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Discovery of Nucho-dorsal Glands in *Rhabdophis adleri*

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Abstract: The nuchal and nucho-dorsal glands are unique defensive organs that have been found in only 13 species of Asian natricine snakes. In *Rhabdophis* such nuchal glands have been described in nine species, whereas the absence of the glands has been also reported in a few species, including *R. adleri*. We reexamined the absence/presence of the nuchal glands of *R. adleri* by dissection of two freshly dead individuals and a recently preserved specimen, as well as by careful reobservation of six type specimens, in which the absence of the glands had been reported. The glands were found both in the neck and along the entire length of the body in the three new specimens. We also confirmed the presence of the glands, at least in the anterior part of the body, in three type specimens. We conclude that the glands were simply overlooked in the original description and that *R. adleri* possesses nucho-dorsal glands that extend over the full length of the body, including the neck region.

Key words: *Rhabdophis adleri*; Nuchal glands; Hainan Island; China; Defensive organ

Introduction

Rhabdophis adleri is a natricine snake species endemic to Hainan Island, China (Zhao, 1997). As one of the diagnostic characters of this species, Zhao (1997) noted the absence of nuchal glands. The nuchal and nucho-dorsal glands are unique organs that have been found only in 13 species of Asian natricine snakes (Mori et al., 2012). A series of behavioral, chemical, morphological, and physiological studies of *R. tigrinus*, in which the nuchal glands were first described (Nakamura, 1935), have revealed that cardiotonic steroids sequestered from toads consumed as prey are stored in the glands and are used as chemical weapons to deter predators, accompanied by several peculiar defensive displays (see Hutchinson et al., 2012; Mori et al., 2012 for reviews). During an integrative study of the

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evolution of this unique defensive system, we had an opportunity to dissect fresh specimens of *R. adleri* and discovered that this species actually possesses gland-like structures along the entire length of the body, structures Smith (1938) referred to as nuchodorsal glands. Here, we present a detailed description of the glands. We also examined the type specimens of *R. adleri* to investigate whether the glands are present in the type series.

Materials and Methods

Three specimens of *R. adleri* collected in Diaoluoshan, Hainan in April 2015 (Id. Nos. TJ2015-001 and TJ2015-002) and in Wuzhishan, Hainan in July 2012 (ZGX52) were examined. Identification of these specimens was made based on the description by Zhao (1997). The former two specimens were measured and dissected immediately after the snake died (TJ2015-001) or after the snake was euthanized with pentobarbital (TJ2015-002). The last specimen was measured and dissected after it had been fixed in 10% formalin and preserved in 70% ethanol. Snout–vent length and tail length were measured to the nearest 1 mm. The number of ventral scales was counted following Dowling's (1951) method. The numbers of subcaudal pairs and scale rows at mid-body also were counted.

We carefully peeled the dorsal skin off of each specimen from the posterior edge of the parietal scales to the anterior region of the tail. We counted the number of glands in the neck and trunk regions separately (see Results for the definition of the neck and trunk series of glands). Maximum length (measured along the major body axis) and maximum width (measured perpendicular to the major body axis) of each gland were measured to the nearest 0.05 mm using calipers for all glands in the neck region and for the first, last, and every 25 pairs from the first in the trunk region. The relative anteroposterior position of these glands was evaluated by counting the number of ventral scales from the anteriormost one. The position of the glands in relation to body scale rows was recorded as follows: mid-dorsal scale row, along the vertebral line, was defined as zero, and the number of each scale row was counted in both ventrolateral directions until reaching the scale under which the glands were positioned.

Six type specimens of *R. adleri* deposited in the museum of Chengdu Institute of Biology, Chinese Academy of Sciences were examined to confirm the absence/presence of the glands. These specimens included holotype (CIB64III5917), allotype (CIB64III5228), and four paratypes (CIB64III5112, 5114, 5115, 5883).

Results

Examination of new specimens

Based on meristic characters, we identified the three specimens as *R. adleri*, although the third specimen (ZGX52) had only 17 scale rows at mid-body (Table 1). We confirmed the presence of two longitudinal rows of glands

Table 1. Body size, scale counts, and the numbers of neck and trunk glands of three specimens of *Rhabdophis adleri*. SVL: snout-vent length (mm), TL: tail length (mm), V: number of ventral scales, SC: number of subcaudal pairs, SR: number of scale rows at midbody, SL: number of supralabials (left/right), NG: number of neck glands (left/right), TG: number of trunk glands (left/right). See text for definitions of neck and trunk glands.

