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Anatomy of the nuchal glands in red-necked keelback (*Rhabdophis subminiatus*)

The Red-necked keelback, *Rhabdophis subminiatus* (Schlegel, 1837), occurs in Indonesia on Java, Sumatra, Nias, and possibly Sulawesi (David & Vogel 2021). This species exhibits a suite of defensive displays, including body flattening, neck flattening, neck arching, neck butting, and dorsal-facing postures (Mori & Burghardt 2008, Anita *et al.* 2022). Body flattening enlarges the body profile to intimidate predators, while neck flattening accentuates the bright red coloration of the neck, a visual warning linked to the location of the nuchal glands.

In addition to venom glands, *R. subminiatus* possesses integumentary toxin glands in the dorsal neck skin (Smith 1938, Mori *et al.* 2012). These nuchal glands are unusual defensive organs that sequester bufadienolides from ingested toads (Akizawa *et al.* 1985, Hutchinson *et al.* 2007). The stored cardiotonic steroids render the snake unpalatable, and behavioral studies confirm a dietary preference for toxic anurans, further reinforcing their defensive role (Bergman 1956, Anita *et al.* 2022, 2024). Among *Rhabdophis*, about 20 species are now recognized to possess nuchal or nucho-dorsal glands, although their number and distribution vary considerably (Mori *et al.* 2023).

Nuchal glands were first described by Nakamura (1935) in *Natrix tigrina* (= *R. tigrinus*), which bears 13–16 pairs of sacculated glands. Smith (1938) expanded this work to ten species of *Natrix*, describing the sacculated form as chains of spherical units. His account of "*Natrix subminiata*" was based on material from northern Vietnam, now referred to *R. helleri* (David & Vogel 2021), leaving the morphology of true *R. subminiatus* poorly documented. More recent studies have emphasized interspecific variation in gland number and morphology (Mori *et al.* 2016a,b, Zhu *et al.* 2020). Although some *Rhabdophis* species also develop dorsal or trunk glands, the present study focuses exclusively on the nuchal glands of *R. subminiatus*.

Six adults of *Rhabdophis subminiatus* were collected in West Java: four from Bandung (specimens A, B, E, and F) and two from Bogor (specimens C and D). Specimens were euthanized with an intracardial injection of ketamine (350 mg/kg, 10%) and xylazine (14 mg/kg, 2%). Following confirmation of death, the ventral body wall was dissected from the cardiac region to the cloaca. Preservation differed by set: four specimens were fixed in 10% neutral buffered formalin (two weeks), and two in Bouin's solution (24 h), before transfer to 70% ethanol. Morphometric data taken included snout–vent length (SVL), tail length (TL), ventral scales (V), subcaudals (SC), supralabials (SL), infralabials (IL), and mid-body dorsal scale rows (SR).

Nuchal glands (NG) were examined by reflecting the dorsal neck skin, separating it from the musculature, and observing the glands under a stereo microscope (Olympus SZX12, Tokyo, Japan). Photographs were taken with a Canon EOS 700D, and gland dimensions were measured using ImageJ (Abramoff *et al.* 2004, Schneider *et al.* 2012). To determine gland orientation, digital radiographs were obtained for specimens B and D with a Poskom POX-100BT mobile X-ray unit (45 kVp, 3.60 mAs). A pin needle inserted at the caudal margin of the glands marked their posterior extent, and vertebral counts from the first cervical to the cloaca were recorded from lateral and dorsoventral views. The six examined *Rhabdophis subminiatus* measured 486–587 mm in total length (SVL+TL). Other morphometric and meristic data are summarized in Table 1. The dorsal scales were diamond-shaped, green on the head, and grading to yellow and red on the neck, while the ventrals were rectangular and white (Figs. 1A–C).

In four specimens where glands could be examined, the nuchal glands lay beneath the dorsomedial neck scales, beginning posterior to the parietals and extending caudally to the level of the 13th ventral scale, corresponding to the 13th vertebra (Figs. 1D–E, 2). The anterior pairs were rounded, reaching maximum size at the 4th–5th pairs, and becoming more oval and uniform posteriorly. Preserved specimens showed the glands as translucent and whitish, whereas freshly killed specimens displayed a yellowish hue (Figs. 2A–B). Counts revealed 16 pairs of glands in three specimens (A, B, and D) and 17 pairs in one (specimen F). Two individuals (specimens C and E) were used for histology, preventing gland counts, though external morphology confirmed the expected position beneath the first and second dorsal scale rows adjacent to the vertebral column. Gland diameters averaged 0.67 ± 0.09, 0.77 ± 0.14, and 0.72 ± 0.17 mm in the three measured specimens. Maximum diameters occurred at the 4th pair (0.89 ± 0.11 and 1.01 ± 0.06 mm) or the 5th pair (0.96 ± 0.18 mm). Mean gland areas were 0.7 ± 0.2, 1.1 ± 0.3, and 0.8 ± 0.4 mm².

