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Evolution of a special organ, nuchal gland, based on a molecular phylogeny of the Eurasian natricine snakes (Serpentes: Colubridae)

Hirohiko Takeuchi^{1*}, Akira Mori¹, Li Ding², Ansem de Silva³, Indraneil Das⁴, Tao Thien Nguyen⁵, Tein-Shun Tsai⁶, Teppei Jono⁷, Guang-xiang Zhu⁸, Dalshani Mahaulpatha⁹, Ye-zhong Tang², Alan H. Savitzky¹⁰

1. Kyoto University, Japan
2. Chengdu Institute of Biology, Chinese Academy of Sciences, China
3. Gampola
4. University of Malaysia, Sarawak, Malaysia
5. Vietnam National Museum of Nature, Vietnam
6. National Pingtung University of Science and Technology, Taiwan, China
7. University of Ryukyus, Japan
8. Sichuan Agricultural University, China
9. University of Sri Jayawardenepura, Sri Lanka
10. Utah State University, USA

*Presenting author's e-mail: hirohiko@zoo.zool.kyoto-u.ac.jp

An Asian natricine snake, *Rhabdophis tigrinus*, has a unique series of organs, called nuchal glands, which contain cardiac steroidal toxins known as bufadienolides. *Rhabdophis tigrinus* sequesters bufadienolides from its toad prey and stores them in the nuchal glands as a defensive substance. Among more than 3400 species of snakes, only 18 Asian natricine species are known to have the nuchal glands. These 18 species belong to three genera, *Balanophis*, *Macropisthodon*, and *Rhabdophis*. In *Macropisthodon* and *Rhabdophis*, however, species without the nuchal glands also exist. This evidence suggests multiple independent origin and/or secondarily loss of the nuchal glands. To infer the evolutionary history of the nuchal glands, we investigated the molecular phylogenetic relationships among Eurasian natricine species with and without the nuchal glands, based on variations in partial sequences of the oocyte maturation factor Mos (c-mos) gene, the recombination-activating gene 1 (Rag 1), and the mitochondrial cytochrome b (cyt.b) gene (total 2.6 kbp). The results supported that species with the nuchal glands were all contained in a single clade. Therefore, based on principle of parsimony, it is inferred that the common ancestor of the species within this clade has obtained the nuchal glands and that multiple independent evolution has not occurred. *Rhabdophis swinhonis*, some individuals of which are known to lack nuchal glands, is considered to have emerged as newly detected position in the nuchal gland clade. Therefore, it is strongly suggested that secondarily loss of the nuchal glands has occurred at least in some individuals of *R. swinhonis*.

Keywords: nuchal glands, *Rhabdophis*, *Macropisthodon*, *Balanophis*, Eurasian Natricinae