# The life aquatic: an association between habitat type and skin thickness in snakes

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An aquatic animal faces challenges not encountered by its terrestrial counterparts, promoting adaptive responses in multiple traits. For example, a thicker dermis might protect snakes when they are pushed against sharp objects by water currents, and might enable a snake to shed fouling organisms attached to its skin. We thus predicted that marine snakes should have thicker skin than terrestrial species, and that smaller sea snakes should have relatively thicker skin (because absolute, not relative, thickness determines vulnerability to fouling). Measurements of 192 snakes of 44 species supported those predictions. Many (but not all) sea snakes have skins 50% thicker than those of terrestrial and amphibious snake species, representing multiple independent evolutionary origins of thicker skin (in acrochordids, *Laticauda* sea kraits and both main clades of hydrophiine sea snakes). Marine snakes showed different allometries of skin thickness compared with their terrestrial counterparts; larger snakes had thicker skin within and among species of amphibious and terrestrial snakes, but larger aquatic snake species had thinner skin compared with smaller taxa. Interspecific variation in skin thickness was primarily due to increased collagen in the deep dermis, a physical barrier well suited to protecting against physical injury and to resisting penetration by epibionts.

ADDITIONAL KEYWORDS: adaptation – cutaneous – morphology – reptile – underwater.

## INTRODUCTION

The skin is the largest organ in the body, and plays a critical role in buffering internal conditions (of temperature, hydration, pH, salinity, etc.) against external fluctuations (Montagna, 2012). The skin's functions range from immune defence to sensory perception, and variation in skin structure among taxa plausibly reflects adaptive responses to selective forces imposed by ambient conditions (e.g. Daly *et al.*, 2008; Wilde *et al.*, 2014). The structure of skin varies considerably within squamate lineages. For example, sensory papillae and osteoderms occur in the scales of some squamate taxa but not others (Price, 1982; Crowe-Riddell *et al.*, 2016). Function of the epidermis in snakes

has attracted intensive research (e.g. Hazel *et al.*, 1999; Rivera *et al.*, 2005; Klein *et al.*, 2010; Klein & Gorb, 2012; Baum *et al.*, 2014), but the overall thickness of skin has been less well studied. Nonetheless, adaptive bases for interspecific differences in skin thickness may include reducing water loss in arid environments (Abo-Eleneen & Allam, 2011), resisting abrasion while burrowing (e.g. Klein & Gorb, 2012), and as armour against retaliation by prey (Han & Young, 2018).

We propose an additional influence on skin thickness: the challenges posed by aquatic life. First, aquatic snakes may be pushed against sharp objects (such as coral edges) by turbulent water. Second, sea snakes frequently are colonized by commensal (fouling) organisms such as algae, barnacles, hydrozoans, polychaetes, molluscs and bryozoans (e.g. Darwin, 1851, 1854; Zann *et al.*, 1975; Jeffries & Voris, 1979; Key

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*et al.*, 1995; Badrudeen, 2000; Alvarez & Celis, 2004; Ohba *et al.*, 2005; Pfaller *et al.*, 2012). These epibionts can impair hydrodynamics and reduce swimming speeds (Shine *et al.*, 2010). Sloughing (shedding) the outer layers of the skin can eliminate most fouling organisms (Fig. 1), perhaps explaining why sea snakes slough their skins more frequently than terrestrial snakes (Kropach & Soule, 1973; Heatwole, 1999; Lillywhite & Menon, 2019). However, sloughing does not always eliminate fouling organisms that penetrate deeply into the skin (Foster, 1987; Heatwole, 1999). Epibionts cling to substrates using chemical adhesives (e.g. pedal glue in gastropods, byssus in bivalves) and etching of the substrate to provide traction (Fletcher & Callow, 1992; Bromley & Heinberg, 2006). Fouling algae can produce extensive rhizoid systems that penetrate deep into the substrate, causing physical breakdown of the surfaces to which they attach (Moss & Woodhead, 1970). A thicker skin might enable a sea snake to divest itself of fouling organisms that would otherwise impede its movements.

Previous studies on the skin of sea snakes have focused on aspects such as micro-ornamentation (Price, 1982), sensory papillae (Povel & Van Der Kooij, 1996; Westhoff *et al.*, 2005; Crowe-Riddell *et al.*, 2016), rugosity (Avolio *et al.*, 2006a, b), water retention (Lillywhite & Menon, 2019), and rates of gas and water exchange (Dunson & Robinson, 1976; Lillywhite & Sanmartino, 1993; Lillywhite *et al.*, 2009), and have looked at a small number of taxa (but see Price, 1982; Han & Young, 2018). To test the prediction that aquatic snakes have relatively thick skins, as an adaptation against physical damage and to expel fouling organisms during sloughing, we describe skin morphology in a range of aquatic, amphibious and terrestrial snakes.



**Figure 1.** A turtle-headed sea snake (*Emydocephalus annulatus*) showing algal fouling removed with sloughing. Photograph by Claire Goiran.

## MATERIAL AND METHODS

### STUDY SPECIES

Most snakes are terrestrial, but aquatic habits have evolved in several lineages (Heatwole, 1999). Exposure to fouling organisms is most relevant to two major clades (the *Aipysurus* and *Hydrophis* lineages) within the fully marine Hydrophiinae (Sanders *et al.*, 2013; Lee *et al.*, 2016). These two clades have adapted to marine habitats along different trajectories (Sanders *et al.*, 2012). We sampled species from both groups. We also sampled one marine and one primarily freshwater species of fully aquatic filesnakes (*Acrochordus*, Acrochordidae), and an insular taxon of sea krait (*Laticauda*) that we also scored as 'aquatic'. All sea kraits are amphibious to some degree, foraging at sea but returning to land to slough, digest, mate and lay eggs (e.g. *L. colubrina*: Shetty & Shine, 2002) although *L. schistorhynchus* rarely leaves the water (Guinea, 1994). In keeping with that characterization, a close relative of *L. schistorhynchus*, the primarily aquatic *L. semifasciata*, supports more epibiont species compared with more terrestrial sea kraits (Pfaller *et al.*, 2012). Another subset of species in our sample were categorized as 'amphibious' because they show varying degrees of association with aquatic habitats. *Ephalophis*, *Parahydrophis* and *Hydrelaps* (all within the Hydrophiinae) often crawl on exposed mudflats as well as swim (Storr *et al.*, 1986; Sweet, 1989; Nagelkerken *et al.*, 2008), as do the homalopsids *Cerberus*, *Enhydris*, *Fordonia* and *Myron* (Murphy, 2007). All other taxa within our sample were classed as terrestrial, although some frequently enter the water (e.g. *Tropidonophis mairii*). Phylogenetically, our sample includes terrestrial species from the families Colubridae ($N = 2$), Elapidae ($N = 8$) and Pythonidae ($N = 1$), semi-aquatic members of the Homalopsidae ($N = 4$), Hydrophiinae ($N = 3$) and *Laticauda* sea kraits ($N = 1$), and aquatic members of the Acrochordidae ($N = 2$), *Laticauda* sea kraits ($N = 1$) and Hydrophiinae ($N = 22$).

