Initial cytoarchitectonic characterization of the squamate forebrain: Case studies of the Western Diamondback rattlesnake (*Crotalus atrox*) and two distinct chameleon species (*Trioceros jacksonii*; *Rieppeleon kerstenii*)

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Introduction

Compared to the mammalian brain, the available data on the cytoarchitecture of the squamate brain is woefully deficient. Furthermore, most cytoarchitectonic studies do not draw comparisons across diverse taxa, ignoring a critical component of brain evolution. The comparative cytoarchitectonics among animals of common descent can be used to determine if the distributional patterns of neurons reflect primitive or derived features.

Sensory drive can be a powerful evolutionary force and consists of two major components: motor and perceptual. The motor aspect relates to the physical ability of an animal to communicate, interact, or interpret the natural world. The perceptual aspect relates to the ability of an animal to detect signal variation, which is a result of the brain's capability to process signal information.

Thermosensation in rattlesnakes may be an example of how novel sensorimotor abilities permitted the use of new ecological niches, and this expansion of ecological space could have driven speciation. Color change in chameleons may be an example of how evolutionary changes in a brain region responsible for the analysis of communication signals improved the detection of signal variation, and this expansion of perceptual space could have driven speciation.

Here we characterize the neural architecture of three squamate species with markedly different sensory specializations. Further, we compare the visual systems of two closely related lizard species and a distantly related snake species to assess the evolutionary influence of sensory secpializations upon the squamate visual system.

Materials & Methods

Animal Preparation. Animals were exanguinated under deep sedation. Chameleons were transcardially perfused with saline (2 ml) followed by 4% paraformaldehyde (PFA) (2 ml). Rattlesnake brains were immersion fixed in 4% PFA. In all cases, brains were cryoprotected in sucrose/PFA, frozen in hexane and coronally cut into 20-30 µm-thick sections.

Nissl Staining. Series were stained with thionin to ascertain the cytoarchitecture of various brain regions.

Imaging. Nissl stained sections were stitched at 20x with a Zeiss M2 Axioimager equipped with Volocity Software. The appropriate fluorophores were used for immuno stained sections.

Cytoarchitecture Comparisons. The rattlesnake sections were aligned with cytoarchitecture data published for the related species *Crotalus viridis* (Gruberg *et al.*, 1979). Similarly, sections from both chameleon species were aligned with the related species *Chamaeleo chamaeleon* (Bennis *et al.*, 1994).

Conclusions

Cytoarchitectonics revealed forebrain and midbrain differences among the squamates in this study.

Laminar organization of the optic tectum among squamates is variable, and may reflect evolutionary relationships.

Future work includes creating brain atlases for these squamates.

References

Bennis, M., J. Reperant, J. Rio, and R. Ward. 1994. An experimental re-evaulation of the primary visual system of the European Chameleon, *Chameleo chameleon*. *Brain, Behavior and Evolultion* 43:173-188.

Gruberg, E.R., E. Kicliter, E.A. Newman, L. Kass, and P.H. Hartline. 1979. Connections of the tectum of the rattlesnake *Crotalus viridis*: An HRP study. *The Journal of Comparative Neurology* 188:31-42.

Pyron, A.R., F.T. Burbrink, and J.J. Wiens. 2013. A phylogeny and revised classification of Squamata, including 4161 species of lizards and snakes. *BMC Evolutionary Biology* 13:93.

Ramón, P. 1896. El structura de encéfalo del cameléon. *Rev. Trimest. Micrograf* 146-82.

