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1 From matte banded to glossy black: Structures underlying colour
2 change in the caudal lures of southern death adders (*Acanthophis*
3 *antarcticus*, Reptilia: Elapidae)

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17 Running head: Colour change in death adder lures

18

19 Abstract

20 Many ambush-foraging snakes move their tails to entice prey within striking range (caudal luring).
21 During ontogeny, the conspicuous hues of caudal lures change to match the cryptic patterning of the
22 body/head coinciding with decreased luring behaviour; reflecting the trade-off between prey
23 acquisition and camouflage as the snake grows. Australo-Papuan death adders (*Acanthopis*,
24 Elapidae) are unique in that both juveniles and adults use caudal luring, but ontogenetic colour
25 change has not been investigated. We examined the spectral reflectance, microstructure, and
26 pigmentation of caudal skin in wild-sourced and captive bred *Acanthopis antarcticus* ranging in
27 body size (snout-vent length 116-674 mm; mass 3-832 g; $n = 33$) to test whether colour properties
28 change as snakes grow. We found that lure colour is distinct from the cryptic body skin across life
29 history, and changes from a matte banding pattern (grey/black) in neonates/juveniles, to uniform
30 and glossy black with a yellow ventral stripe in larger snakes. These colour changes are caused by
31 increases in dermal pigmentation and a transition to a smooth, interlocking epidermal
32 microstructure. To understand the selection pressures that might be driving ontogenetic colour
33 change in this species, further studies should test how different prey types might respond to distinct
34 lure morphologies.

35

36 **Keywords:** caudal luring, glossiness, microstructure, ontogenetic colour change, reflectance,
37 structural colour

38

39 INTRODUCTION

40 Ontogenetic colour change (OCC), unrelated to sexual selection, is a poorly understood aspect
41 of animal biology. In snakes, OCC is associated with gaining or maintaining body camouflage as larger
42 individuals occupy new habitats and adopt different behaviours (Wilson, Heinsohn, & Endler, 2007).
43 The mechanisms underlying OCC in snakes are poorly known but are likely to involve changes in a
44 combination of structure (*e.g.* collagen, epidermal microstructure) and pigmentation (Olsson, Stuart-
45 Fox, & Ballen, 2013; Spinner *et al.*, 2013).

46 Many ambush-foraging snakes move their tails to attract prey within striking range (caudal
47 luring), and often undergo OCC in the distal portion of the tail responding to shifting selection
48 pressures during ontogeny. The conspicuous green/yellow hues of juvenile caudal lures in Viperidae,
49 Crotalidae, Pythonidae and Boidae, for example, become indistinguishable from the camouflaged
50 patterning of the body, coinciding with a declining frequency of luring in adults (Neill, 1960;
51 Heatwole & Davison, 1976). The smaller and conspicuous tails of juvenile snakes are thought to be
52 selected for attracting ectothermic vertebrates (*e.g.* lizards and frogs) that are typically eaten by
53 smaller snakes (Reiserer, 2002; Rabatsky & Waterman, 2005; Nelson, Garnett, & Evans, 2010). In
54 contrast, adult ambush snakes typically eat larger, endothermic vertebrates and the hues of caudal
55 lure are under positive selection to match the cryptic colouration of the body and head.

56 Australo-Papuan death adders (Elapidae: *Acanthophis*) are unusual among ambush snakes
57 because both juveniles and adults use caudal luring to attract prey (McPhee, 1959; Neill, 1960;
58 Carpenter, Murphy, & Carpenter, 1978). *Acanthophis* (ca. 8 species) are ecologically convergent with
59 vipers, sharing many traits pertaining to their ambush foraging mode including a flat, heavy body,
60 triangular head shape, enlarged fangs that partially rotate with the maxilla, body camouflage and
61 caudal luring (Shine, 1980) (Figure 1). To lure prey, death adders use a combination of lateral and
62 vertical tail movements including rapid thrashing, slow undulations, lifting and waving (Carpenter *et*
63 *al.*, 1978; Chiszar *et al.*, 1990; Hagman, Phillips, & Shine, 2008; McDonald, 2010) (Video S1). An

64 'incomplete' ontogenetic shift in diet composition is thought to contribute to the persistence of
65 caudal luring in adult death adders, *i.e.* ectotherms remain important prey items of adults (Heatwole
66 & Davison, 1976; Shine, 1980). Adult *Acanthophis* have conspicuous caudal lures with hues ranging
67 from pale cream, white, yellow and black (Cogger, 2000; Wilson & Swan, 2013; Mirtschin,
68 Rasmussen, & Weinstein, 2017) that contrast with their camouflaged body skin (Figure 1). Although
69 juvenile death adders are known to use caudal luring too, the variation and ontogeny in the
70 colouration of caudal lures has not been investigated.

71 In this study, we examine the colouration of caudal lures in a population southern death adders
72 (*Acanthophis antarcticus*) at different life stages (neonate, juvenile, subadult and mature adult) to
73 test how and when OCC might occur in this population. Because luring appears to be retained in
74 adults to attract prey, we expect there to be minimal to no difference in colour properties across
75 body sizes (*i.e.* no OCC). Alternatively, if the adult lure is under positive selection for crypsis, we
76 would expect OCC that causes the lure to become indistinguishable from the colour and patterning
77 on the body, and from the presumably conspicuous juvenile caudal lure (as in many vipers).