### METHODS FOR MEASUREMENTS

We examined snakes (formalin-fixed, alcohol-preserved) in the collection of the Northern Territory Museum ($N = 142$), supplemented by specimens from our private collections (G.P.B. and R.S., $N = 50$). On each animal, we recorded snout–vent length (SVL) as a measure of overall body size, and scored thickness of the skin three times, at each of three sites evenly spaced along the body (at 25, 50 and 75% of snake SVL). We measured scalar, not interscalar, sites on the skin. To do so, we made a small incision midway between the ventral shields and the uppermost part of the body (i.e. mid-lateral) and inserted the tip of a micrometer dial thickness gauge (Peacock G-1A, Ozaki Manufacturing;

accurate to 0.01 mm) beneath the resulting flap of skin. We then measured thickness under three adjacent scales by moving the tip of the micrometer around beneath the flap of skin. This procedure generated nine measures of skin thickness per specimen.

Where possible (14 species), we obtained measurements from five males and five females per species (sexed by tail shape and, if necessary, dissection) and encompassing a range of body sizes. We also obtained data on an additional 30 species represented by fewer specimens (one to three specimens per species), to broaden our sampling. Pilot analyses showed no effect of including vs. excluding these additional animals on our conclusions, so for simplicity we only report analyses of the total data set, except for intraspecific analyses comparing body length to skin thickness (conducted only on species for which we had ten specimens).

## METHODS FOR HISTOLOGICAL ANALYSIS

We selected five species of aquatic snakes with relatively thick skin (*Acrochordus arafurae*, *Aipysurus laevis*, *Emydocephalus annulatus*, *Hydrophis curtus*), four aquatic snakes with thin skin (*Hydrophis kingii*, *Hydrophis peronii*, *Hydrophis elegans*, *Hydrophis pacificus*), one amphibious species (*Laticauda colubrina*), and three terrestrial species (*Antaresia childreni*, *Demansia vestigiata*, *Tropidonophis mairii*). Each skin sample was taken from the small flap at the lateral mid-body made for thickness measurements (above). Each sample was approximately 1 cm long by 0.5 cm wide and incorporated the length of at least two scales. Samples were placed in 10% neutral buffered formalin, trimmed along the long (anterior–posterior) axis, processed in standard fashion for histology, embedded in paraffin wax on the thin lateral edge, sectioned at 5 μm, and stained with haematoxylin and eosin. Thicknesses of the epidermis, superficial dermis and deep dermis were measured at the mid-scale region (mid-way between two interscalar hinges) of two adjacent scales using a calibrated digital photomicroscope camera and associated software (Olympus DP 70 with Cellsense software). The superficial dermis was defined as the area of dermis containing relatively loose, fine, collagen bundles, and the deep dermis the region with thicker, more densely packed collagen bundles, ending at the hypodermis (Dubansky & Close, 2018; Han & Young, 2018; see Fig. 2 inset). In *Acrochordus arafurae*, the skin was covered in variably sized raised bumps separated by relatively thin skin. Measurements for this species were taken from the mid-regions of two medium-sized bumps (Fig. 3D).

## METHODS FOR STATISTICAL ANALYSIS

To quantify intraspecific allometry, we correlated an individual's skin thickness with its SVL within each species (for the subsample of species for which we had

ten specimens). To explore interspecific variation, we calculated mean SVLs, and skin thickness at 25, 50 and 75% of SVL, for each species, and used these means as the units in our analyses. Preliminary tests revealed no significant differences between conspecific males and females (except that $P = 0.02$ for *Cerberus australis* and *Hydrophis curtus*, but the differences disappeared when SVL was included as a covariate) so sex was not included as a factor in subsequent analyses. We then conducted analysis of covariance (ANCOVA) with the response variable being skin thickness (in micrometres) and the predictor variables being habitat type (terrestrial–amphibious–aquatic), location along the body (anterior–middle–posterior) and their interaction.

To compare interspecific allometries of skin thickness among snakes from different habitats, we treated data on skin thickness measurements at each location along the body as replicates of the dependent variable, and conducted ANCOVA with habitat type and mean SVL as factors, plus their interaction. To examine the impacts of habitat and location on the body, we used analysis of variance (ANOVA) with these predictor variables (and their interaction), and with species included as a random variable (because we had three measurements for each species). The above analyses were conducted in JMP 13.0 (SAS Institute).

To explore phylogenetic changes in skin thickness, we used the phylogenetic tree of Zheng & Wiens (2016), which we pruned to include only species in our database. We ran a phylogenetic generalized least square analysis (pgls) implemented in the R package 'caper' (Orme *et al.*, 2014). For this we used the maximum likelihood value of the λ parameter to scale the variance–covariance phylogenetic distances matrix and compute contrasts. The phylogenetic model included skin thickness as the response variable and habitat (aquatic, amphibious and terrestrial) and SVL as predictors. Skin thickness and SVL were $\log_{10}$ transformed prior to analyses.

For analysis of histological data, we calculated species mean values for the 12 taxa for which we had samples (total of 21 specimens; see above). Most taxa were represented by two specimens, but only single specimens were available for *Hydrophis elegans*, *H. kingii*, *H. pacificus*, *H. peronii* and *Laticauda colubrina*. However this leaves $7 \times 2 + 5 = 19$, not 21 specimens

## RESULTS

### EFFECT OF LOCATION ON THE BODY ON SKIN THICKNESS

The scales of terrestrial snakes were thinner towards the rear of the body, whereas this pattern was less pronounced in amphibious and aquatic snakes (Fig. 4). ANOVA showed a significant interaction between habitat and location
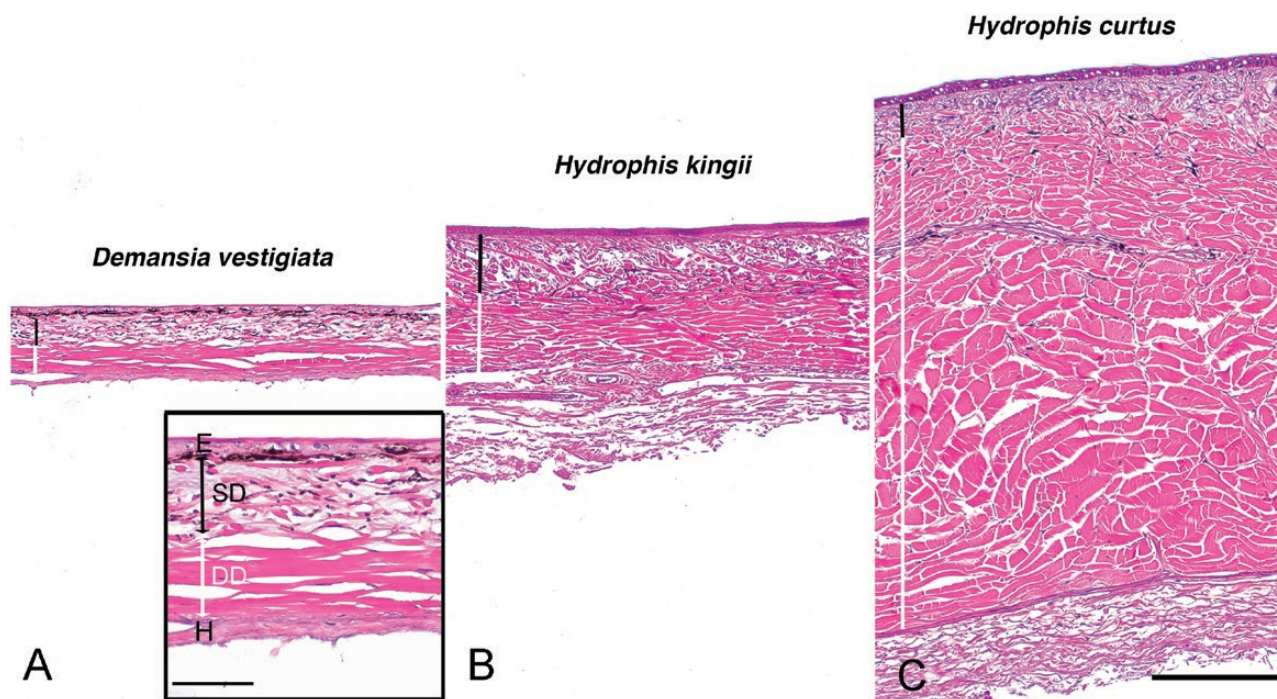
**Figure 2.** Morphology of the skin of (A) a terrestrial elapid snake (*Demansia vestigiata*), and two species of sea snakes, (B) *Hydrophis kingii*, and (C) *H. curtus*, demonstrating variation in skin thicknesses and relative thickness of different layers. The photomicrographs show transverse histology sections with the epidermis upmost. The inset in panel A shows the location of the epidermis (E), superficial dermis (SD), deep dermis (DD) and hypodermis (H). The vertical bars in each panel show the margins of the superficial dermis (black bar) and deep dermis (white bar). The horizontal scale bar in panel C represents a length of 200 µm and applies to all images except the inset in panel A, in which the scale bar represents a length of 50 µm. Haematoxylin and eosin stain.