Radiography of two specimens confirmed that the posterior limit of the glands coincided with the 13th vertebra and ventral scale (Figs. 2C–D). Vertebral counts from the first cervical to the cloaca were 142 and 138, exactly matching the number of ventrals in each specimen (Table 1).

Table 1. Morphometric data, nuchal gland counts, and scale counts of six specimens of the Red-necked keelback, Rhabdophis subminiatus, from West Java, Indonesia; F, female; M, male; NE, not examined.

Specimen No.	A	B	C	D	E	F
Sex	F	F	M	F	F	F
NG (pairs)	16	16	NE	16	NE	17
SVL (mm)	462	392	355	428	370	373
TL (mm)	125	121	131	106	118	115
V	NE	142	137	138	149	144
SR	19	19	19	19	19	19
SC (pairs)	62	69	71	62	64	75
SL (L/R)	8/8	8/8	8/8	8/8	8/8	8/8
IL (L/R)	10/10	10/10	10/10	10/10	10/10	10/10

This study provides the first detailed description of nuchal gland morphology in true *Rhabdophis subminiatus*. Although limited to a single species, the data contribute to a broader understanding of interspecific variation in gland number and structure within the genus. The 16–17 pairs of nuchal glands observed in *R. subminiatus* are comparable to *R. tigrinus* (13–16 pairs; Nakamura 1935) but exceed those of *R. adleri* (8–11), *R. nuchalis* (7–10), *R. pentasupralabialis* (10–11), and *R. guangdongensis* (9–10; Mori *et al.* 2016a,b, Zhu *et al.* 2020). These species also possess nucho-dorsal glands along the trunk, which were not considered here. In contrast, *R. helleri* and *R. himalayanus* possess substantially more glands (35 and 25–40, respectively; Smith 1938). Smith also noted that the southern form (*R. subminiatus*) had less developed glands than the northern *R. helleri*, now recognized as a distinct species (David & Vogel 2021).

Takeuchi *et al.* (2018) demonstrated that all species with nuchal glands fall within a single, well-supported clade, implying a unique evolutionary origin for this defensive system. The observed variation—ranging from sacculated nuchal glands to extensive nucho-dorsal or elongated nonsacculated forms—likely reflects alternative adaptations that evolved within this lineage following that singular origin. Based on gland number and distribution, *R. subminiatus* appears closer to *R. himalayanus* and *R. tigrinus* than to species with nucho-dorsal glands (*R. adleri*, *R. nuchalis*, *R. pentasupralabialis*, *R. guangdongensis*).

Our data also clarify the relationship between gland position and axial morphology. The posterior edge of the glands consistently aligned with the 13th ventral scale, which corresponded to the 13th vertebra. Vertebral counts matched ventral counts exactly, confirming a 1:1 relationship in *R. subminiatus*. While this ratio is widely documented in many snake families (Alexander & Gans 1966, Dowling 1951, Szyndlar & Georgalis 2023), it had not been demonstrated for *Rhabdophis*. Similar correlations have been noted in *Natrix tessellata* and other colubrids (Çömden *et al.* 2024), suggesting that this pattern is conserved in species with sacculated nuchal glands.

Finally, the bright yellow-to-red neck coloration, enhanced by neck flattening, likely functions as an aposematic signal directing predators' attention to the nuchal region. This display, in combination with neck arching, neck