78 MATERIALS AND METHODS

79 *Study species*

80 The genus *Acanthophis* (Australo-Papuan death adders) has been the source of taxonomic
81 uncertainty but currently comprises approximately eight species which occur across mainland
82 Australia, New Guinea and associated offshore islands (Mirtschin *et al.*, 2017). Southern death
83 adders (*A. antarcticus*, Shaw & Nodder, 1802) occur across temperate southern Australia into the
84 sub-tropical reaches of the eastern coast where they live in undisturbed habitats of mallee, spinifex
85 grasslands, coastal dunes and sclerophyll forest (Figure 1). Two colour forms appear to be under
86 selection for crypsis in local substrate: red body patterns are found in northern and eastern
87 Australia, and grey body patterns in southern Australia (Johnston, 1996).

88 *Animal husbandry and euthanasia*

89 We examined lure properties in 33 captive *A. antarcticus* that ranged in snout to vent length
90 (SVL; 116-674 mm) and mass (3-832 g) (Table S1). Adults were wild-collected, sourced from coastal
91 dune populations from Smoky Bay, South Australia (Figure 1 inset), and offspring were captive bred
92 progeny of these wild-collected individuals. Colour patterns were noted for the wild-caught *A.*
93 *antarcticus* from our study site and determined to be a brownish body colour and dark black caudal
94 lure (Figure 1). Three females gave birth to litters of 22, 24 and 30 neonates, respectively. We fed
95 half of the neonates weekly and the other half every two weeks to manipulate snake growth
96 patterns allowing us to determine if change of caudal lures were size or age dependent. Individuals
97 from our two 'feeding groups' were randomly selected from all three litters. Thirty-one progeny
98 were euthanized at different body sizes and ages (from 1 day old to 8 months old) and two adult
99 snakes (between 15 and 20 years old). Details of animal husbandry and housing are in the
100 supplementary materials.

101 Snakes were euthanized via intramuscular injection of pentobarbitone (see ethics statement).
102 Deceased snakes were frozen and kept for several months in the freezer (-20 degrees Celsius). After
103 reflectance measurements were taken on thawed snakes, the specimens were persevered in 10%
104 formalin and stored in 100% ethanol at the South Australian Museum (unregistered specimens).

105 *Dissection and microscopy*

106 We removed the tail from an adult and juvenile, and processed for microscopy. The tails were
107 dorsally bisected: one half was fixed by emersion in 4% paraformaldehyde (PFA) and 1.25%
108 glutaraldehyde in phosphate-buffered saline (PBS) and 4% sucrose (pH 7.2) for 48 h for scanning
109 electron microscopy and one half in 10% formalin in PBS for 48 h for histology and light
110 microscopical examination. Tails from each animal were further dissected according to their
111 anatomical position (ventral, dorsal) and epidermal colouration (white, black or yellow). A portion of
112 dorsal skin on the midbody, remote from the lure, was also removed from the adult and dissected

113 according to the crossband colouration (light brown, dark brown, pale cream) and fixed by emersion
114 in 4% PFA and 1.25% glutaraldehyde in PBS, for 48 h for electron microscopy. Fixed tissues were
115 stored in a refrigerator (4°C) for 48 h.

116 For scanning electron microscopy (SEM), samples were rinsed in a PBS solution containing
117 4% sucrose (pH 7.2) and post-fixed in 2% osmium tetroxide for 1 h before immersion in a
118 consecutive series of ethanol solutions (70%, 90%, 100%). Dried specimens were then immersed in
119 1:1 solution of hexamethyldisilazane (HDMS) and 100% ethanol, before immersion in 100% HDMS
120 for 20 to 40 minutes. Samples were subsequently left to air-dry for 30 min before being mounted
121 with an epoxy resin on platinum-coated aluminium stubs. The coated samples were viewed with a
122 high-vacuum, 10 kV SEM (XL30, Philips, Japan). The colour of skin was noted prior to processing for
123 SEM, but we found no clear difference in microstructure recorded.

124 For histology, samples were immersed in PBS solution (pH 7.4) and decalcified using
125 ethylenediaminetetraacetic acid (EDTA) for 24 h. Samples were subsequently dehydrated by
126 successive immersion in ethanol (70%, 80%, 95%, 100%), rinsed in xylene, immersed and embedded
127 in paraffin. Transverse sections (10 µm thickness) were mounted onto slides and left unstained to
128 view pigmentation. Slides were imaged using a mounted camera (LC30, Olympus, Australia) and light
129 microscope (BX51, Olympus, Australia).