Specimen No.	Sex	SVL	TL	V	SC	SR	SL	NG	TG
TJ2015-001	F	562	197	152	81	19	8/8	10/10	144/144
TJ2015-002	F	630	260	151	83	19	8/8	8/8	144/144
ZGX52	M	609	288+	159	74+	17*	8/8	11/10	151/150

* The numbers of scale rows around the neck and at the anterior 1/3 of the body were 19.

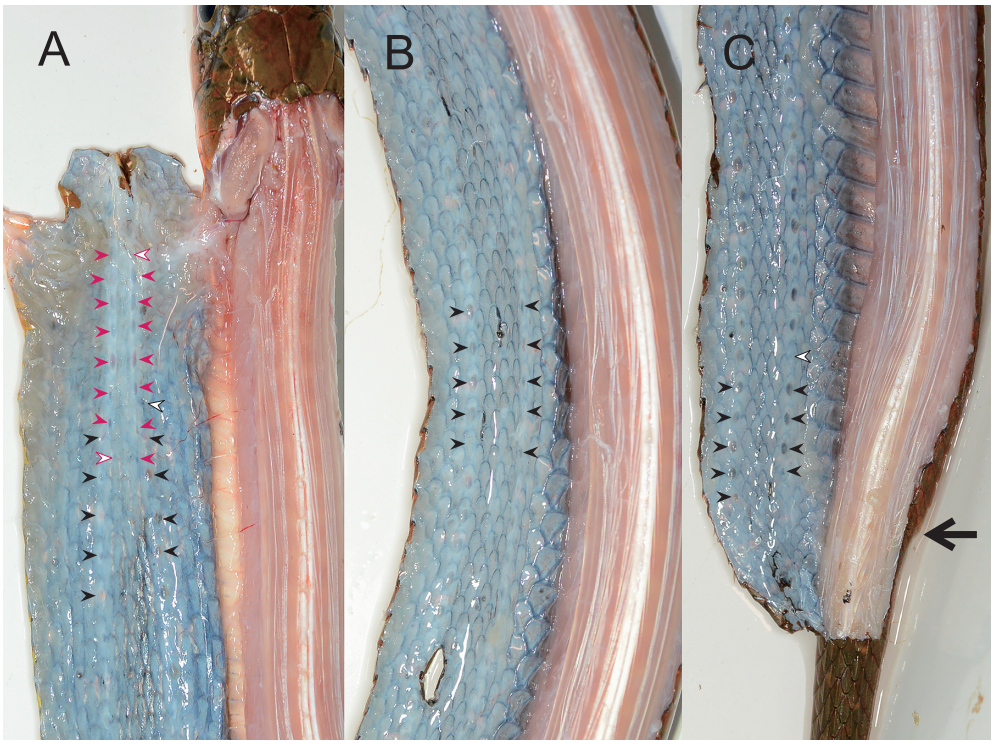

FIG. 1. Nucho-dorsal glands of *Rhabdophis adleri* (TJ2015-002) in the neck and anterior regions of the body (A), in the middle part of the body (B), and in the posterior part of the body (C). A: All neck glands and the first five trunk glands in each row are indicated by red and black arrowheads, respectively. Positions of the first left and the 8th right neck glands and the first left trunk gland, which are not visible in the photo, are indicated by white arrowheads. Note the partial overlap between the neck and trunk gland rows. B: Five pairs of trunk glands are indicated by black arrowheads as examples. C: The five posteriormost trunk glands in each row indicated by arrowheads. The position of a gland that is not visible in the photo is indicated by a white arrowhead. Large black arrow indicates position of the cloaca. Also see Fig. 2.

that extended anteroposteriorly throughout the entire length of the body. No glands were present on the tail.