Plate 28

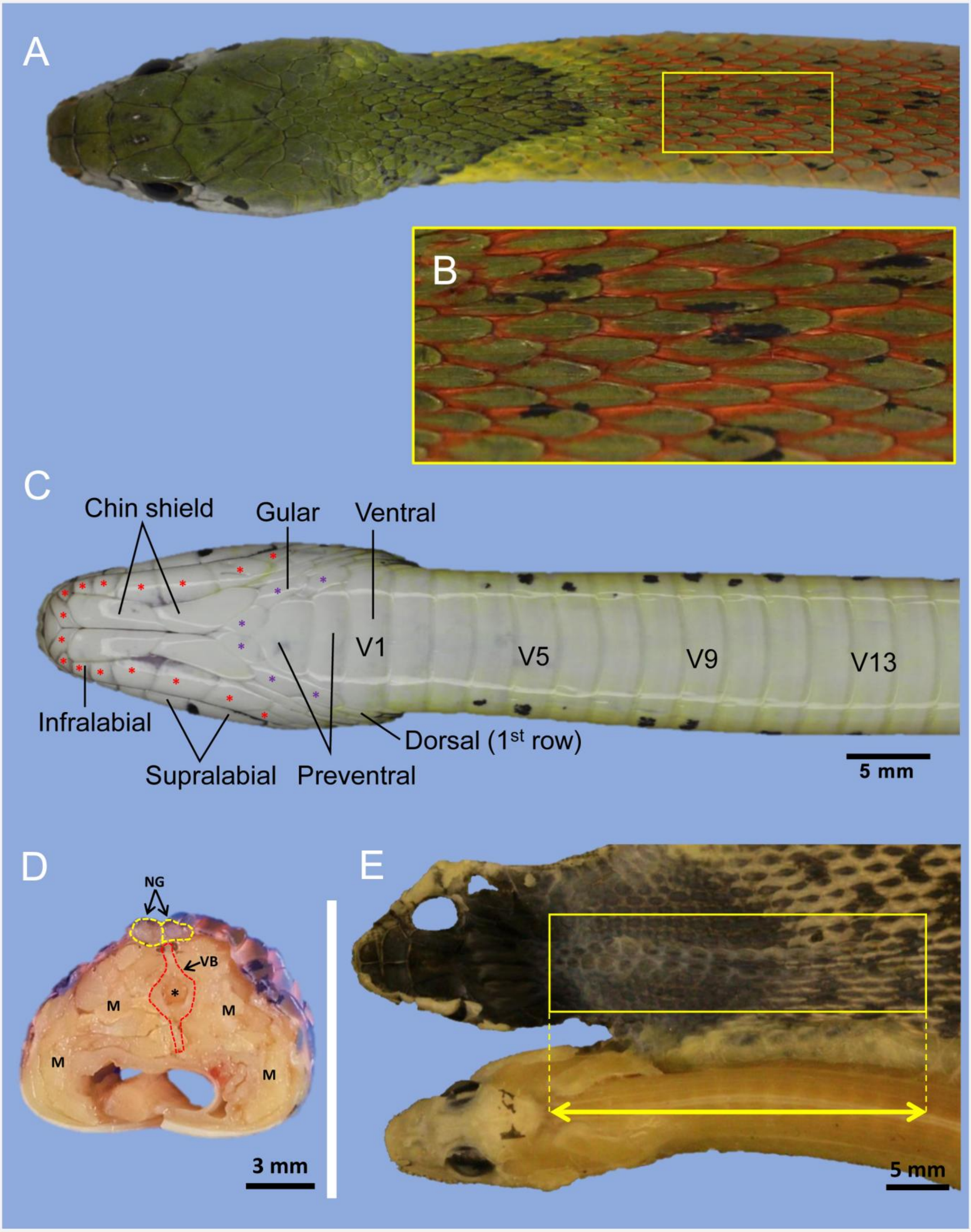

Figure 1. External morphology of *Rhabdophis subminiatus*: **(A)** dorsal head scales and colouration grading from olive green to yellow and red on the neck; **(B)** close-up of diamond-shaped dorsal scales; **(C)** ventral head scales and rectangular ventral scales; **(D)** transverse cervical section showing nuchal glands situated beneath the skin; and **(E)** dorsal dissection illustrating their position from the caudal margin of the parietals to approximately the 13th ventral scale (see close-up on the next plate); NG = nuchal gland, VB = vertebra, M = muscle, * = spinal cord

