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Discriminating Populations of *Paleosuchus trigonatus* (Caimaninae: Alligatoridae) through Microsatellite Markers Retrieved by Next Generation Sequencing

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The development of microsatellites was very laborious, time consuming and with no success guaranteed. Currently, the use of next-generation sequencing to develop specific microsatellite loci for non-model species have revolutionized the fields of population genetics and evolutionary biology. We isolated and characterized 10 new microsatellites loci for *Paleosuchus trigonatus* using ION TORRENT Personal Genome Machine. We tested the transferability of these loci to three related species of Caimaninae and used these bi-parental markers to test population structure and investigated the genetic diversity of two populations of *P. trigonatus* that are under the impact of hydroelectric powerplants on the Madeira and Xingu rivers. We used an adapted ddRADseq protocol to obtain a reduced representation of the genomes of four dwarf caimans sampled across the distribution of the species and after we filtered putative polymorphic microsatellites loci. We screened 32 *P. trigonatus* from two populations, Madeira (N = 16) and Xingu (N = 16). We investigated the transferability for three related species: *Paleosuchus palpebrosus* (N = 5), *Caiman crocodilus* (N = 6) and *Melanosuchus niger* (N = 6),

cross-amplifying successfully with good levels of polymorphism. The genetic diversity of *P. trigonatus* was low for both Madeira (*He*: 0.535 ± 0.148) and Xingu (*He*: 0.381 ± 0.222) populations, similar what was reported for other crocodilian species. The set of these 10 loci were sufficiently polymorphic to be used in future mating systems or kinship studies in *P. trigonatus*. Using DAPC analysis with a set of nine microsatellites loci we were able to separate the four species of Caimaninae studied and with all 10 loci we detected a shallow genetic structure between Madeira and Xingu populations of *P. trigonatus*. The AMOVA and STRUCTURE analysis using *locprior* model corroborate the putative shallow genetic structure between these populations. For the first time, specific microsatellites loci were developed for a crocodilian species using next-generation sequencing. Our set of microsatellites also represent the first toolkit of bi-parental molecular marker that will be available for *P. trigonatus*, since the species have no specific microsatellites nor have they been cross-amplified using loci developed to other species. These novel molecular markers will be also useful in conservation genetics and phylogeographic studies of *P. trigonatus*, since they improve our ability to monitor the putative effects of dams on the loss of genetic diversity.

Keywords Cross-amplification; Genetic structure; Madeira River; Xingu River.