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

Abstract

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

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

INTRODUCTION

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

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

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

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, Fl, USA) and analysed using Ocean View software v1.6.7

135   (Ocean Optics, Dunedin, Fl, 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 200µm) 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. antarticus* 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. antarticus* 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. obvs.), 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 "transitionary" 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.

Author contributions: L.A., J.M.C-R, B.S. and K.L.S. conceived of the study. S.D., L.P., J.H.N., M.F., B.S.

and J.M.C-R collected reflectance data and tissue samples. L.A. and N.D. were responsible for animal

husbandry. L.P. and J.M.C-R conducted microscopy analyses. J.M.C-R, A.L. and J.C.P. analysed

reflectance data. J.M.C-R wrote the manuscript with input from all authors.

Data accessibility: Supplementary methods, tables and figures are provided as Supplementary

Materials. Images of caudal lures for specimens used in this study are available at

doi/10.25909/13239497. Reflectance data for individual specimens, diet data, and code used to plot

diet and reflectance data available at https://github.com/jcroweriddell/death-adder-lure-

reflectance. Supplementary video S1 of luring behaviour available at https://vimeo.com/462914012.

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Figures

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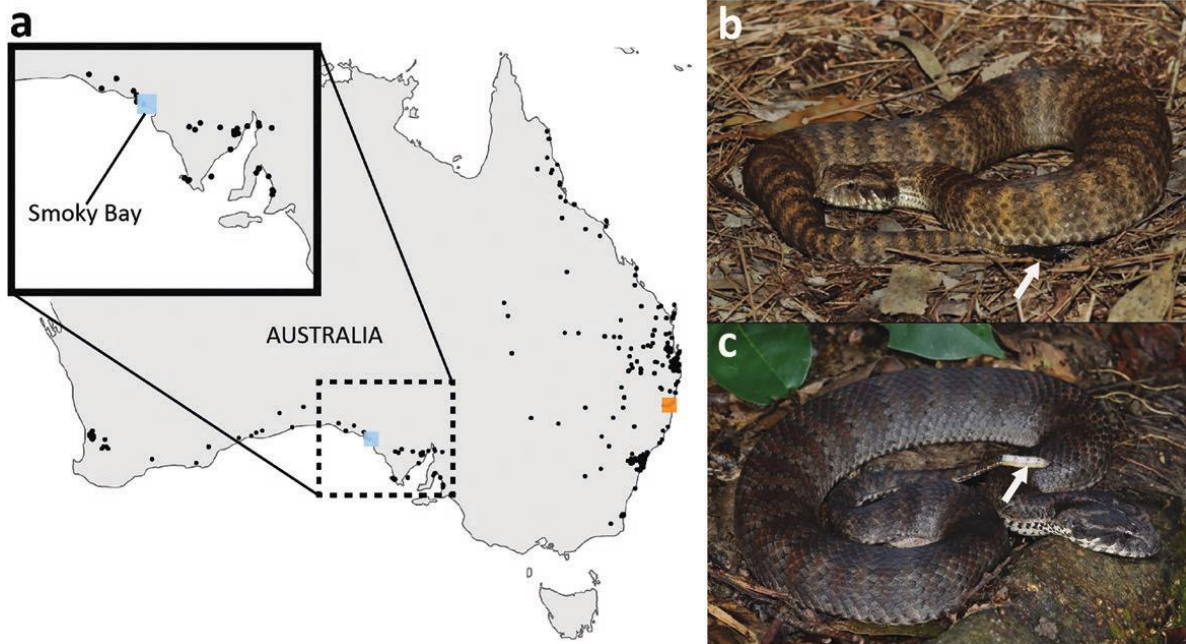
Figure 1. Distribution and colour variation in *Acanthophis antarcticus*. a) Disjunct distribution of *A. antarcticus* in Australia; inset shows the locality of source population in Smoky Bay, South Australia. b) Image of adult *A. antarcticus* showing black caudal lure that is typical for snakes from southern Australia. c) Image of adult *A. antarcticus* showing white caudal lure that is typical for individuals from the eastern coast of Australia. White arrows indicate location of the caudal lure; coloured squares on map show capture site for snakes shown in images: Smoky Bay, South Australia (b, blue), and Bellingen, New South Wales (c, orange). Occurrence data from the Atlas of Living Australia occurrence downloaded at https://doi.org/10.26197/5e44150a40c6a accessed on 13 February 2020. Image credits: Luke Allen, Shane Black.

