Phylogenetics and Integrative Taxonomy of African Water Snakes (Squamata: Colubridae: Grayia)

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Phylogenetics and Integrative Taxonomy of African Water Snakes (Squamata: Colubridae: *Grayia*)

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Abstract

Grayia is a genus of relatively large (1.5 – 2.5 m) aquatic Afrotropical snakes that is currently comprised of four species. Recent molecular phylogenies recovered *Grayia* in its own distinct subfamily (Grayiinae), which was strongly supported as the sister group to Colubrinae. Because tropical African snakes are generally understudied, the relationships within *Grayia* are poorly known. Due to morphological conservatism, identification is often difficult and previous studies involving *Grayia* included misidentified specimens in other genera. The goal of this study is to build a phylogenetic tree that can be used to understand the relationships and taxonomy of *Grayia* via an integrative taxonomic approach that combines molecular and morphological data. One nuclear (BDNF) and four mitochondrial genes (COI, cyt *b*, 16S and ND4) were used to construct a phylogenetic tree with Maximum likelihood methods; outgroups included the genera *Calamaria, Sibynophis* and *Masticophis*. Preliminary trees suggest *G. ornata* and *G. smithii* are sister taxa, whereas *G. caesar* (originally described as the sole member of the genus *Xenurophis*) is sister to *G. tholloni*. At least two divergent lineages of *G. ornata* suggest cryptic species are likely present in Democratic Republic of the Congo (DRC) and Republic of Congo.

Introduction

- The genus *Grayia* is comprised of four species: *Grayia caesar, G. ornata, G. smithii*, and *G. tholloni* (Fig. 1). These large Afrotropical watersnakes superficially resemble natricines and water cobras. Given their unique position in several recent molecular phylogenetic/phylogenomic studies, the genus has been placed in its own unique subfamily, Grayiinae, [1][2][3] and at least two of these studies have shown support for Colubrinae as the sister taxon to *Grayia* [2][3]. Although these large snakes are hunted by local people for “bushmeat” food and various medicinal uses [4][5], species within *Grayia* are generally understudied and poorly sampled in recent collections. As a result, relationships within the genus are poorly known. Moreover, morphological conservatism often makes identification difficult, and several “*Grayia*” samples on GenBank included misidentified specimens in other genera. The goal of this study is to build a phylogenetic tree that reconciles the current taxonomy of *Grayia* via an integrative taxonomic approach that combines molecular and morphological data. Herein we present preliminary molecular data to guide future morphological studies.

Figure 1: Selected voucher specimens (in life) of *Grayia* from the phylogeny in Figure 2 and an example of *G. caesar*.

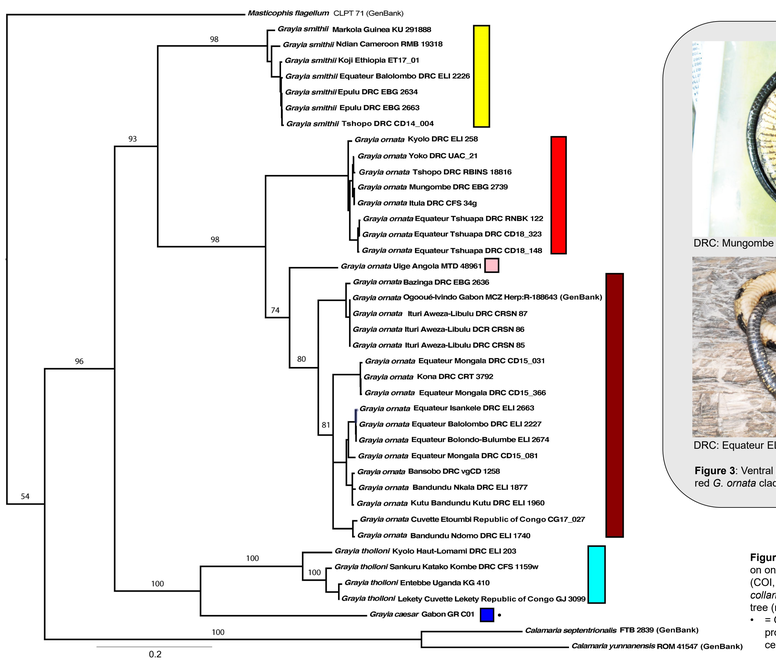

Figure 2: Maximum likelihood tree of *Grayia* based on one nuclear (BDNF) and four mitochondrial (COI, cyt *b*, 16S and ND4) genes. *Sibynophis collaris* and *S. subpunctatus* were used to root the tree (not shown).
• = Only country-level locality information provided by collector. Locality was placed in the center of the country in Fig. 4.

