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1 **Phototactic tails: Evolution and molecular basis of a novel sensory trait in sea snakes**

2 Running title: Phototactic tails in sea snakes

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22

23 **Abstract**

24 Dermal phototaxis has been reported in a few aquatic vertebrate lineages spanning fish, amphibians
25 and reptiles. These taxa respond to light on the skin of their elongate hind-bodies and/or tails by
26 withdrawing under cover to avoid detection by predators. Here, we investigated tail phototaxis in sea
27 snakes (Hydrophiinae), the only reptiles reported to exhibit this sensory behaviour. We conducted
28 behavioural tests in 17 wild-caught sea snakes of eight species by illuminating the dorsal surface of
29 the tail and mid-body skin using cold white, violet, blue, green and red light. Our results confirmed
30 phototactic tail withdrawal in the previously studied *Aipysurus laevis*, revealed this trait for the first
31 time in *A. duboisii* and *A. tenuis*, and suggested that tail photoreceptors have peak spectral sensitivities
32 between blue and green light (457-514 nm). Based on these results, and an absence of photoresponses
33 in five *Aipysurus* and *Hydrophis* species, we tentatively infer that tail phototaxis evolved in the
34 ancestor of a clade of six *Aipysurus* species (comprising 10% of all sea snakes). Quantifying tail
35 damage, we found that the probability of sustaining tail injuries was not influenced by tail phototactic
36 ability in snakes. Gene profiling showed that transcriptomes of both tail skin and body skin lacked
37 visual opsins but contained melanopsin (*opn4x*) in addition to key genes of the retinal regeneration and
38 phototransduction cascades. This work suggests that a non-visual photoreceptor (e.g. Gq rhabdomeric)
39 signalling pathway underlies tail phototaxis, and provides candidate gene targets for future studies of
40 this unusual sensory innovation in reptiles.

41 **Keywords:** extraocular, dermal phototaxis, sea snakes, dermal photoreception, melanopsin

42

43 **Introduction**

44 Most organisms use non-visual light detection to regulate essential physiological and behavioural
45 functions (Wolken 1995). Prominent roles of non-visual photoreception include colour changes in the
46 skin that facilitate camouflage, communication or thermoregulation, phototactic orientation and
47 movement, and the circadian and seasonal timing of key biological events (Peirson et al. 2009; Foster
48 & Soni 1998). Various cephalic or ‘extraocular’ tissues have been linked to non-visual photoreception,

49 such as the parietal organ and pineal complex (Foster & Soni 1998). In organisms lacking fur or
50 feathers, the skin also provides a primary site for non-visual photoreception (Kelley & Davies 2016).

51 Dermal photoreception or the ‘dermal light sense’ mediates dermal phototaxis, defined here as the
52 movement, including the whole body or a body part of an organism, towards or away from light
53 (Kelley & Davies 2016; Steven 1963; Millott 1968). This sensory modality is best known among
54 marine invertebrates, of which many species migrate along vertical light gradients and show abrupt
55 withdrawal responses to sudden changes in light intensity (reviewed in Wolken 1988; Ramirez et al.
56 2011). Among vertebrates, dermal phototaxis have been described in lampreys (Young 1935; Steven
57 1950; Ronan & Bodznick 1991), hagfish (Patzner 1978; Steven 1955), aquatic salamanders, and a
58 single frog (*Xenopus laevis* tadpole) (Alder 1976; Baker et al. 2015; Pearse 1910; Reese 1906; Sayle
59 1916). Olive sea snakes (*Aipysurus laevis*) are the only reptiles reported to show dermal phototaxis
60 (Zimmerman & Heatwole 1990), but this species’ phototactic behaviour is strikingly similar to that of
61 the other elongate, aquatic vertebrates.