**Figure 3.** Morphology of the skin of four aquatic snakes: (A) *Aipysurus laevis*; (B) *Emydocephalus annulatus*; (C) *Laticauda colubrina*; and (D) *Acrochordus arafurae*, demonstrating variation in skin thicknesses and relative thickness of different layers. The photomicrographs show transverse histology sections with the epidermis upmost. The vertical bars in each panel show the superficial dermis (black bar) and deep dermis (white bar). The horizontal scale bar in panel (D) represents a length of 200 µm and applies to all images. Haematoxylin and eosin stain.

**Figure 4.** Overall thickness of skin on the lateral bodies of snakes belonging to three habitat-use categories (aquatic, terrestrial, amphibious) at 25, 50 and 75% of body length (anterior, middle, posterior). The figure shows mean values and associated standard errors, calculated from species means.

on the body ($F_{4,82} = 2.96$, $P < 0.03$). Mean thickness of the skin was 556–580 μm in aquatic snakes at all three body locations, whereas amphibious species averaged 374–397 μm (Fig. 4). The skin of terrestrial snakes was thicker than that of amphibious species in the forepart and middle of the body (447–457 μm), but was similar to that of amphibious species in the rear part of the body (361 μm; Fig. 4).
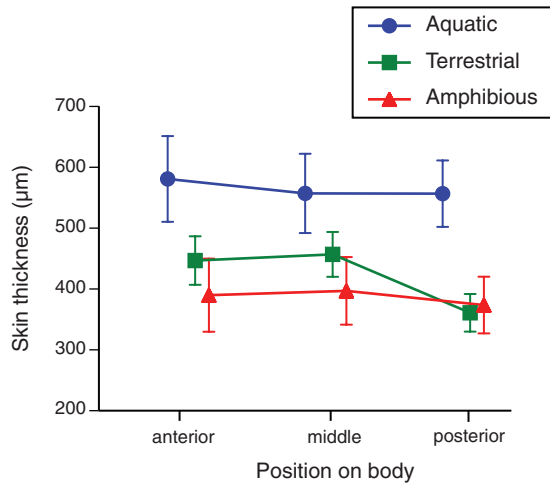
### EFFECT OF HABITAT ON SKIN THICKNESS

Despite the above-mentioned significant interaction term, the effect of habitat type ($F_{2,41} = 4.53$, $P < 0.02$) meant that aquatic snakes had thicker skin than terrestrial and amphibious snakes at all sites along the body (Fig. 4). Mean skin thickness averaged 564.8 μm in aquatic species, 421.5 μm in terrestrial species and 386.8 μm in amphibious species (Fig. 4). Despite this divergence in mean values, some aquatic snakes had skin as thin as that of most terrestrial and amphibious species (Table 1). Mean skin thickness per species ranged from 260 to 586 μm in terrestrial snakes, from 286 to 554 μm in amphibious snakes, and from 274 to 982 μm in aquatic snakes (Table 1). Considerable variation was evident even between species within genera, e.g. 456–867 μm in *Aipysurus*, 591–982 μm in *Acrochordus* and 274–866 μm in *Hydrophis* (Table 1).

### EFFECTS OF HABITAT AND BODY LENGTH ON SKIN THICKNESS

Within species for which we had data on ten specimens each, intraspecific correlations between SVL and skin thickness were positive and significant in five of six terrestrial species, one of two amphibious taxa, but just one of six aquatic species. An ANOVA on the strength of these correlations (combining amphibious with terrestrial to attain adequate sample sizes) showed a non-significantly higher mean $r^2$ value in non-aquatic taxa (mean $r^2 = 0.47$, range 0.02–0.71) than in aquatic taxa (mean $r^2 = 0.26$, range 0.07–0.47; $F_{1,12} = 3.35$, $P = 0.09$); that result becomes statistically significant ($F_{1,11} = 8.29$, $P < 0.02$) if we omit one terrestrial outlier (*Notechis scutatus*, $r^2 = 0.02$). Unlike all of the other taxa, our sample of this species comprised long-term captives collected from a very wide geographical area.

Interspecifically, an ANCOVA revealed that the effect of SVL on mean skin thickness differed among snakes from different habitat types (interaction, habitat × SVL, $F_{2,38} = 4.89$, $P < 0.015$; see Fig. 5). Mean skin thickness increased with mean SVL in both terrestrial and amphibious snakes (terrestrial $N = 11$, slope = 2.95, $r^2 = 0.49$, $P < 0.02$; amphibious $N = 8$, slope = 7.66, $r^2 = 0.83$, $P < 0.002$), but tended to decrease with increasing SVL in aquatic snakes ($N = 25$, slope = −3.15, $r^2 = 0.13$, $P = 0.08$; see Fig. 5). Over the same SVL range, larger species of terrestrial snakes had thicker skin than larger species of aquatic snakes (Fig. 5). Unfortunately, low overlap in mean SVLs between amphibious species (generally small; mean 495 mm SVL) and the other categories (generally large; 912 and 853 mm SVL for marine and terrestrial taxa, respectively) makes it difficult to compare them (Fig. 5). Over the small range where SVLs overlap, aquatic and amphibious snakes had similarly thick skins (two longest amphibious snakes 540 ± 20 μm; five shortest aquatic snake species 514 ± 138 μm). Nonetheless, within the entire data set (44 species), the 11 species with the thickest skin (>590 μm) are all marine.

### PHYLOGENETIC CHANGES IN SKIN THICKNESS

Phylogenetic trees (e.g. Zheng & Wiens, 2016; Sherratt *et al.*, 2018) indicate multiple transitions in skin thickness. Our taxonomic sampling is insufficient to detect all changes, but we can identify at least five transitions between thin and thick skins (arbitrarily, >200 μm divergence between sister-groups), as follows:

1. amphibious hydrophiines (*Ephalophis*, *Hydrelaps*, *Parahydrophis* 286–311 μm) vs. the *Aipysurus* clade (*Aipysurus–Emydocephalus* 455–867 μm);
2. *Aipysurus fuscus* (455) vs. *Aipysurus laevis* (867 μm);
3. *Aipysurus duboisii* (458) vs. *Aipysurus foliosquama* (723 μm);
4. *Hydrophis elegans* (369) vs. *H. curtus* (866 μm); and
5. *Acrochordus arafurae* (982) vs. *Acrochordus granulatus* (591 μm).