Figure 3: Ventral patterns of specimens from the red and dark red *G. ornata* clades.

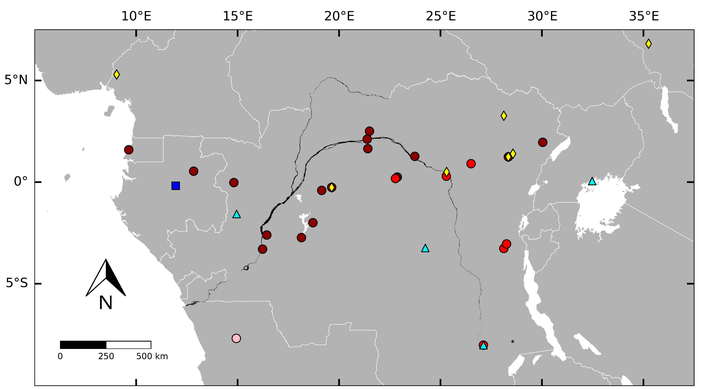

Figure 4: Map of Central Africa showing the localities of genetic samples used in this study. The confluence of the western Congo and Ubangi Rivers are shown in black. Bodies of water are white. Symbol colors match the color-coded clades in Fig. 2. Circles = *G. ornata*, squares = *G. caesar*, triangles = *G. tholloni* and diamonds = *G. smithii*.

Materials and Methods

- DNA extractions were conducted on tissue samples from each of the four *Grayia* species with the Qiagen DNeasy blood and tissue kit. One nuclear (BDNF) and four mitochondrial (16S, cyt *b*, COI, ND4) genes were amplified using standard PCR techniques with an ABI 3130xl automated sequencer at the University of Texas at El Paso (UTEP) Genomic Analysis Core Facility. The resulting sequences, supplemented with data acquired from GenBank (except for misidentified samples), were used to construct a phylogenetic tree with Maximum likelihood methods via the CIPRES Science Gateway (https://www.phylo.org/). Based on previous studies, *Masticophis* (Colubrinae), *Calamaria* (Calamariinae) and *Sibynophis* (Sibynophiinae) were chosen as outgroups; the latter genus was used to root the tree[1].

Results & Discussion

- The molecular data set included 1 *Grayia caesar*, 26 *G. ornata*, 7 *G. smithii,* and 4 *G. tholloni* (Fig. 2). Including outgroups, a total of 43 sequences (37 generated for the first time in this study) were used to build our phylogenetic tree of *Grayia.* Our data set included the mitochondrial genes 16S (511 base pairs [bp]), cyt *b* (1014 bp), ND4 (681 bp), COI (668 bp), and the nuclear gene BDNF (670 bp), for a total of 3,544 bp. Our preferred tree (Fig. 2) recovered two well-supported clades, including *Grayia ornata* + *G. smithii*, and *G. caesar* + *G. tholloni*. One sample of *G. smithii* from Ethiopia represents a new country record. The *G. ornata* specimen from Angola (pink clade) was found ~200 km from the type locality (likely topotypic), but it is markedly divergent from the other *G. ornata* samples (DRC and Republic of Congo) in a clade with moderate support (74). Striking color pattern differences are evident between some specimens from the red and dark red *G. ornata* clades (Fig. 3) of the phylogeny.
- The clade shown in the color red is widespread and includes samples from both sides of the eastern Congo River (Fig. 4). The *G. ornata* sample (seen in the dark red clade) from Bandundu (DRC) is genetically identical to a sample from Cuvette Etoumbi in the Republic of Congo, suggesting these populations cross the western Congo regularly. The western portion of both the Congo and Ubangi rivers often act as a barrier to terrestrial and arboreal snakes (e.g., *Toxicodryas*); however, *Grayia* are exclusively aquatic and we hypothesize that rivers likely serve as dispersal routes rather than barriers[6]. The biogeographic barriers responsible for speciation patterns in *Grayia* remain unknown and require further study.
- Future efforts will include the addition of a second nuclear gene (NT3), acquisition of more samples, additional phylogenetics analyses, morphological analyses, and species-delimitation analyses.

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