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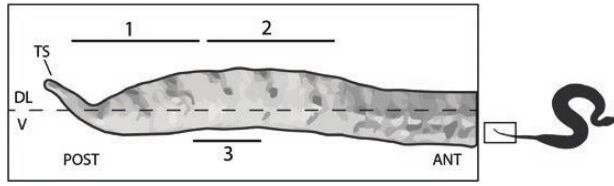
Figure 2. Schematic of the caudal lure in *Acanthophis antarcticus* showing locations where reflectance was measured. One measurement was taken each at the darkest and lightest point on dorsal-lateral scales, location of measurement was recorded as closest to posterior end of tail (position 1) or closest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

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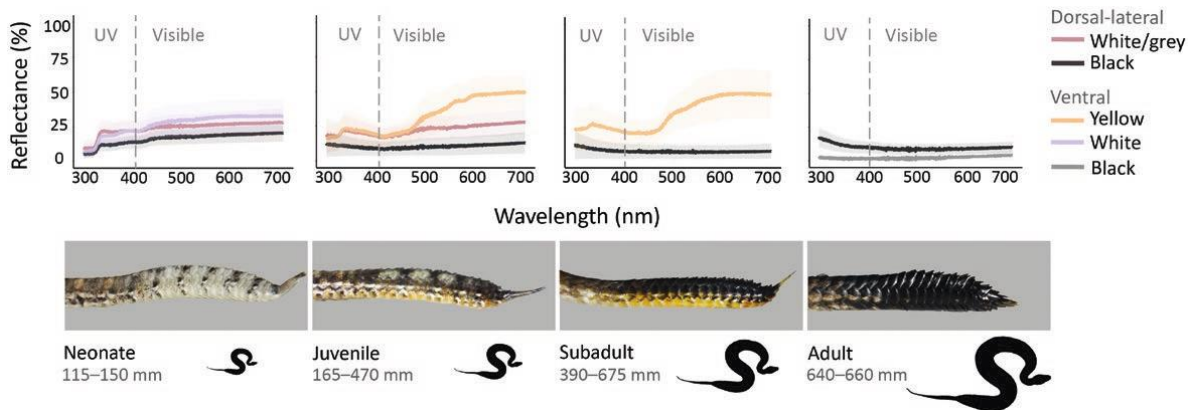
Figure 3. Ontogenetic colour change of caudal lures in *Acanthophis antarcticus*. Representative images of lure morphologies are shown at the different life stages, which correspond to snout to vent length. Plots show spectral reflectance of each colour recorded for the different lure morphologies; division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

