore structures. **C** Representative micro CT scans of each eggshell morphotype showing the outer surface and internal pore structures structures.