

## ORIGINAL RESEARCH

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

Hirohiko Takeuchi<sup>1</sup>  | Alan H. Savitzky<sup>2</sup> | Li Ding<sup>3</sup> | Anselm de Silva<sup>4</sup> | Indraneil Das<sup>5</sup> | Tao Thien Nguyen<sup>6,7</sup> | Tein-Shun Tsai<sup>8</sup> | Teppei Jono<sup>3</sup> | Guang-Xiang Zhu<sup>9</sup> | Dharshani Mahaulpatha<sup>10</sup> | Yezhong Tang<sup>3</sup> | Akira Mori<sup>11</sup>

<sup>1</sup>Seto Marine Biological Laboratory, Field Science Education and Research Center, Kyoto University, Shirahama, Japan

<sup>2</sup>Department of Biology, Utah State University, Logan, Utah

<sup>3</sup>Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu, China

<sup>4</sup>Gampola, Sri Lanka

<sup>5</sup>Institute of Biodiversity and Environmental Conservation, University Malaysia Sarawak, Sarawak, Malaysia

<sup>6</sup>Vietnam National Museum of Nature, Vietnam Academy of Science and Technology, Hanoi, Vietnam

<sup>7</sup>Graduate University of Science and Technology, Vietnam Academy of Science and Technology, Hanoi, Vietnam

<sup>8</sup>Department of Biological Science and Technology, National Pingtung University of Science and Technology, Neipu Township, Taiwan

<sup>9</sup>College of Life Science, Sichuan Agricultural University, Ya'an, China

<sup>10</sup>University of Sri Jayawardenepura, Nugegoda, Sri Lanka

<sup>11</sup>Department of Zoology, Graduate School of Science, Kyoto University, Kyoto, Japan

## Correspondence

Akira Mori, Department of Zoology, Graduate School of Science, Kyoto University, Kyoto, Japan.  
Email: gappa@ethol.zool.kyoto-u.ac.jp

## Present address

Hirohiko Takeuchi, College of Bioresource Science, Nihon University, Fujisawa, Kanagawa, Japan

## 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 cardiotoxic 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.