

EVO-DEVO AND THE STUDY OF MORPHOLOGICAL DIVERSITY IN SQUAMATES: EXAMPLES OF METHODOLOGICAL APPROACHES

Theme: Metagenomics and biodiversity

Fernando Cipriano Andrade Oliveira, Marina Elisa Singarete, Mariana Grizante, Tiana Kohlsdorf

Biodiversity is often inferred from a taxonomic perspective based on the description of populations or species and their spatial distribution, which is extremely relevant for conservation strategies. A complementary approach for understanding the evolution of biodiversity is to focus on the mechanisms related to the origin of different morphologies from a basal body plan, which is assumed to involve changes in developmental processes involved in the formation of a given structure. One pattern that is particularly striking is the recurrent evolution of elongated limbless morphologies from a pentadactyl body shape, a case very well represented in Squamata, a group that includes lizards, snakes and amphisbaenias: changes in body morphology, from an ancestral form with four limbs to elongated and limbless body shapes, have occurred independently several times during the evolution of the clade. In this group, even within the elongated-limbless body shape there is a considerable diversity in body proportions, mostly related to variation in trunk and tail (absolute and relative) lengths. The investigation of developmental mechanisms underlying the evolution of morphological diversity in Squamates, however, is constrained by restrictions imposed by the use of non-model organisms, such as limited embryo availability and impossibility of molecular manipulations (like gene silencing and mutant organisms). In order to overcome these limitations, we use two complementary approaches to investigate changes in developmental mechanisms that may be associated to the evolution of morphological diversity in Squamata:

- 1) identification of patterns of molecular evolution in developmental genes, and
- 2) tests of the functional role of these patterns in developmental pathways.

We illustrate the first approach with data from bioinformatic analyses on sequence evolution of Hox genes among squamates (coding regions from *Hoxa-13* and regulatory regions of the Hox-d cluster), where specific patterns of nucleotide substitutions seem associated to the evolution of elongated and limbless (or digit-reduced) morphologies in the group. The functional relevance of these molecular footprints is then tested *in vitro* using mutated plasmids to verify if nucleotide substitutions actually modify the network of molecular interactions involved in limb development. With these two methodological approaches we exemplify how another layer, related to developmental processes, can be incorporated to the investigation of origin and evolution of morphological diversity in animal groups.