130 *Spectrophotometry and photography*

131 We photographed the caudal lures of 33 specimens (Table S1) using a DSL camera (Canon
132 EOS 7D) fitted with a macro lens (f/2.8 Canon EF-S 60mm). We then measured the spectral
133 reflectance of the lateral-dorsal and ventral scales on each caudal lure using an Ocean Optics
134 spectrometer (MAYA2000 Pro, Dunedin, FI, USA) and analysed using Ocean View software v1.6.7
135 (Ocean Optics, Dunedin, FI, USA). The mean reflectance was calculated for three locations on the
136 caudal lure representing different colours (white/grey, yellow, black; Figure 2) and plotted in R
137 v3.6.2 (R Core Team, 2019; Wickham *et al.*, 2019). Each reflectance measurement was expressed

138 relative to a Spectralon 99% white reflectance standard (WS-1-SL, Ocean Optics, Dunedin, FL, USA).
139 The probe (QR200-7-UV-BX 200 μ m) was mounted to maintain a constant angle (90°) and distance (4
140 mm) from the surface of the skin. Specimens were mounted in white plasticine to secure tails flat.
141 Measurements were taken in a dim-lit room to minimise scattered light. Both the UV (deuterium)
142 and visible (halogen) lamps were used simultaneously for each measurement. The wavelength and
143 reflectance values of these two references were saved by accessing the file data in the Ocean View
144 v1.6.7 schematic window (Ocean Optics, Dunedin, FL, USA). Following calibration, a reflectance
145 reference was taken using the Spectralon standard to ensure that calibration was completed
146 successfully, indicated by reflectance values of approximately 100% reflectance across all
147 wavelengths. Reference measurements were also recorded of the white plasticine as a 'background'
148 reference, and of the shuttered probe for a 'dark' reference.

149 RESULTS

150 We found ontogenetic changes in colour and specular properties of lures that broadly
151 coincide with a shift in prey types (Figure 3; Figure S1). Although this change was continuous, we
152 identified four distinct phenotypes approximately corresponding to neonate, juvenile, subadult and
153 adult life histories. The "neonate banded" lure phenotype (SVL 116-147 mm; mass 3.1-9.8 g; $n = 13$)
154 was a matte texture with lateral-dorsal bands that alternated black (4-5 bands) and white/grey (4-5
155 bands) with ventral white stripe; each colour displayed a UV signature (peak 340-360 nm). The
156 "juvenile transition" lure phenotype (SVL 164-467 mm; mass 11.5-172.7 g; $n = 10$) was a matte
157 texture with lateral-dorsal bands of black (3-4 bands) and light grey (3-4 bands) with a lateral ventral
158 yellow stripe (peak 500-700 nm); the grey and yellow scales displayed a UV signature (peak 340-360
159 nm). The "subadult striped" lure phenotype (SVL 414-674 mm; mass 92.2-524.9 g; $n = 8$) was black
160 on the lateral-dorsal scales with a ventral yellow stripe (peak 500-700 nm); only the yellow scales
161 showed a UV signature (peak 340-360 nm). The yellow stripe was variable from pale yellow to
162 orange (range 500-580 nm) among juvenile/subadult phenotypes (Figure S3). The "adult uniform"

163 lure phenotype (SVL 414-674 mm; mass 92.2-524.9 g; $n = 2$) was entirely black across the lure. The
164 black colouration of the subadult/adult lure phenotypes was spectrally uniform with low average
165 reflectivity (<20%) but highly reflective when viewed at angles relative to predominant light sources
166 (e.g. the sun) creating a glossy specular reflection (i.e. high, angular dependent, reflectance across
167 visible wavelengths). The lighter colour bands of the neonate/juvenile lures were only twice as
168 reflective as the black colouration, and appeared as a middle grey colour rather than an absolute
169 white (Figure 3). The silhouette of the lure also changed: overlapping scales created a serrated edge
170 and the terminal spine was sharper in the subadult/adult phenotypes (Figure 4).

171 Adult epidermal microstructure consisted of a series of interlocking *oberhautchen* (outer
172 epidermal cells) that created a “smooth” platelike surface (Figure 4). In contrast, *oberhautchen* of
173 juvenile skin was sculpted into frayed edges and deep divots that created imbricate and “rough”
174 surface. The microstructure of the dorsal body skin in both adults and juveniles was also imbricate
175 with numerous deep divots (Figure 5). In the skin of the adult lure, pigment-containing cells were
176 densely distributed in both the dermis (melanophores) and the epidermis (melanosomes). In the skin
177 of the juvenile lure, the black bands contained some melanophores in the dermis; the grey/white
178 bands contained sparsely distributed melanosomes in the epidermis and completely translucent
179 layers of outer epidermis (β -layer).

180 DISCUSSION

181 *Mechanisms underlying colour change*

182 This study reveals changes in epidermal microstructure and dermal pigmentation underlying
183 previously undescribed OCC in caudal lures of *A. antarcticus*. There are two main mechanisms of
184 animal colouration: pigments that selectively absorb visible wavelengths of light, and (sub)surface
185 microstructures that interfere with light to selectively reflect light, in an angular-dependent way,
186 resulting in either highly coloured iridescence or spectrally neutral glossiness (Maia, D’Alba, &
187 Shawkey, 2011).