**Table 1.** Thickness of lateral skin in preserved snakes. SVL = snout–vent length

| Habitat | Family | Species | Mean SVL (cm) | Habitat | Mean skin thickness (µm) | SE for skin thickness |
|---|---|---|---|---|---|---|
| Amphibious | Homalopsidae | *Cerberus australis* | 55.1 | Mangroves | 409.6 | 35.6 |
| Amphibious | Homalopsidae | *Enhydris polylepis* | 63.1 | Freshwater | 554.2 | 35.6 |
| Amphibious | Homalopsidae | *Fordonia leucobalia* | 43.0 | Mangroves | 393.3 | 79.6 |
| Amphibious | Elapidae | *Ephalophis greyae* | 50.0 | Mangroves | 311.1 | 133.0 |
| Amphibious | Elapidae | *Hydrelaps darwiniensis* | 42.0 | Mangroves | 305.2 | 65.0 |
| Amphibious | Elapidae | *Laticauda colubrina* | 69.0 | Coral reefs | 525.6 | 112.6 |
| Amphibious | Homalopsidae | *Myron richardsoni* | 30.9 | Mangroves | 309.4 | 79.6 |
| Amphibious | Elapidae | *Parahydrophis mertoni* | 43.0 | Mangroves | 285.6 | 79.6 |
| Aquatic | Acrochordidae | *Acrochordus arafurae* | 106.4 | Freshwater | 982.1 | 42.0 |
| Aquatic | Acrochordidae | *Acrochordus granulatus* | 63.5 | Mangroves | 590.6 | 94.0 |
| Aquatic | Elapidae | *Aipysurus apraefrontalis* | 75.5 | Coral reefs | 690.6 | 94.0 |
| Aquatic | Elapidae | *Aipysurus duboisii* | 90.8 | Coral reefs | 458.9 | 94.0 |
| Aquatic | Elapidae | *Aipysurus eydouxii* | 73.7 | Coral reefs | 867.0 | 42.0 |
| Aquatic | Elapidae | *Aipysurus foliosquama* | 76.0 | Coral reefs | 723.3 | 94.0 |
| Aquatic | Elapidae | *Aipysurus fuscus* | 62.5 | Coral reefs | 455.6 | 94.0 |
| Aquatic | Elapidae | *Aipysurus laevis* | 97.0 | Coral reefs | 856.1 | 42.0 |
| Aquatic | Elapidae | *Emydocephalus annulatus* | 72.2 | Coral reefs | 769.2 | 42.0 |
| Aquatic | Elapidae | *Hydrophis atriceps* | 115.0 | Coral reefs | 347.8 | 133.0 |
| Aquatic | Elapidae | *Hydrophis curtus* | 93.5 | Coral reefs | 866.2 | 42.0 |
| Aquatic | Elapidae | *Hydrophis cyanocinctus* | 143.0 | Coral reefs | 437.8 | 133.0 |
| Aquatic | Elapidae | *Hydrophis czeblukovi* | 107.0 | Coral reefs | 425.6 | 133.0 |
| Aquatic | Elapidae | *Hydrophis elegans* | 119.0 | Coral reefs | 369.4 | 94.0 |
| Aquatic | Elapidae | *Hydrophis harduicki* | 78.3 | Coral reefs | 823.3 | 94.0 |
| Aquatic | Elapidae | *Hydrophis kingii* | 124.0 | Coral reefs | 346.7 | 94.0 |
| Aquatic | Elapidae | *Hydrophis macdowelli* | 69.0 | Coral reefs | 350.6 | 94.0 |
| Aquatic | Elapidae | *Hydrophis major* | 101.4 | Coral reefs | 478.8 | 42.0 |
| Aquatic | Elapidae | *Hydrophis ornatus (=ocellatus)* | 93.3 | Coral reefs | 363.3 | 94.0 |
| Aquatic | Elapidae | *Hydrophis pacificus* | 136.0 | Coral reefs | 274.4 | 133.0 |
| Aquatic | Elapidae | *Hydrophis peronii* | 87.2 | Coral reefs | 368.5 | 76.8 |
| Aquatic | Elapidae | *Hydrophis platurus* | 57.0 | Open ocean | 466.7 | 94.0 |
| Aquatic | Elapidae | *Hydrophis shistosa* | 85.8 | Coral reefs | 508.9 | 94.0 |
| Aquatic | Elapidae | *Hydrophis stokesii* | 92.0 | Coral reefs | 591.1 | 94.0 |
| Aquatic | Elapidae | *Laticauda schistorhyncus* | 61.7 | Coral reefs | 708.5 | 65.0 |
| Terrestrial | Elapidae | *Acanthophis praelongus* | 54.3 | Savanna woodland | 377.4 | 20.6 |
| Terrestrial | Pythonidae | *Antaresia childreni* | 71.3 | Savanna woodland | 379.7 | 20.6 |
| Terrestrial | Elapidae | *Austrelaps superbus* | 71.0 | Cool-temperate | 461.1 | 65.2 |
| Terrestrial | Elapidae | *Demansia vestigiata* | 90.3 | Savanna woodland | 260.7 | 20.6 |
| Terrestrial | Elapidae | *Notechis scutatus* | 87.1 | Cool-temperate | 457.6 | 20.6 |
| Terrestrial | Elapidae | *Oxyuranus scutellatus* | 141.0 | Savanna woodland | 585.6 | 65.2 |
| Terrestrial | Elapidae | *Pseudechis australis* | 115.0 | Savanna woodland | 572.2 | 65.2 |
| Terrestrial | Elapidae | *Pseudonaja nuchalis* | 109.0 | Savanna woodland | 542.2 | 65.2 |
| Terrestrial | Colubridae | *Stegonotus australis* | 83.4 | | 318.4 | 20.6 |
| Terrestrial | Elapidae | *Tropidechis carinatus* | 60.0 | | 426.7 | 65.2 |
| Terrestrial | Colubridae | *Tropidonophis mairii* | 55.7 | | 260.2 | 20.6 |

Our criterion (>200 μm divergence) was also satisfied for at least one sister-group comparison among terrestrial elapids (*Demansia vestigiata* 261 μm vs. *Oxyuranus scutellatus* 586 μm).

A quantitative phylogenetically based analysis of our data [of the 37 species present in the tree of Zheng & Wiens (2016)] reveals a medium-to-strong phylogenetic signal in the data ($\lambda = 0.47$). The intercept

(i.e. skin thickness when log SVL = 0, or SVL = 1 mm) was significantly higher for aquatic species than for amphibious ones. Mean SVL, however, was marginally positively associated with thicker skin in amphibious snakes (slope $0.84 \pm 0.43$ SE; $P < 0.06$), but with a decrease in skin thickness in aquatic species (slope = $-0.26$; so for the log SVL × habitat interaction, $P < 0.036$). The model explained 32% of the variation in skin thickness.
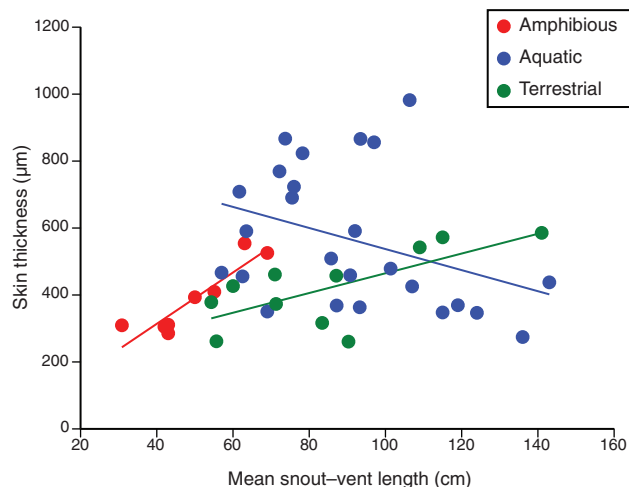


**Figure 5.** Interspecific allometry of overall mean skin thickness (one value per species, based on $N = 1$–10 specimens) relative to mean snout–vent lengths of the animals measured. The different colours show data for amphibious species, aquatic species and terrestrial species, and regression lines fitted to these data. See Table 1 for the list of species in each category.

