

Divergence of *Crocodylus acutus* in the Central American Isthmus

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Abstract: Climatic and geological events have shaped life on Earth throughout its history. The rise of the Central American Isthmus (CAI) is an event that changed global circulation patterns, initiating glaciations, connecting the terrestrial biotas of South and North America and establishing the Caribbean Sea. The nature of this event makes it an excellent natural scenario to test vicariance and divergence by allopatry. Many studies have shown the effect of the formation of this land bridge on marine and terrestrial species, but no studies have been made on semi-aquatic ones. The American crocodile (*Crocodylus acutus*) is a semi-aquatic species that arrived to the Neotropics before the complete closure of the CAI, and a candidate to test how genomic divergence accumulated through time after a recent isolation event. We used Restriction Site Associated DNA sequencing markers (RADseq) on populations on both sides of the Isthmus of Panama to: a) understand how genomic variation is partitioned between different populations of *C. acutus*, b) infer the demographic history of *C. acutus*, and c) evaluate the effect of the recent opening of the Panama Canal. We recovered more than ~17,000 SNPs per population. We found three clear genetic clusters: 1) Caribbean and the Panama Canal, 2) Pacific Coast, and 3) Coiba. We suggest that the biology of the species played an important role on the resilience of the species to the rise of the CAI. Rather, the divergence coincides with the Last Glacial Maximum (LGM), an event that caused a global sea level drop. The LGM potentially affected the nesting and nursery sites isolating crocodile populations in Panama. We did not find alterations in the population structure caused by the reconnection of the Pacific and Caribbean, but mutation rates and long generation times of crocodiles may be masking this process.

Keywords: *Crocodylus*, evolution, genomics, Central American Isthmus.

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