188 The matte banded pattern in the neonate/juvenile lure morphologies are created by sparse
189 epidermal and dermal pigments, combined with small depressions and irregular surface structure
190 (Figure 4). This matte microstructure closely resembles the body scales of *A. antarcticus* (Figure 5)
191 and textured scales of other snakes (e.g. rattlesnakes) (Stille, 1987; Price & Kelly, 1989), but is much
192 shallower than the ridged nanostructures of the variably coloured and “velvety” scales in Gaboon
193 vipers (*Bitis* spp.) (Spinner *et al.*, 2013). The glossy texture of the subadult/adult lure morphologies is
194 created by an interlocking and smooth epidermal microstructure, that differs from the body scales
195 (Figure 4; Figure 5), and causes a highly glossy broadband reflection resembling other biological
196 materials such as feathers, eggs, cuticles and petals (Maia *et al.*, 2011; van der Kooi *et al.*, 2014; Igic
197 *et al.*, 2015; Maurer, Kohl, & Gebhardt, 2017). These “glossy” lure morphologies also have dense
198 pigmentation that uniformly absorbs wavelengths to create a black hue that is not influenced by
199 viewing angle relative to light source (cf iridescence). During caudal luring, this combination of
200 structure and pigmentation may result in a rapid change in luminance when seen by an observer.
201 These results have implications for future research on caudal luring, as previous studies have
202 manipulated tail colour and/or behaviour (Hagman *et al.*, 2008; Nelson *et al.*, 2010; Farrell, May, &
203 Andreadis, 2011), but did not consider the interaction between epidermal microstructure and the
204 perceiver’s angle of view during caudal luring.

205 *What is the relationship between colour and motion during caudal luring?*

206 To understand the significance of OCC in caudal lures, it must be viewed in context of snake
207 and prey behaviour. This study is the first to describe a distinct neonate/juvenile lure morphologies
208 in *Acanthophis*, but many field guides detail that the colour of adult lures is highly variable among
209 species and geographically separated populations (Cogger, 2000; Mirtschin *et al.*, 2017; Wilson &
210 Swan, 2013). During caudal luring, the body of *A. antarcticus* will lay motionless while the tail is
211 positioned beside or in front of the jaw and moved laterally in a series of rapid thrashing movements
212 and/or slow undulations and “rippling waves”; the tail may also be lifted vertically and waved in the

213 air or else moved in rapid “busts” to position the tail back-and-forth from beside the jaw to above
214 the head (Carpenter *et al.*, 1978; Chiszar *et al.*, 1990; Hagman *et al.*, 2008; McDonald, 2010; Nelson
215 *et al.*, 2010). Carpenter *et al.* (1978) categorised the luring behaviour of *A. antarcticus* in to two
216 alternating phases: (I) slow, fine motor movements of the tail tip, and (II) fast, gross motor
217 movements of the entire tail. The conspicuous colouration of the caudal lure, which contrasts the
218 camouflaged patterning of the body, is likely to draws an observer’s attention towards the tail while
219 it is positioned against the cryptic head/body of the snake (Neill, 1960). In addition to contrasting
220 colouration of the lure and body, we describe distinct colours within the caudal lure itself, especially
221 in medium-sized *A. antarcticus* that have caudal lures with bright yellow and UV signatures on the
222 ventral scales (cf banded or glossy black on the lateral-dorsal scales; Figure 3). This ventral
223 colouration is hidden from view during the slow undulations in phase I, but is revealed by a series of
224 vertical lifting movements in phase II (Video S1). How colour might enhance or augment the
225 movement of the tail during caudal luring needs to be investigated further.

226 *Ecological significance of colour change in caudal lures*

227 The signalling effects of OCC in *A. antarcticus* lures on prey attraction are unknown.
228 Response of ectothermic prey to caudal luring has been tested in the northern death adder (*A.*
229 *praelongus*) and indicated that smaller lures are more effective in eliciting a response in lizards, but
230 only ectothermic prey were tested (Hagman *et al.*, 2008). Given that mammals and birds comprise a
231 portion of the subadult/adult diet in this species (Figure S1), however, response of endothermic prey
232 to a range of lure phenotypes and lighting conditions needs to be tested. Previous authors proposed
233 that luring is retained in adult *Acanthophis* because ectothermic prey (consumed by juveniles of this
234 species) remains an important part of the adult diet (Heatwole & Davison, 1976). If this were the
235 case, lure morphology should remain unchanged as the snake grows (*i.e.* no OCC), or else acquire
236 similar colour patterning to the body if camouflage is favoured by selection. On the contrary, we find
237 that the caudal lure transitions between different colours, all of which are distinct from the body

238 colour pattern. Based on our results, we assert a new hypothesis that lure morphology is under
239 differential positive selection for attracting ectothermic prey types (e.g. diurnal lizards) in juvenile
240 snakes and endothermic prey (e.g. nocturnal mammals) in adult snakes.