Plate 29

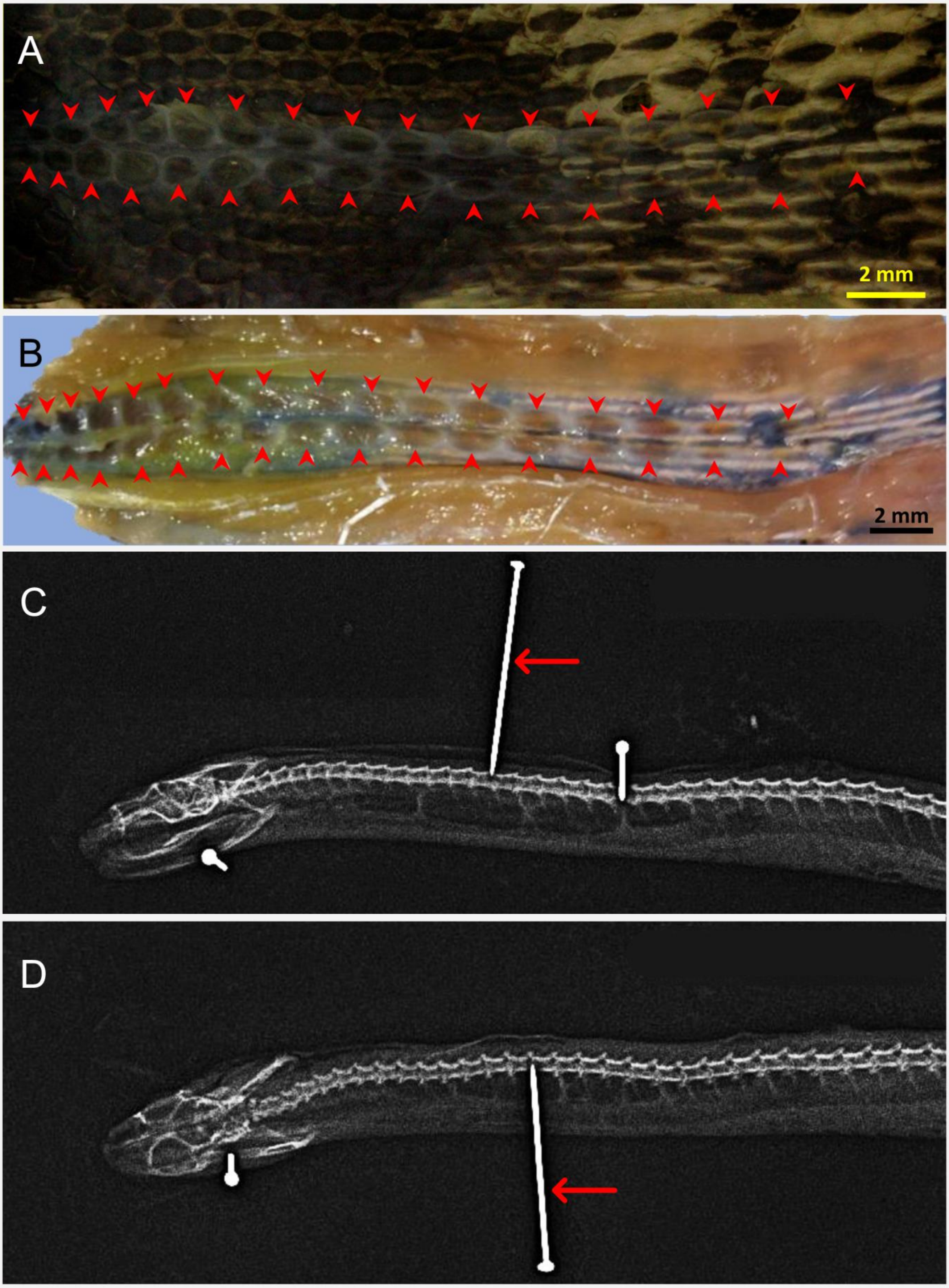

Figure 2. Nuchal gland morphology of *Rhabdophis subminiatus*: **(A)** preserved specimen showing whitish, cloudy glands; **(B)** freshly killed specimen showing yellowish glands; Radiographs of *R. subminiatus* (specimen D): **(C)** lateral and **(D)** dorsoventral views of the posterior extent of the nuchal glands (red arrows) at the level of the 13th vertebra; white pins indicate markers; red arrow heads indicate gland pairs

butting, and dorsal-facing postures (Mori & Burghardt 2001, 2008, Anita *et al.* 2022), integrates visual and chemical defenses into a coordinated antipredator strategy. Such coupling of morphological and behavioral adaptations underscores the complexity of the nuchal gland system. These findings emphasize the importance of morphological data for interpreting the evolution of defensive strategies in natricine snakes.

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