### MICROSCOPIC STRUCTURE OF SKIN

The epidermis was thin in all snakes, typically composed of a prominent basal layer of epidermal cuboidal cells followed by one to three less distinct, thin layers of flattened cells beneath a thin surface stratum corneum (see all photomicrograph figures, high-magnification detail in Fig. 2A inset). The margin delineating the superficial and deep dermis was abrupt in some species (e.g. *D. vestigiata*, *H. kingii* and *H. curtus*), in which the superficial dermis contained small collagen bundles widely separated by ground substance (evident histologically as clear space to pale eosinophilic or basophilic amorphous material; Fig. 2). Species with intermediate distinction between superficial and deep dermis included *Aipysurus laevis* (Fig. 3A) and *L. colubrina* (Fig. 3C). The two terrestrial species, *T. mairii* and *Antaresia childreni*, exhibited distinct margins between superficial and deep dermis (Fig. 6). Species with a gradual and indistinct transition between the size of collagen bundles of the superficial and deep dermis included *E. annulatus* (Fig. 3B), *H. elegans* (Fig. 7B) and *H. pacificus* (Fig.



**Figure 6.** Morphology of the skin of two terrestrial snakes: (A) the colubrid *Tropidonophis mairii*; and (B) the python *Antaresia childreni*. The photomicrographs show transverse histology sections with the epidermis upmost. The vertical bars in each panel show the superficial dermis (black bar) and deep dermis (white bar). The horizontal scale bar in panel (B) represents a length of 200 μm and applies to all images. Haematoxylin and eosin stain.
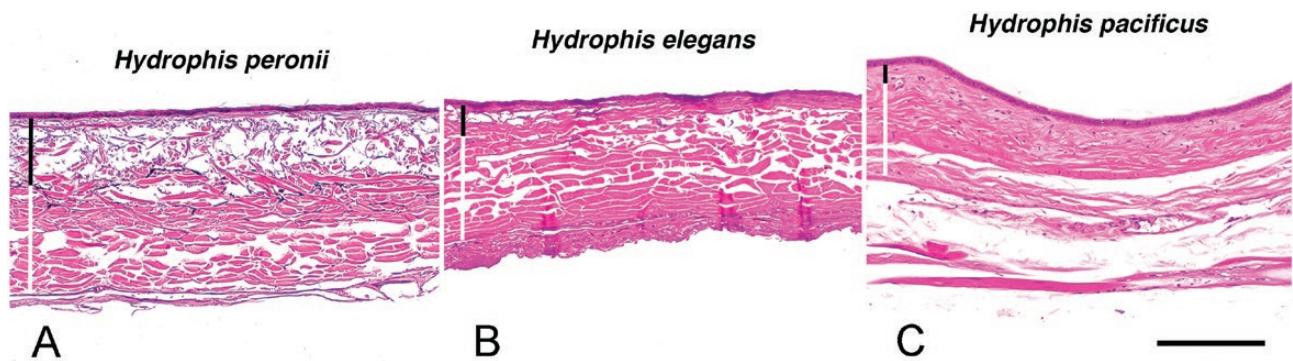
**Figure 7.** Morphology of the skin of three species of sea snakes: (A) *Hydrophis peronii*; (B) *Hydrophis elegans*; and (C) *Hydrophis pacificus*. The photomicrographs show transverse sections with the epidermis upmost. The vertical bars in each panel show the superficial dermis (black bar) and deep dermis (white bar). The horizontal scale bar in panel (C) represents a length of 200 μm and applies to all images. Haematoxylin and eosin stain.

7C). In all species, the distinction between the deep dermis and loose hypodermal connective tissue was obvious, frequently delineated by a thin continuous line of collagen (e.g. see Figs 3A, C, 7A, C). While melanocytes were evident in the basal epidermis in most species, the predominant location of pigment was the dermis. In some species, for example *D. vestigiata* (Fig. 2A) and *Antaresia childreni* (Fig. 6B), pigment was primarily present in melanophores in the superficial dermis immediately beneath the epidermal basement membrane. This situation contrasts with other species such as *H. kingii* (Fig. 2B) and *H. peronii* (Fig. 7A) in which melanophores were densest at the margin of the superficial and deep dermis, and others such as *H. curtus* (Fig. 2C) and *L. colubrina* (Fig. 3C) in which melanophores continued into the upper third of the deep dermis.

Histological data on 12 species of snakes (21 specimens total) showed that the deep dermis was by far the thickest layer, comprising an average of 74% of the total thickness of the skin (range 44–95%). Hence, variation in the thickness of the deep dermis explained most of the variation in total skin thickness ($N = 12$, $r^2 = 0.98$, $P < 0.0001$; see Fig. 8C; for epidermis vs. total skin thickness $N = 12$, $r^2 = 0.03$, $P = 0.60$; for superficial dermis vs. total skin thickness $N = 12$, $r^2 = 0.01$, $P = 0.91$). Our measurements of skin thickness from histology were positively correlated with those from the micrometer method [$N = 12$ species, $r^2 = 0.53$, $P < 0.008$; without one outlier (*Acrochordus arafurae*), the fit improves to $r^2 = 0.85$, $P < 0.0001$].

## DISCUSSION

Correlations between environmental factors and organismal traits are found widely (e.g. Lynch, 2018), but most studies on aquatic snakes have focused on physiological issues such as adaptations to diving (e.g. Heatwole, 1999). Our data suggest that the evolutionary transition from terrestrial to aquatic habits in snakes has been accompanied – in some but not all species – by an increase in thickness of the lateral skin. That thickening is due primarily to an increase in the deep dermis layer, which is far thicker than other layers [Figs 2, 3; consistent with Han & Young's (2018) report that in 14 species of terrestrial snakes, the epidermis averaged 17.4 μm, the superficial dermis 45.3 μm and the deep dermis 253.4 μm].

We suggest that, because the earliest snakes were burrowers (Brandley *et al.*, 2008), they may have had thick skin as an adaptation to resist abrasion underground (Klein & Gorb, 2012). However, most modern snakes are surface-active, not fossorial. They have relatively thin skin: about 400 μm thick based on our data and those of Han & Young (2018). Skin thickness varies with body size (and with location on the body, in terrestrial snakes), and among even closely related species from similar habitats (Table 1). Some of that variation may reflect ecological factors: for example, the Calabar burrowing python (*Calabaria reinhardtii*) may have evolved very thick skin (>2000 μm) in response to the threat posed by retaliating rodent prey (Han & Young, 2018).