Phylogenetic tree of squamates

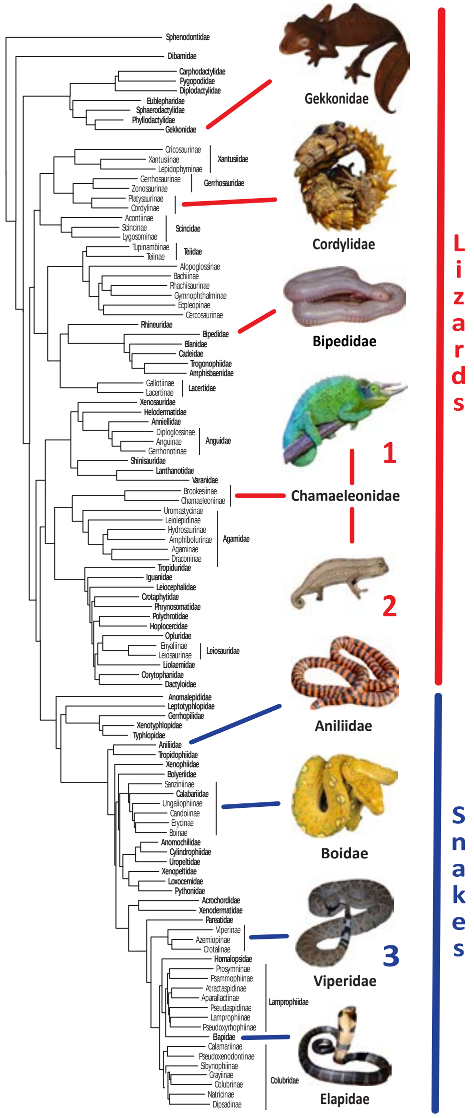
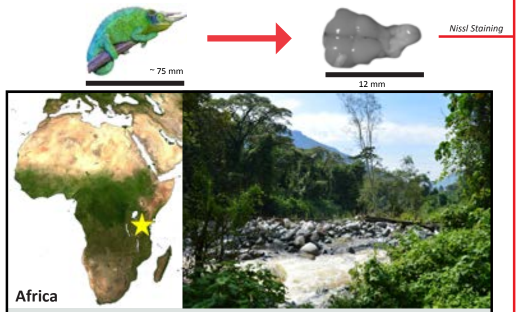


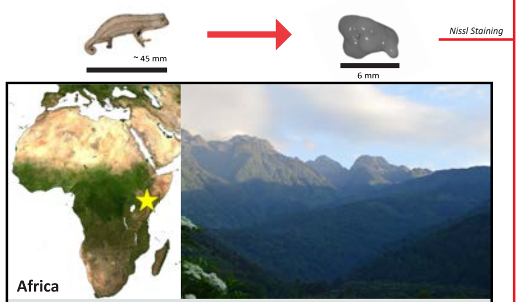
FIGURE 1. Family-level molecular phylogeny of the amniote order Squamata and representative squamates. Modified from Pyron *et al.* (2013).

1. Squamata: Chamaeleonidae: *Trioceros jacksonii*



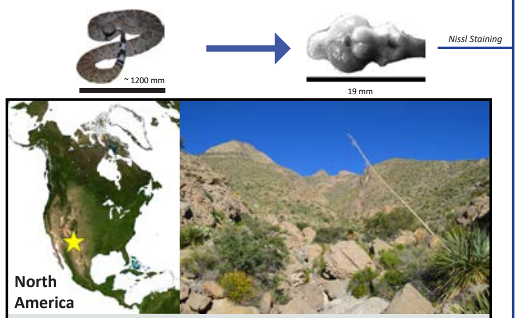
The Jackson's chameleon inhabits tropical forests in the highlands of Tanzania, East Africa. This typical chameleon is a lizard in the family **Chamaeleonidae**, and has an extensive ability to change body colors.