241 Previous analysis of stomach contents in museum specimens of *A. antarcticus* (throughout
242 their Australian geographic range) reveal that larger snakes tend to eat more endotherms, e.g. birds
243 and mammals, than smaller snakes (summarised in Figure S1 and Table S2) (Shine, 1980; Shine,
244 Spencer, & Keogh, 2014). The mammalian prey that could be identified to family level were mostly
245 rodents (Muridae, 52%), followed by a single dunnart (*Sminthopsis* spp.) and antechinus (*Antechinus*
246 spp.); skinks comprise the majority of reptilian prey (74%) followed by agamid and varanid lizards;
247 *Litoria* tree frogs were the majority of amphibian prey (33%) (Shine, 1980), and most birds could not
248 be identified (Table S2). The difference in prey types consumed during ontogeny likely indicate a
249 shift in foraging patterns, with smaller snakes potentially targeting diurnal reptiles and frogs and
250 larger snakes targeting crepuscular/nocturnally active mammals.

251 The glossy lure phenotypes of larger snakes (subadult/adult) may be used to exploit sensory
252 biases of various prey to attract attention or enhance contrast of adjacent scales on the tail/head.
253 The specular reflections may be more effective at reflecting moonlight, catching the attention of
254 mammals that are active at dusk/night. The black colour, glossy appearance and imbricate silhouette
255 might also be mimicking the carapace and/or appendage of an arthropod (e.g. carabid beetles),
256 which are prey items for nocturnally active marsupials that adult *A. antarcticus* that historically
257 consumed. Indeed, insect parts have been recorded in the secondary stomach contents of adult *A.*
258 *antarcticus* (which swallow their prey whole). We do not have data on the spectral reflectance or
259 microstructure of local arthropods or skinks for comparison, thus an 'aggressive mimicry' hypothesis
260 is tentative. Regardless, previous work on *A. antarcticus* behaviour indicates that caudal luring
261 closely matches the velocity speeds of common invertebrates (Nelson *et al.*, 2010), suggesting that
262 the morphology and motion of the caudal lure are likely exploiting receiver bias in their

263 insectivorous prey. Finally, the positioning of the lure beside the head may also be salient. The labial
264 scales have bright white spots with a UV signature (peak 330-420 nm; Figure S2) that, when the dark
265 lure is undulated, may create a strobe effect of UV light, which are likely visible to birds and
266 mammals that have the capacity to detect UV light (Jacobs, 1992).

267 The morphology of the neonate caudal lure (white/grey and black bands) and the juvenile
268 caudal lure (grey bands) also display UV signatures, which are likely visible to skinks that are active
269 during the day (Fleishman, Loew, & Whiting, 2011). This lure morphology resembles the banding
270 pattern of some rattlesnakes, which use their tails in aposematic rattling displays. Given that captive
271 neonate/juvenile *A. antarcticus* readily lure at feeding times (L. Allen, pers. obs.), however, an
272 aposematic function is unlikely. The juvenile/subadult caudal lures have features of both the
273 neonate lures (e.g. matte banded) and adult lures (glossy, black, imbricate scales). These lure
274 morphologies also have a yellow ventral stripe with a UV signature, which is absent in the other lure
275 morphologies. This yellow stripe is reminiscent of local skinks (e.g. *Hemiergis peronii*), and is likely
276 only revealed to potential prey during rapid vertical lifting of the tail during luring (Video S1). These
277 medium-sized snakes consume both endothermic and ectothermic prey (Figure S1; Table S2). Thus,
278 these “transitional” lure morphologies may be effective at luring ectothermic prey (lizards, frogs)
279 during the day, and endothermic prey (mammals, birds) at dusk and during the night.

280 To understand the ecological significance of OCC in *A. antarcticus*, behavioural experiments
281 should test how potential prey types respond to different lure morphologies. Future behavioural
282 studies will need to consider visual modelling of spectral reflectance to infer how lure colours are
283 perceived by prey, as well as the influence of light intensity/spectral quality and background features
284 (e.g. leaf litter) of microhabitats where *A. antarcticus* forage (Endler, 1992; Leal & Fleishman, 2002).
285 Furthermore, the activity patterns of juveniles and adults snakes likely differ, which would
286 dramatically alter how caudal lure signalling properties are perceived by potential prey. Such studies
287 will be important in understanding the conservation threats to death adders in Australia and New

288 Guinea, especially the impact of invasive species (e.g. *Rhinella marnia*) (Brown, Phillips, & Shine,
289 2011), and population fluctuations in local prey types.

290 *Implications for evolution of luring in snakes*

291 The prevalence of OCC in caudal lures and variation in luring behaviour is poorly
292 documented across life history stages in snakes. Recent studies suggest that luring is more prevalent
293 in adult viperids than previously thought. The lure of the spider-tailed viper (*Pseudocerastes*
294 *urarachnoides*), for example, only develops only in adulthood and superbly mimics the movement
295 and limbs of a spider to attract bird-prey (Fathinia *et al.*, 2015). Other examples include the Cantil
296 snake (*Bothrops bilineatus smaragdinus*) and related viperids (e.g. *B. insularis*, *Cerastes vipera*,
297 *Vipera latastei*) that appear to lure at dusk/night to entice birds and/or mammals (da Fonseca,
298 Correa, & Oliveira, 2019). Luring may also be context-dependent: studies in wild puff adders (*Bitis*
299 *arietans*), which use both lingual and caudal luring, found that snakes use lingual luring only when
300 anuran prey are nearby (Glaudas & Alexander, 2017). Other behavioural studies have also shown
301 that snakes can discriminate between prey types and potential predators, and alter their luring
302 behaviour accordingly (Reiserer, 2002; Reiserer & Schuett, 2008). Broad behavioural testing and
303 morphological descriptions of captive and wild populations will reveal how ecological and
304 environmental factors have influenced the evolution of caudal luring among convergent ambush-
305 foraging snakes.

