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ORIGINAL RESEARCH

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Evolution of nuchal glands, unusual defensive organs of Asian natricine snakes (Serpentes: Colubridae), inferred from a molecular phylogeny

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Abstract

A large body of evidence indicates that evolutionary innovations of novel organs have facilitated the subsequent diversification of species. Investigation of the evolutionary history of such organs should provide important clues for understanding the basis for species diversification. An Asian natricine snake, *Rhabdophis tigrinus*, possesses a series of unusual organs, called nuchal glands, which contain cardiotonic steroid toxins known as bufadienolides. *Rhabdophis tigrinus* sequesters bufadienolides from its toad prey and stores them in the nuchal glands as a defensive mechanism. Among more than 3,500 species of snakes, only 17 Asian natricine species are known to possess nuchal glands or their homologues. These 17 species belong to three nominal genera, *Balanophis*, *Macropisthodon*, and *Rhabdophis*. In *Macropisthodon* and *Rhabdophis*, however, species without nuchal glands also exist. To infer the evolutionary history of the nuchal glands, we investigated the molecular phylogenetic relationships among Asian natricine species with and without nuchal glands, based on variations in partial sequences of Mt-CYB, Cmos, and RAG1 (total 2,767 bp). Results show that all species with nuchal glands belong to a single clade (NGC). Therefore, we infer that the common ancestor of this clade possessed nuchal glands with no independent origins of the glands within the members. Our results also imply that some species have secondarily lost the glands. Given the estimated divergence time of related species, the ancestor of the nuchal gland clade emerged 19.18 mya. Our study shows that nuchal glands are fruitful subjects for exploring the evolution of novel organs. In addition, our analysis indicates that reevaluation of the taxonomic status of the genera *Balanophis* and *Macropisthodon* is required. We propose to assign all species belonging to the NGC to the genus *Rhabdophis*, pending further study.