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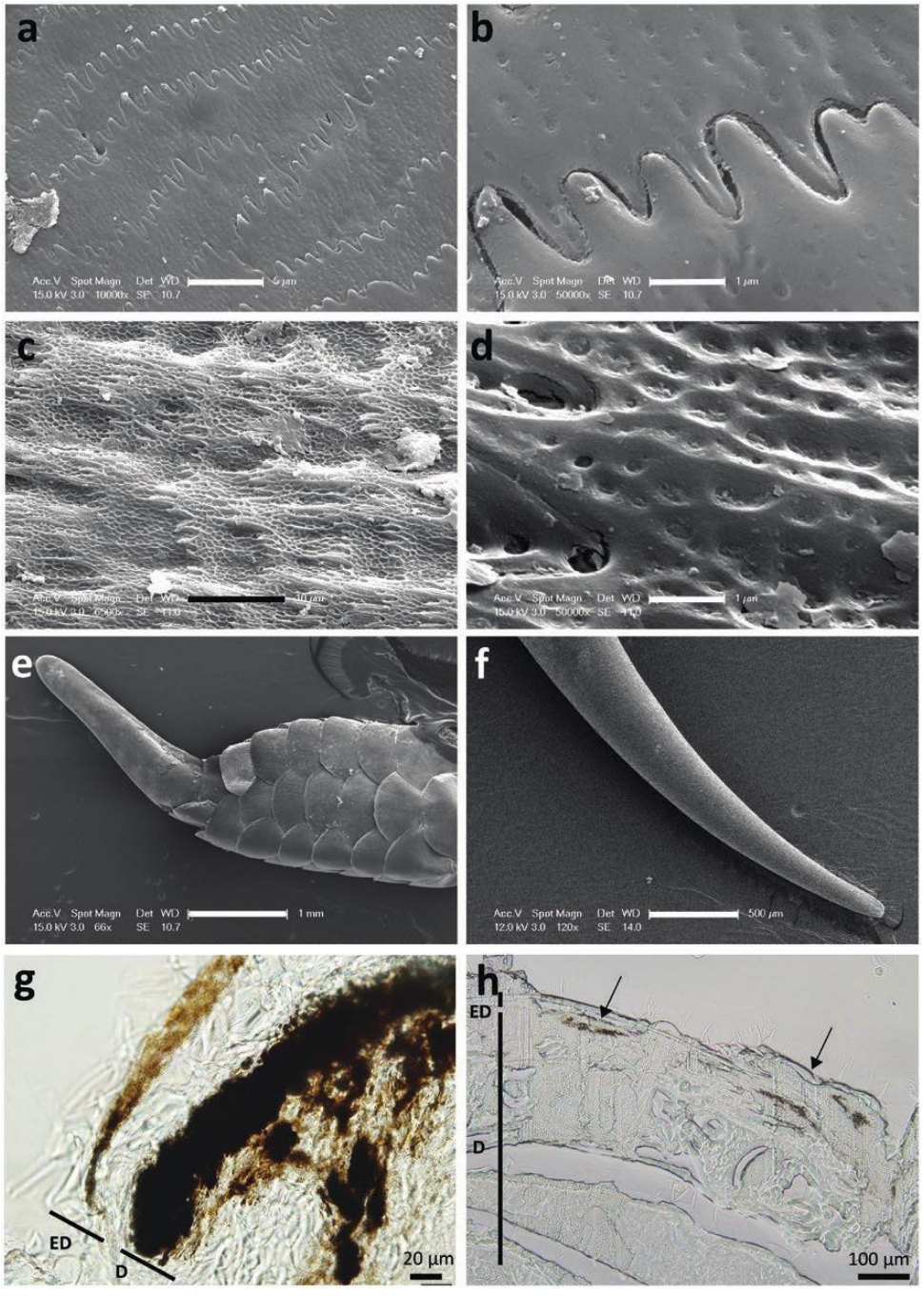
Figure 4. Skin structures underlying ontogenetic colour change in caudal lures in *Acanthophis*

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

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

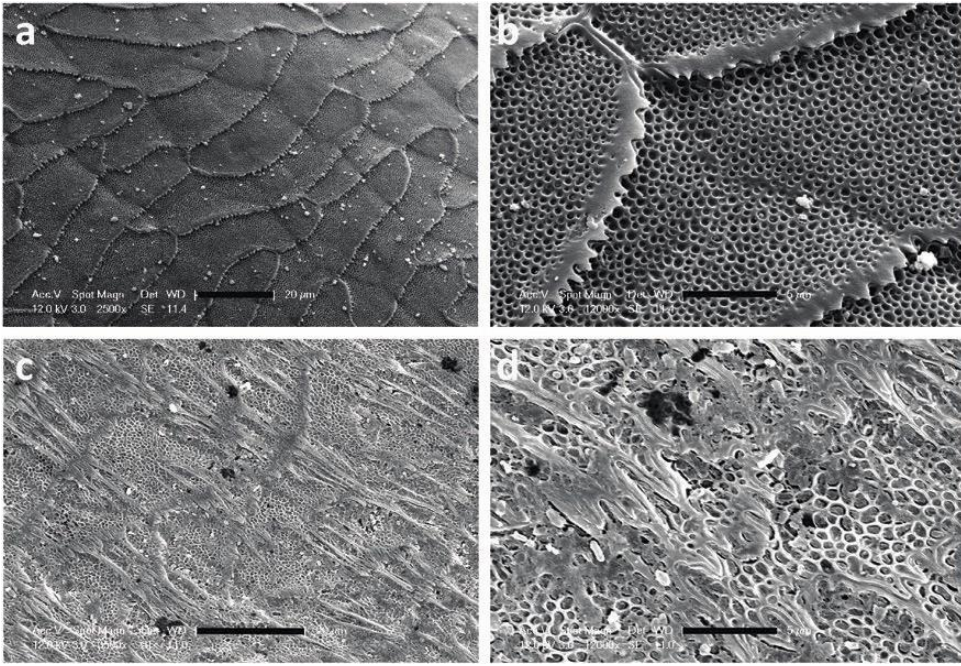images of unstained slides show pigments cells the epidermis (ED) and dermis (D) in an g) adult lure

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

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

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

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