306 CONCLUSION

307 This study is the first to report OCC in caudal lures and demonstrate the underlying structural
308 changes to the skin in snakes. We propose that juvenile and adult lures are under different positive
309 selection pressures for the attraction of diurnal ectothermic or nocturnal endothermic prey,
310 respectively. This hypothesis needs to be tested using behavioural experiments that measure prey
311 response to different lure phenotypes, as well as recordings of how caudal luring behaviour might
312 change during ontogeny for different *Acanthophis* populations and species.

313

314 **Ethics:** All interactions with animals and collection of samples were conducted under the
315 requirements of the Department for Environment and Water and the institutional guidelines of
316 Venom Supplies, and was undertaken in conformance with Animal Welfare Act 1985 (South
317 Australia). All measurements were taken from deceased animals that were alcohol-preserved and
318 housed as unregistered specimens at the South Australian Museum, no ethical considerations apply.

319 **Competing interests:** We declare no competing interests.

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328 and J.M.C-R collected reflectance data and tissue samples. L.A. and N.D. were responsible for animal
329 husbandry. L.P. and J.M.C-R conducted microscopy analyses. J.M.C-R, A.L. and J.C.P. analysed
330 reflectance data. J.M.C-R wrote the manuscript with input from all authors.

331 **Data accessibility:** Supplementary methods, tables and figures are provided as Supplementary
332 Materials. Images of caudal lures for specimens used in this study are available at
333 [doi/10.25909/13239497](https://doi.org/10.25909/13239497). Reflectance data for individual specimens, diet data, and code used to plot
334 diet and reflectance data available at [https://github.com/jcrowerriddell/death-adder-lure-](https://github.com/jcrowerriddell/death-adder-lure-reflectance)
335 [reflectance](https://github.com/jcrowerriddell/death-adder-lure-reflectance). Supplementary video S1 of luring behaviour available at <https://vimeo.com/462914012>.

336

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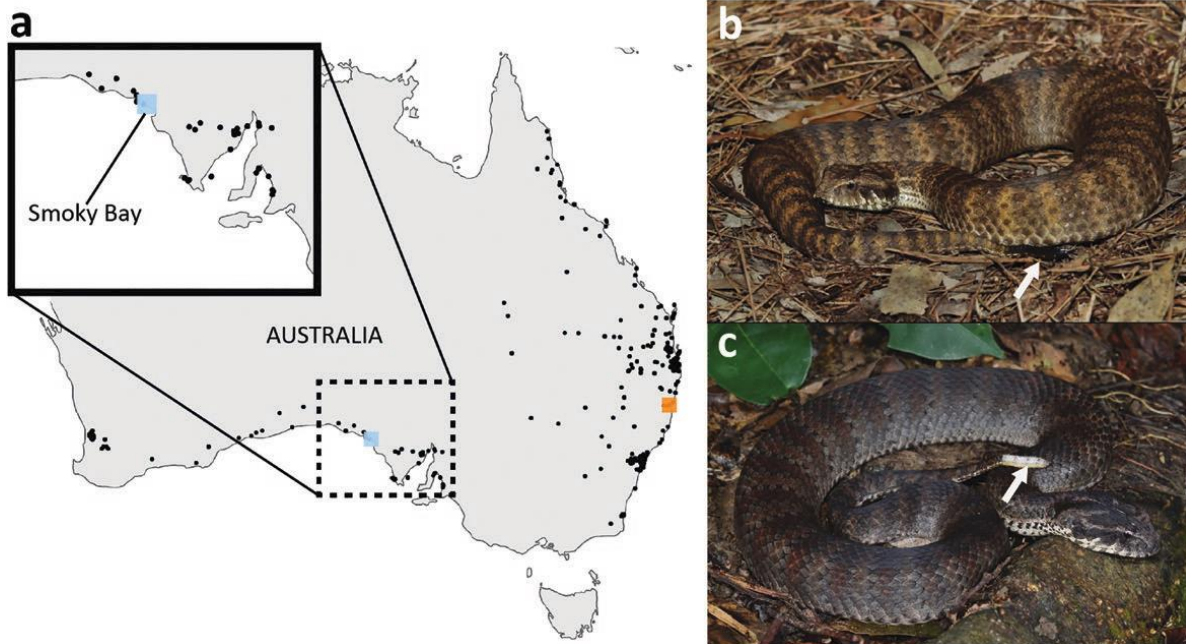
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415 **Figures**



416

417 **Figure 1.** Distribution and colour variation in *Acanthophis antarcticus*. a) Disjunct distribution of *A.*
418 *antarcticus* in Australia; inset shows the locality of source population in Smoky Bay, South Australia.