In the neck region, eight to 11 glands were arranged along each side of the mid-dorsal scale row (Fig. 1). Hereafter, we refer to them as neck glands. These glands were not conspicuous and were less developed compared to the nuchal glands of other species (see photographs in Mao and Chang [1999] and Hutchinson et al. [2007]). The neck glands started from near the posterior edge of the parietal plates (a level that corresponds to the first or second ventral scales) to the level of the 8th or 9th ventral scales. Each gland within a row was separated anteroposteriorly from the adjacent glands. Mean lengths of the neck glands were 1.96 mm (range, 1.6–2.4), 1.68 mm (1.25–2.1), and 2.21 mm (1.5–3.0), and their mean widths were 1.27 mm (0.6–1.7), 0.69 mm (0.45–0.85), and 1.37 mm (0.7–2.1) for specimens TJ2015-001, TJ2015-002, and ZGX52, respectively. The size of the glands in a row varied irregularly, but the two posteriormost neck glands tended to be smaller than the other glands.

Another series of paired glands were arranged in rows from the ventrolateral side of the one or two posteriormost neck glands to the position corresponding to the last or the second last ventral scales. Hereafter, we refer to these as trunk glands (Fig. 1).

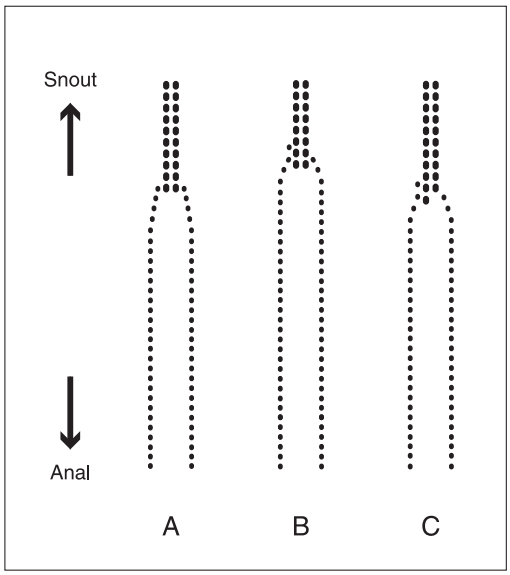

Fig. 2. Schematic dorsal view of the arrangement of the neck glands (large black ellipses) and the trunk glands (small black ellipses) in the anterior body of three individuals of *Rhabdophis adleri*. Note that the drawing shows only relative spatial arrangement of glands and does not reflect the actual size of each gland or the distance between glands. A: TJ2015-001, B: TJ2015-002, C: ZGX52.

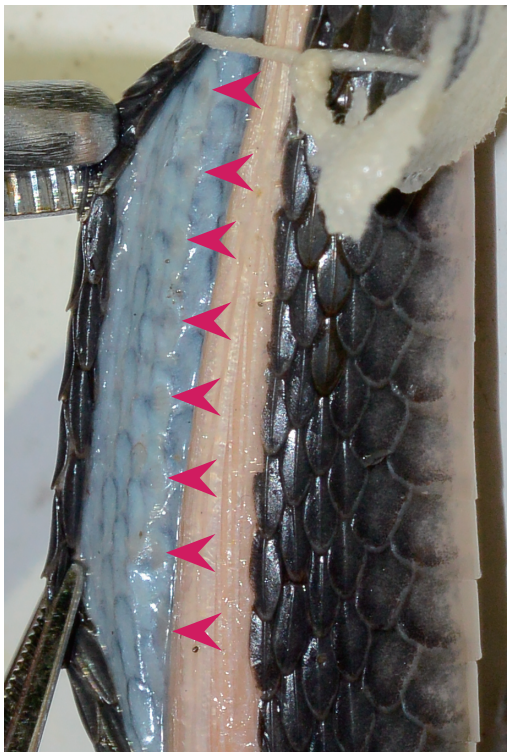

Fig. 3. A part of the right row of trunk glands in the anterior region of the body of a paratype (CIB64III5114). Arrowheads indicate the glands.