Our study reveals broader habitat-associated divergences in skin thickness. Although some aquatic snakes retain the ancestral condition of thin skin, species in at least four lineages of snakes that have evolved aquatic habits (acrochordids, *Laticauda* sea kraits, and the *Aipysurus* and *Hydrophis* clades within the hydrophiine sea snakes) evolved skins that are about 50% thicker than that seen in most terrestrial or amphibious snakes (Fig. 4; Table 1). What factors associated with aquatic life might impose selection for thicker skin? The morphological changes seen in our sample falsify several possibilities. For example,
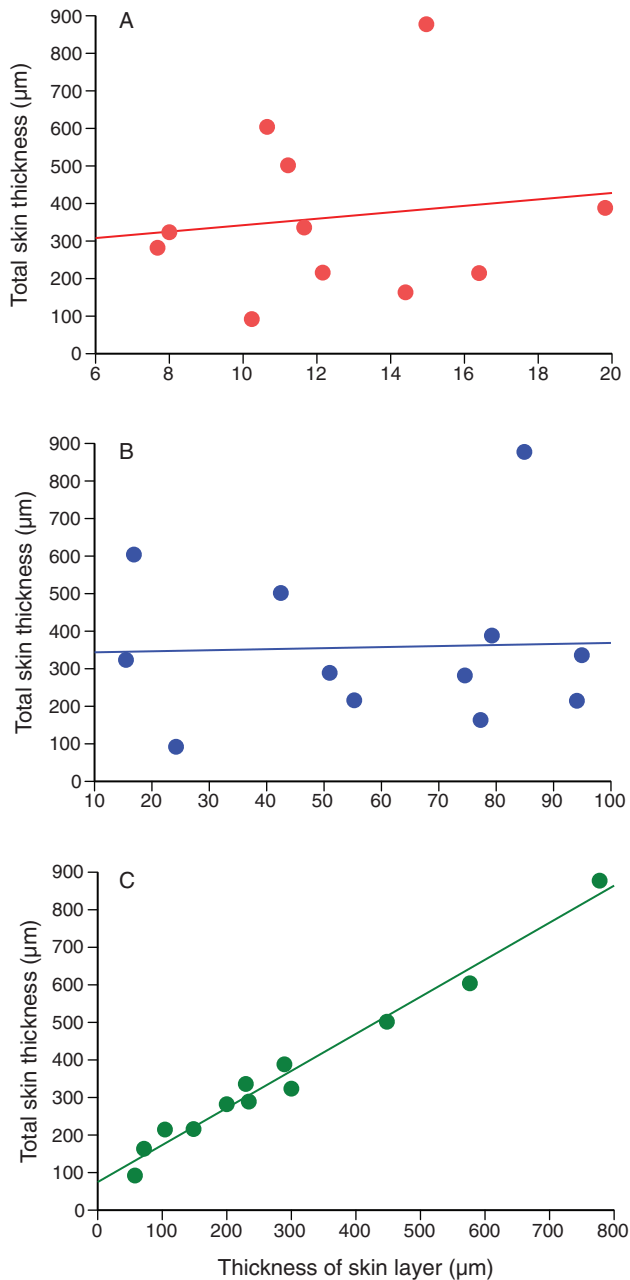
**Figure 8.** Interspecific relationships between the thickness of individual layers within the skin ([A] epidermis, [B] superficial dermis, [C] deep dermis) relative to overall thickness of the skin (i.e. all three layers combined). Each point represents mean value for a single species.

b). Such factors, however, should largely affect the superficial layer of the skin (the epidermis) or the superficial dermis (which contains blood vessels, nerve endings, etc.). Inconsistent with that prediction, the greater thickness of the skin in aquatic snakes is due to elaboration of collagenous material within the deep dermis rather than to modifications of more superficial layers (Fig. 8C). The primary function of the deep dermis is to provide a physical barrier against injury (e.g. Han & Young, 2018).

We suggest that two aspects of aquatic life might impose selection for thicker skin as a defence against injury. Although sea snakes are sometimes consumed by predators (e.g. Heatwole, 1999) and are subject to retaliatory attacks by prey (Goiran & Shine, 2015), we doubt that sea snakes are more vulnerable overall than terrestrial snakes in this respect. Instead, we suggest that aquatic snakes (1) are prone to injuries from water currents (waves, floods, etc.) pushing them against hard objects (such as coral), and (2) are vulnerable to fouling organisms whose attachment systems can penetrate deep within the skin (e.g. Zann, 1975).

An aquatic snake may be pushed against hard objects during periods of strong wave action (especially, cyclonic conditions) or in powerful currents (especially, floods) as it forages or as it surfaces to breathe. Terrestrial snakes would rarely be subject to such an injury. In keeping with that hypothesis, we have recorded frequent wounds on turtle-headed sea snakes (*Emydocephalus annulatus*) after periods of unusually rough seas (R. Shine & C. Goiran, unpubl. data, 2018). Similarly, the relatively thick skins of some sea kraits (*Laticauda* spp.) might protect them as they are thrown against the shore by waves while entering and leaving the water, when they are sometimes smashed against coral repeatedly (R. Shine, unpubl. observation, 2016). In contrast, amphibious snakes that inhabit mudflats (such as *Ephalophis*, *Hydrelaps* and *Parahydrophis*) would be less at risk.

A second factor is the possibility that an enhanced ability to dislodge epibionts (fouling organisms) may be advantageous to aquatic snakes. Algal fouling can reduce swimming speeds of sea snakes by about 20% (Shine *et al.*, 2010). Sloughing rids the snake of most epibionts (Zann *et al.*, 1975; Heatwole, 1999; Shine *et al.*, 2010) but not all of them, because attachment systems can penetrate deeply below the epidermis (Zann, 1975; Heatwole, 1999). A thicker denser skin thus should make it more likely that epibionts cannot penetrate deeply enough to retain their hold on the snake after it sloughs. The primary thickening is in the deep dermis, which is not sloughed; however, reinforcement at that level may weaken epibiont attachments that penetrate through the epidermis. If resistance to epibiont retention depends upon absolute thickness, smaller

an aquatic snake might experience novel pressures associated with hydrodynamics and gas exchange (e.g. Heatwole, 1999; Lillywhite & Menon, 2019), sensory reception (Crowe-Riddell *et al.*, 2016) and shifts in sexually selected traits such as a male's ability to cling to a female during courtship (Avolio *et al.*, 2016a,

species of snakes (and smaller individuals within a species) may benefit from relatively thick skins. Locomotor performance of smaller snakes also may be more adversely affected by epibionts, because a given fouling organism is larger relative to snake body size. This hypothesis is supported by our results (Fig. 5). We note, however, that larger snakes also may carry more epibionts (e.g. Pfaller *et al.* 2012); further research on correlates of epibiont infestation rates would be of great interest. Amphibious snakes should be less vulnerable to epibionts, because their skin can dry out (killing superficial fouling organisms) whenever the snake is on land. Again, this pattern fits with our data.