419 b) Image of adult *A. antarcticus* showing black caudal lure that is typical for snakes from southern
420 Australia. c) Image of adult *A. antarcticus* showing white caudal lure that is typical for individuals

421 from the eastern coast of Australia. White arrows indicate location of the caudal lure; coloured

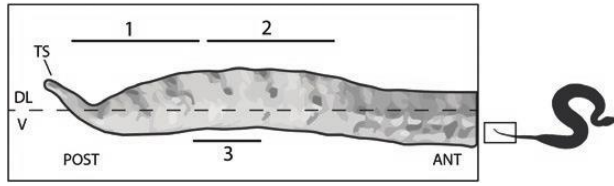
422 squares on map show capture site for snakes shown in images: Smoky Bay, South Australia (b, blue),

423 and Bellingen, New South Wales (c, orange). Occurrence data from the Atlas of Living Australia

424 occurrence downloaded at <https://doi.org/10.26197/5e44150a40c6a> accessed on 13 February 2020.

425 Image credits: Luke Allen, Shane Black.

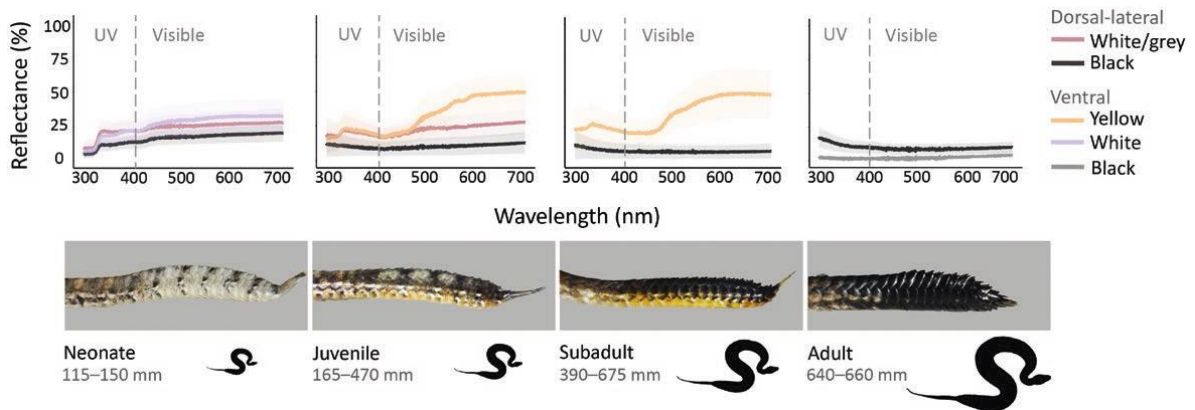
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428 **Figure 2.** Schematic of the caudal lure in *Acanthophis antarcticus* showing locations where
 429 reflectance was measured. One measurement was taken each at the darkest and lightest point on
 430 dorsal-lateral scales, location of measurement was recorded as closest to posterior end of tail
 431 (position 1) or closest to anterior (position 2); one measurement was taken on the ventral scales at
 432 the mid-point of the lure (position 3). DL = dorsal-lateral, V = ventral, POST = posterior, ANT =
 433 anterior, TS = terminal spine.

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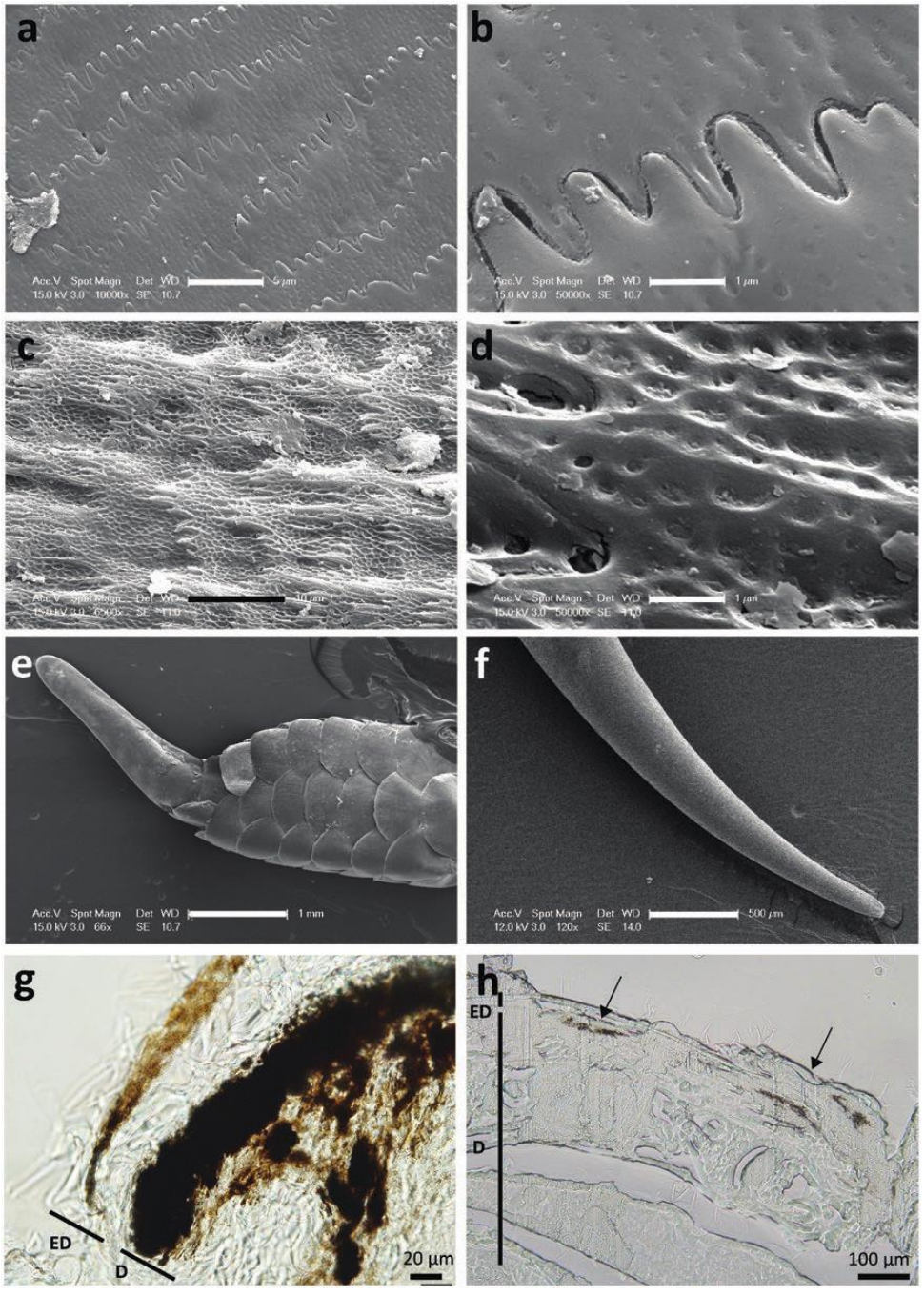