2. Squamata: Chamaeleonidae: *Rieppeleon kerstenii*



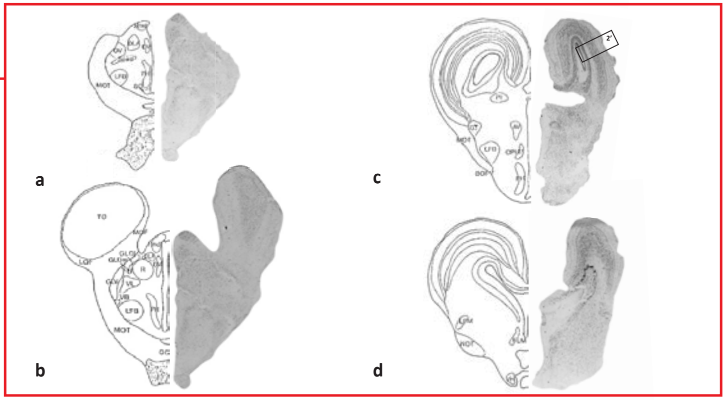
The Kenya pygmy chameleon inhabits tropical forests in the highlands of Kenya, East Africa. This pygmy chameleon is a lizard in the family **Chamaeleonidae**, and has a limited ability to change body colors.

3. Squamata: Viperidae: *Crotalus atrox*



The Western Diamondback rattlesnake occupies deserts throughout southwestern North America. This squamate is a snake in the family **Viperidae**, and has well-developed thermosensing facial pits.

FIGURE 2. Geographic locations, habitats and descriptions of the squamate species (1) *Trioceros jacksonii*, (2) *Rieppeleon kerstenii* and (3) *Crotalus atrox*.



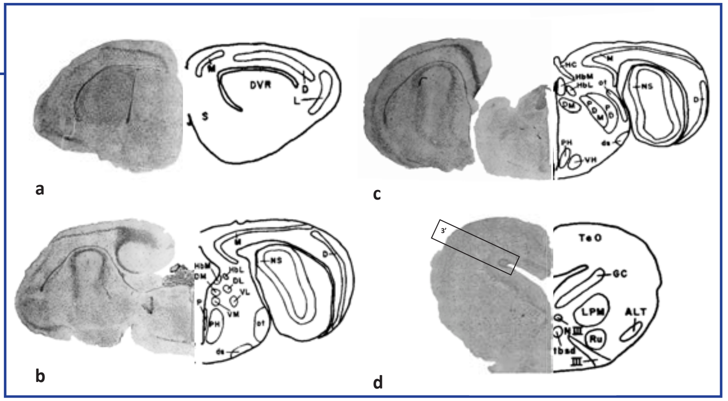
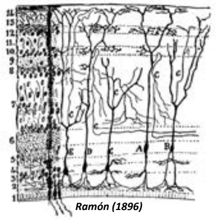


FIGURE 3. Rostral to caudal (a-d) Nissl stained coronal hemi-sections and corresponding drawings of brains for the squamate species (1) *Trioceros jacksonii*, (2) *Rieppeleon kerstenii* and (3) *Crotalus atrox*.

Laminar organization of the optic tectum

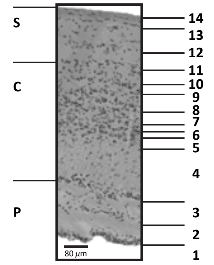


The squamate optic tectum consists of a dorsal cortex formed by alternatiing cellular and fibrous layers.

Stratification of the squamate optic tectum into 14 distinct retinal laminae was first proposed by Ramón (1896) in a chameleon (*left*).

Here we analyze these layers on the basis of Ramón's nomenclature

1'. *Trioceros jacksonii*

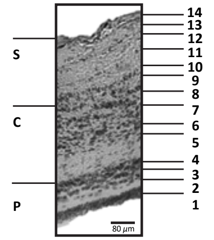


Poorly developed and reduced cellular laminae in periventricular zone

Highly laminated central zone

Sparsely populated superficial zone

2'. *Rieppeleon kerstenii*

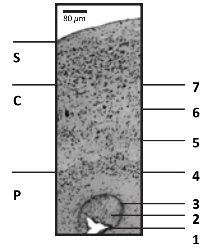


Well-developed periventricular zone

Highly laminated central zone

Thickened and cellularized retinal laminae 1

3'. *Crotalus atrox*



Fibrous periventricular zones 2 and 3

Interdigitated superficial zone

Cellularized retinal laminae 4

S: superficial zone; C: central zone; P: periventricular zone

FIGURE 4. High-magnification photomicrographs of the optic tectum.