We note, however, that many sea snakes have skins no thicker than that of terrestrial species, and that skin thickness is not the only trait that could influence resistance to epibionts. For example, rates of epibiont colonization are exacerbated by clear, shallow waters (Zann *et al.*, 1975), so a sea snake that spent its time in turbid or deep water may experience few problems in this respect. The larvae of many epibionts are highly selective about the substrates upon which they settle, with darker-coloured snakes attracting higher burdens (Shine *et al.*, 2010). Thus, lighter colour might reduce epibiont abundance. The latter hypothesis predicts that darker-coloured snake species may benefit more from thicker skin; subjectively, the thicker-skinned sea snake species (Table 1) tend to be darker in colour than their thinner-skinned counterparts. Another anti-fouling mechanism might be frequent sloughing; snakes could rid themselves of epibionts before the fouling organisms had time to develop deep attachments. The high rates of sloughing in some sea snakes (e.g. Zann, 1975; Heatwole, 1999) may reflect such an adaptation. If some taxa slough more often than others, they might resist epibiont attack without needing thick skin.

The thickness of skin may be important in other contexts too. For example, snakes can rid themselves of toxic pollutants (such as trace metals) when the skin is shed (e.g. Campbell & Campbell, 2001; Jones & Holladay, 2006). Thus, a thicker skin may increase the quantity of pollutants that are lost at sloughing. Because trace elements bind to melanin, a darker skin facilitates loss of those chemicals at sloughing (Goiran *et al.*, 2017). Melanin occurs across different layers of snake skin (in the superficial epidermis, the basal epidermis and the dermis: Lillywhite & Menon, 2019), but only the most superficial of those layers would be sloughed. It is unclear whether an increase in overall skin thickness (which results primarily from thickening of the deep dermis, a component that is not shed) would affect loss of heavy metals at sloughing. Future work could usefully explore the distribution of trace elements across different layers within the skin, to evaluate whether a thicker deep dermis might

increase expulsion of pollutants. Ecological studies on rates of epibiont fouling in free-living snakes would also be of great interest.

Another profitable avenue of research would be histology of epibionts *in situ*, to determine how deeply these organisms penetrate beneath the surface of the skin. More comprehensive sampling of sea snake species would also allow for comparisons of skin thickness to other ecological and morphological traits within this lineage. Species that do not fit the epibiont hypothesis – dark-coloured species that live in clear, shallow water but have thin skins – are of particular interest. For example, the reef shallows sea snake *Aipysurus duboisii* (which sometimes displays dark dorsal colouration) might be expected to experience high rates of settlement from larval epibionts, and yet has relatively thin skin. Does it slough more frequently, or do other aspects of skin morphology or chemistry render it less vulnerable to fouling organisms?

More generally, our data draw attention to how little we know about the morphology of snakes, and especially of aquatic species. The thickness of skin is a fundamental and easily measured trait, and yet we have no information about this trait for the vast majority of snake taxa. Multiple transitions from terrestrial to aquatic habits in snakes provide exciting opportunities to clarify the novel selective forces imposed by aquatic life.

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### AUTHOR CONTRIBUTIONS

R.S. and C.G. initiated the study, R.S., C.G. and G.P.B. gathered data, C.S. conducted and interpreted histology, R.S. and S.M. analysed the data, and all authors contributed to manuscript preparation.

### CONFLICT OF INTEREST

We declare no conflicts of interest.

## REFERENCES

**Abo-Eleneen RE**, **Allam AA. 2011.** Comparative morphology of the skin of *Natrix tessellata* (family: Colubridae) and

*Cerastes vipera* (family: Viperidae). *Zoological Science* **28:** 743–748.

**Alvarez F**, **Celis A. 2004.** On the occurrence of *Conchoderma virgatum* and *Dosima fascicularis* (Cirripedia, Thoracica) on the sea snake, *Pelamis platurus* (Reptilia, Serpentes) in Jalisco, Mexico. *Crustaceana* **77:** 761–764.

**Avolio C**, **Shine R**, **Pile AJ. 2006a.** The adaptive significance of sexually dimorphic scale rugosity in sea snakes. *The American Naturalist* **167:** 728–738.

**Avolio C**, **Shine R**, **Pile AJ. 2006b.** Sexual dimorphism in scale rugosity in sea snakes (Hydrophiidae). *Biological Journal of the Linnean Society* **89:** 343–354.

**Badrudeen M. 2000.** On the occurrence of the cirriped barnacle, *Chelonibia patula* (Ranzani) on the sea snake, *Hydrophis cyanocinctus* (Daudin). *Marine Fisheries Information Service, Technical and Extension Series* **164:** 25.

**Baum MJ**, **Kovalev AE**, **Michels J**, **Gorb SN. 2014.** Anisotropic friction of the ventral scales in the snake *Lampropeltis getula californiae*. *Tribology Letters* **54:** 139–150.

**Brandley MC**, **Huelsenbeck JP**, **Wiens JJ. 2008.** Rates and patterns in the evolution of snake-like body form in squamate reptiles: evidence for repeated re-evolution of lost digits and long-term persistence of intermediate body forms. *Evolution* **62:** 2042–2064.

**Bromley RG**, **Heinberg C. 2006.** Attachment strategies of organisms on hard substrates: a palaeontological view. *Palaeogeography, Palaeoclimatology, Palaeoecology* **232:** 429–453.

**Campbell KR**, **Campbell TS. 2001.** The accumulation and effects of environmental contaminants on snakes: a review. *Environmental Monitoring and Assessment* **70:** 253–301.

**Crowe-Riddell JM**, **Snelling EP**, **Watson AP**, **Suh AK**, **Partridge JC**, **Sanders KL. 2016.** The evolution of scale sensilla in the transition from land to sea in elapid snakes. *Open Biology* **6:** 160054.

**Daly JW**, **Garraffo HM**, **Spande TF**, **Giddings LA**, **Saporito RA**, **Vieites DR**, **Vences M. 2008.** Individual and geographic variation of skin alkaloids in three species of Madagascan poison frogs (*Mantella*). *Journal of Chemical Ecology* **34:** 252–279.

**Darwin C. 1851.** *A monograph on the sub-class Cirripedia. The Lepadidae*. London: Ray Society.

**Darwin C. 1854.** *A monograph of the sub-class Cirripedia. The Balanidae and Verrucidae*. London: Ray Society.

**Dubansky BH**, **Close M. 2018.** A review of alligator and snake skin morphology and histotechnical preparations. *Journal of Histotechnology* **42:** 31–51.

**Dunson WA**, **Robinson GD. 1976.** Sea snake skin: permeable to water but not to sodium. *Journal of Comparative Physiology B* **108:** 303–311.

**Fletcher RL**, **Callow ME. 1992.** The settlement, attachment and establishment of marine algal spores. *British Phycological Journal* **27:** 303–329.

**Foster BA. 1987.** Barnacle ecology and adaptation. In: Southward AJ, ed. *Barnacle biology. Crustacean Issues*, Vol. **5**. Rotterdam: A. A. Balkema Publishers, 113–133.

**Goiran C**, **Shine R. 2015.** Parental defence on the reef: antipredator tactics of coral-reef fishes against egg-eating sea snake. *Biological Journal of the Linnean Society* **114:** 415–425.

**Goiran C**, **Bustamante P**, **Shine R. 2017.** Industrial melanism in the seasnake *Emydocephalus annulatus*. *Current Biology* **27:** 2510–2513.e2.

**Guinea ML. 1994.** Sea snakes of Fiji and Niue. In: Gopalakrishnakone P, ed. *Sea snake toxinology*. Singapore: NUS Press, 212–233.

**Han D**, **Young BA. 2018.** The rhinoceros among serpents: comparative anatomy and experimental biophysics of Calabar burrowing python (*Calabaria reinhardtii*) skin. *Journal of Morphology* **279:** 86–96.

