

Chromosomal microdissection for the synthesis of a specific probe in *Caiman latirostris* for future evaluation of target DNA sequences

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Abstract: The biological impact of pesticides is the result of a cascade of events that can be evident at different levels of biological organization, exerting a marked long-term effect on natural populations. For several years, our research group has been studying the possible effects of massive pesticides in *Caiman latirostris*, using biomarkers of different endpoints. The identification of sensitive early warning markers to be applied in natural biota is indispensable to prevent damage that may affect animal physiology in the long term, leading to possible imbalances in population dynamics. To deepen the characterization of the biological effects of pesticides, we proposed to develop a specific pericentromeric probe to use it later in the micronucleus- and Comet-FISH (fluorescence in situ hybridization) techniques, as new markers of specific genotoxic damage. Microdissection was performed in caiman metaphases using a micromanipulator coupled to an inverted microscope. Two probes have been developed: 1) includes 6 microdissections of the central-pericentromeric region from different chromosomes and 2) includes 12 microdissections of the central-pericentromeric region from chromosome pair 1. Then, were transferred them to different microtubes, and the DNA was amplified using the kit GenomePlex® Single Cell Whole Genome Amplification (WGA4 – Sigma). Following the reactions, the products were verified by agarose gel electrophoresis, and probes were labeled with digoxigenin-11-dUTP using the GenomePlex® WGA Reamplification Kit (WGA3 – Sigma). Positive results were observed for both probes, but they were not consistent in all chromosomes and metaphases. It is necessary to continue improving the probe quality to have more specific results in this type of technique. The micronucleus- and Comet assay-FISH techniques have not been applied in any crocodilian species worldwide and will be integrated to the battery of markers actually used in this species for genotoxic evaluation.

Keywords: Fluorescence in situ hybridization, Pericentromeric probe, Molecular marker, Broad-snouted caiman

Type of presentation: Poster

Thematic area: Research and Knowledge (Systematics, Evolutionary Biology)