435

436 **Figure 3.** Ontogenetic colour change of caudal lures in *Acanthophis antarcticus*. Representative
 437 images of lure morphologies are shown at the different life stages, which correspond to snout to
 438 vent length. Plots show spectral reflectance of each colour recorded for the different lure
 439 morphologies; division between ultraviolet (UV) and visible spectrum shown by vertical dashed line.
 440 Snake silhouette by C. N. Zdenek (Phylopic CC BY-NC-SA 3.0).

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Figure 4. Skin structures underlying ontogenetic colour change in caudal lures in *Acanthophis*

445

antarcticus. Scanning electron microscopy of epidermal microstructure in a, b) adult lure skin; c, d)

446

neonate lure skin, and tip of caudal lure (terminal spine) in d) adult and e) juvenile. Light microscopy

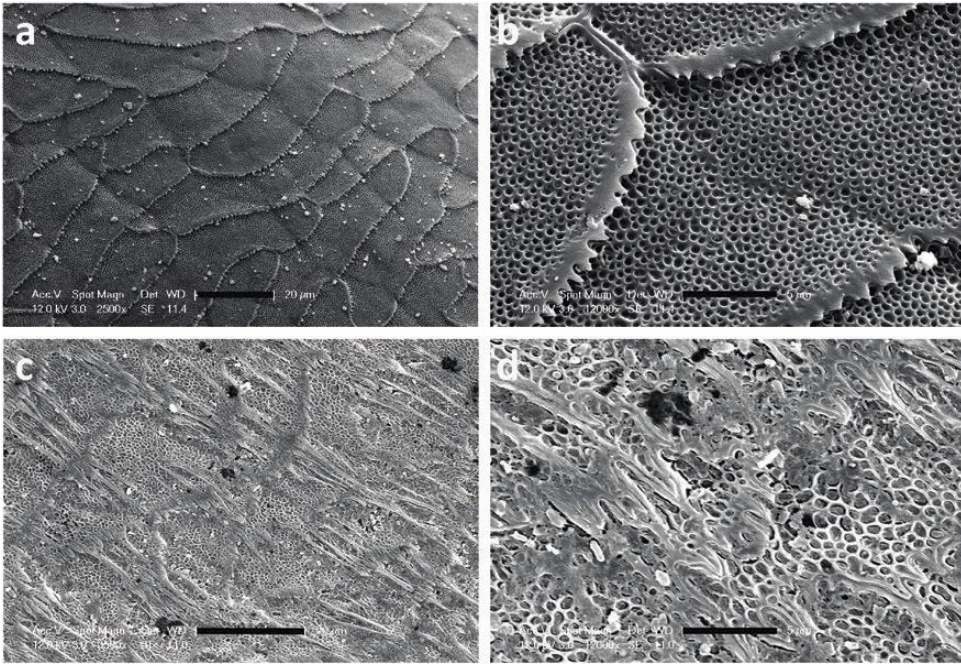
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images of unstained slides show pigments cells the epidermis (ED) and dermis (D) in an g) adult lure

448

and h) neonate lure. Arrows indicate "black" bands in the neonate.

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450

451 **Figure 5.** Epidermal microstructure of skin taken from dorsal scales on the midbody using scanning

452 electron microscopy. a, b) Midbody skin from adult; c, d) midbody skin from neonate.

453