**Hazel J**, **Stone M**, **Grace MS**, **Tsukruk VV. 1999.** Nanoscale design of snake skin for reptation locomotions via friction anisotropy. *Journal of Biomechanics* **32:** 477–484.

**Heatwole H. 1999.** *Sea snakes*, *2nd edn*. Malabar: Krieger Publishing Company.

**Jeffries WB**, **Voris HK. 1979.** Observations on the relationship between *Octolasmis grayii* (Darwin, 1851) (Cirripedia, Thoracica) and certain marine snakes (Hydrophiidae). *Crustaceana* **37:** 123–132.

**Jones DE**, **Holladay SD. 2006.** Excretion of three heavy metals in the shed skin of exposed corn snakes (*Elaphe guttata*). *Ecotoxicology and Environmental Safety* **64:** 221–225.

**Key MM Jr**, **Jeffries WB**, **Voris HK. 1995.** Epizoic bryozoans, sea snakes, and other nektonic substrates. *Bulletin of Marine Science* **56:** 462–474.

**Klein MC**, **Gorb SN. 2012.** Epidermis architecture and material properties of the skin of four snake species. *Journal of the Royal Society, Interface* **9:** 3140–3155.

**Klein MC**, **Deuschle JK**, **Gorb SN. 2010.** Material properties of the skin of the Kenyan sand boa *Gongylophis colubrinus* (Squamata, Boidae). *Journal of Comparative Physiology. A, Neuroethology, Sensory, Neural, and Behavioral Physiology* **196:** 659–668.

**Kropach C**, **Soule JD. 1973.** An unusual association between an ectoproct and a sea snake. *Herpetologica* **29:** 17–19.

**Lynch LM. 2018.** Limb skeletal morphology of North American pine martens, Martes americana and Martes caurina, correlates with biome and climate. *Biological Journal of the Linnean Society* **126:** 240–255.

**Lee MS**, **Sanders KL**, **King B**, **Palci A. 2016.** Diversification rates and phenotypic evolution in venomous snakes (Elapidae). *Royal Society Open Science* **3:** 150277.

**Lillywhite HB**, **Sanmartino V. 1993.** Permeability and water relations of hygroscopic skin of the file snake, *Acrochordus granulatus*. *Copeia* **1993:** 99–103.

**Lillywhite HB**, **Menon GK. 2019.** Structure and function of skin in the pelagic sea snake, *Hydrophis platurus*. *Journal of Morphology* **280:** 544–554.

**Lillywhite HB**, **Menon JG**, **Menon GK**, **Sheehy CM 3rd**, **Tu MC. 2009.** Water exchange and permeability properties of the skin in three species of amphibious sea snakes (*Laticauda* spp.). *The Journal of Experimental Biology* **212:** 1921–1929.

**Montagna W. 2012.** *The structure and function of skin*. New York: Academic Press.

**Murphy JC. 2007.** *Homalopsid snakes: evolution in the mud*. Malabar: Krieger Publishing.

**Nagelkerken ISJM, Blaber SJM, Bouillon S, Green P, Haywood M, Kirton LG, Meynecke JO, Pawlik J, Penrose HM, Sasekumar A, Somerfield PJ. 2008.** The habitat function of mangroves for terrestrial and marine fauna: a review. *Aquatic Botany* **89:** 155–185.

**Moss B, Woodhead P. 1970.** The breakdown of paint surfaces by *Enteromorpha* sp. *New Phytologist* **69:** 1025–1027.

**Ohba H, Fujioka Y, Tottori K, Shibuno T. 2005.** A sea snake wearing green velvet. *Coral Reefs* **24:** 403.

**Orme D, Freckleton R, Thomas G, Petzoldt T, Fritz S, Isaac N, Pearse W. 2014.** *Caper: comparative analyses of phylogenetics and evolution in R*. R package version 0.5.2/r121. Available at: http://R-Forge.R-project.org/projects/caper/.

**Pfaller JB, Frick MG, Brischoux F, Sheehy CM 3rd, Lillywhite HB. 2012.** Marine snake epibiosis: a review and first report of decapods associated with *Pelamis platurus*. *Integrative and Comparative Biology* **52:** 296–310.

**Povel D, Van Der Kooij J. 1996.** Scale sensillae of the file snake (Serpentes: Acrochordidae) and some other aquatic and burrowing snakes. *Netherlands Journal of Zoology* **47:** 443–456.

**Price RM. 1982.** Dorsal snake scale microdermatoglyphics: ecological indicator or taxonomic tool? *Journal of Herpetology* **16:** 294–306.

**Rivera G, Savitzky AH, Hinkley JA. 2005.** Mechanical properties of the integument of the common gartersnake, *Thamnophis sirtalis* (Serpentes: Colubridae). *The Journal of Experimental Biology* **208:** 2913–2922.

**Sanders KL, Rasmussen AR, Elmberg J. 2012.** Independent innovation in the evolution of paddle-shaped tails in viviparous sea snakes (Elapidae: Hydrophiinae). *Integrative and Comparative Biology* **52:** 311–320.

**Sanders KL, Lee MS, Mumpuni, Bertozzi T, Rasmussen AR. 2013.** Multilocus phylogeny and recent rapid radiation of the viviparous sea snakes (Elapidae: Hydrophiinae). *Molecular Phylogenetics and Evolution* **66:** 575–591.

**Sherratt E, Rasmussen AR, Sanders KL. 2018.** Trophic specialization drives morphological evolution in sea snakes. *Royal Society Open Science* **5:** 172141.

**Shetty S, Shine R. 2002.** Activity patterns of yellow-lipped sea kraits (*Laticauda colubrina*) on a Fijian island. *Copeia* **2002:** 77–85.

**Shine R, Brischoux F, Pile AJ. 2010.** A seasnake's colour affects its susceptibility to algal fouling. *Proceedings of the Royal Society B* **277:** 2459–2464.

**Storr GM, Smith LA, Johnstone RE. 1986.** *Snakes of Western Australia*. Perth: Western Australian Museum.

**Sweet S. 1989.** Foraging behavior in the primitive sea snake *Hydrelaps darwiniensis*. In: Annual Meeting of the American Society of Ichthyologists and Herpetologists, San Francisco, California, USA, 17–23 June 1989.

**Westhoff G, Fry BG, Bleckmann H. 2005.** Sea snakes (*Lapemis curtus*) are sensitive to low-amplitude water motions. *Zoology* **108:** 195–200.

**Wilde S, Timpson A, Kirsanow K, Kaiser E, Kayser M, Unterländer M, Hollfelder N, Potekhina ID, Schier W, Thomas MG, Burger J. 2014.** Direct evidence for positive selection of skin, hair, and eye pigmentation in Europeans during the last 5000 y. *Proceedings of the National Academy of Sciences of the United States of America* **111:** 4832–4837.

**Zann LP. 1975.** Biology of a barnacle (*Platylepas ophiophilus* Lanchester) symbiotic with sea snakes. In: Dunson A, ed. *The biology of sea snakes*. Baltimore: University Park Press, 267–286.

**Zann LP, Cuffey RJ, Kropach C. 1975.** Fouling organisms associated with the skin of sea snakes. In: Dunson A, ed. *The biology of sea snakes*. Baltimore: University Park Press, 251–265.

**Zheng Y, Wiens JJ. 2016.** Combining phylogenomic and supermatrix approaches, and a time-calibrated phylogeny for squamate reptiles (lizards and snakes) based on 52 genes and 4162 species. *Molecular Phylogenetics and Evolution* **94:** 537–547.