Approximately two posteriormost pairs of neck glands and two anteriormost pairs of trunk glands overlapped, although the condition varied among individuals (Fig. 2). The first trunk glands were located on the second body scale row from the mid-dorsum (i.e., three scale rows between the glands in a pair). The width between each of the paired glands gradually increased, and eventually trunk glands were positioned on the fourth body scale row (seven scale rows between the gland rows) at the third or fifth glands (Fig. 2). Thereafter, the trunk glands were always located on the fourth body scale row. The trunk glands were basically symmetrical, forming a row consisting of 144 to 151 glands in each side (Table 1). Each gland in a row was anteroposteriorly separated from adjacent glands. Approximately one gland in each row was associated with each ventral scale. Mean lengths of trunk glands were 1.24 mm (range, 0.9–1.5), 1.35 mm (0.5–1.9), and 1.59 mm (1.1–2.2), and its mean widths were 0.67 mm (0.6–0.8), 0.82 mm (0.5–1.8), and 1.05 mm (0.7–1.7) for specimens TJ2015-001, TJ2015-002, and ZGX52, respectively. The pattern of changing size of the glands in a row varied among the three individuals, but those from 25th to 125th were relatively large, whereas the posteriormost glands tended to be small.

Examination of type specimens

All six type specimens had been partially dissected previously by Zhao (1997) to determine the occurrence of the nuchal glands. However, because of the strict regulations of the Chengdu Institute of Biology for handling type specimens, we were unable to extend the dissection of the specimens. In addition, string had been tied tightly around the neck of the snakes to attach the tags, which we were

not allowed to cut. Therefore, we were not able to reflect the skin around the neck to look at the underside of the skin. Consequently, our examination was limited to the visual observation of a small area of the body several centimeters posterior to the neck region.

Three type specimens (holotype, allotype, and one paratype, CIB64III5112) had sections of skin peeled back, presumably to expose nuchal glands. These skin sections extended from the right mid-lateral level to the ventral region, instead of mid-lateral to mid-dorsal. Hence, the dorsal region, where nuchal glands should be present, was never examined by Zhao or by us. In the remaining three paratypes, we confirmed the presence of a few glands under the dorsal skin of the trunk region (Fig. 3). We could not confirm the presence of glands under the dorsal skin of the neck region because museum regulations precluded examination of that area.

Discussion

Our examination clearly demonstrated the presence of nucho-dorsal glands along the entire length of the body in the three recently collected specimens of *R. adleri*. We extracted some fluid from the glands of two specimens (TJ2015-001 and 002), and a chemical analysis confirmed the presence of cardiotonic steroids similar to those detected in the nuchal glands of *R. tigrinus* (Hutchinson et al., 2007; chemical results will be presented elsewhere). Because our species identification based on meristic characters and body coloration largely agreed with the original description of *R. adleri* (Zhao, 1997), and because three of the type specimens were confirmed to have the glands, at least in the anterior part of the body, it is certain that absence of nucho-dorsal glands is not a character of *R. adleri*. Zhao's (1997) failure to find them undoubtedly stemmed in part from examining the wrong region of the skin in three of the type specimens and in part from the relative inconspicuousness and small size of the glands.

Smith (1939) reported that three natricine species (*R. nuchalis*, *R. nigrocinctus*, and *Macropisthodon plumbicolor*) have glands along the full length of the body (nucho-dorsal glands). Mori et al. (2012) presumed that two additional species, *R. leonardi* and *R. pentasupralabialis*, previously included under *R. nuchalis*, would also have the nucho-dorsal glands (note that in Table 1 of Mori et al. [2012] *R. nigrocinctus* was erroneously listed as a species having the glands only in the neck). Accepting this presumption, *R. adleri* is the sixth species known to possess nucho-dorsal glands and the 14th species that has nuchal glands, sensu lato (Mori et al., 2012).

It should be noted that the definition of "neck" of snakes has been a controversial issue and currently there is no consensus as to whether snakes have a neck. In addition, the nuchal glands, at least those of *R. tigrinus*, are not structurally and functionally considered true "glands" (see Fukada, 1958; Mori et al., 2012). Therefore, "nuchal gland" is not an appropriate name for this organ. Nonetheless, we tentatively employ that traditional term in this study, awaiting future solution for the terminological problems.

Reevaluation of the taxonomic status of *R. adleri* may be necessary because the absence of nuchal glands was used as one of its diagnostic characters (Zhao, 1997). Phylogenetic study focusing on nuchal gland morphology would be a fruitful approach for elucidating the evolution of this unusual defensive organ. Investigation of the ontogenetic development of the organs and the genetic mechanism that underlies their development would shed light on the evolutionary origin of the organs and may provide a clue for appropriate terminology for these structures.

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