62 Sea snakes, lampreys, hagfish and aquatic salamanders all exhibit dermal photosensitivity that is
63 most pronounced at the dorsal tips of their tails and stimulates negative phototaxis. Lamprey larvae
64 and hagfish respond to tail illumination by deflecting their tails, swimming and/or burrowing to
65 conceal themselves in river and lake beds (Ullén et al. 1993; Steven 1955; Deliagina et al. 1995;
66 Young 1935; Binder & McDonald 2008; Patzner 1978). Resting olive sea snakes and aquatic
67 salamanders respond with localised tail movements, often retracting their tail-paddles under reef or
68 rock overhangs.

69 The convergent innovation of phototactic tails in elongate aquatic taxa that diverged relatively
70 early in the >400 million-year evolutionary history of vertebrates suggests similar selection for
71 concealment from predators. These selection pressures may be particularly strong in animals with
72 vulnerable hind-bodies and tail paddles that are anatomically remote from the concentration of sensory
73 organs on the head. Sea snakes have various predators, such as sharks and marine mammals, and
74 specimens often have bite injuries to their tails, sometimes resulting in partial loss of the paddle
75 (Heatwole 1975; Masunaga et al. 2008). Tail paddles are vital to efficient underwater locomotion so

76 tail damage must impact feeding, mating success and vulnerability to predation (Aubret & Shine
77 2008). Zimmerman and Heatwole (1990) demonstrated that captive *A. laevis* sea snakes concealed
78 their tails under artificial reef during daylight more often than night, when tails were more likely to be
79 protruding while the rest of the body was concealed. Hence, phototactic responses are expected to
80 provide protection during daytime (and possibly dim-light) resting periods.

81 The genetic and physiological mechanisms underlying dermal phototaxis remain largely unknown
82 for any vertebrate taxon (Kelley & Davies 2016). Hindering research progress is a conspicuous
83 absence of photoreceptive structures such as stacked membranes or lenses within photoreceptive skin
84 (Ramirez et al. 2011). However, gene expression studies have revealed visual opsins in colour-
85 changing cells within the skin of cephalopods (Kingston et al. 2015; Ramirez & Oakley 2015;
86 Mäthger et al. 2010), teleosts (Schweikert et al. 2018; Ban et al. 2005; Chen et al. 2013) and gekkonid
87 lizards (Fulgione et al. 2014). This shows that the dermal photoreceptors involved in colour change
88 likely evolved by co-opting existing visual photoreceptor pathways of the eye, despite lacking
89 structures found in classical photoreceptors (Kingston & Cronin 2016; Ramirez et al. 2011). Other
90 studies in teleosts (Bertolesi & McFarlane 2018) and amphibians (Provencio et al. 1998; Moriya et al.
91 1996) have identified a role for non-visual opsins in colour change, implying that independent, non-
92 visual photoreceptor pathways underlie dermal photoreception in these diverse taxa.

93 In this study, we sought to better understand the evolution and molecular basis of tail phototaxis in
94 sea snakes. We first used behavioural tests of tail (caudal) phototaxis in wild-caught sea snakes from
95 eight species with the aim to better resolve the evolutionary origin of the trait. We then screened for
96 candidate phototaxis genes expressed in the skin of two phototactic species. Because the dermal
97 photoreceptive structures and genes involved in phototaxis are entirely unknown, we comprehensively
98 profiled genes related to visual and non-visual photoreceptors in whole transcriptomes of tail and body
99 skin, eye and other available organs. Finally, we quantified injuries on the tails of species with and
100 without phototactic abilities, with the expectation that phototactic species might have lower bite rates
101 indicating greater protection from attacks or have increased bite rates due to an intrinsically higher
102 vulnerability to predation.

103 **Materials and Methods**

104 **Specimens**

105 Sea snakes are fully marine squamate reptiles that are phylogenetically nested within the Australo-
106 Papuan terrestrial front fanged snakes (Elapidae: Hydrophiinae). Phototactic responses were measured
107 in 17 wild-caught, captive individuals of eight species that spanned all major lineages of sea snakes:
108 *Aipysurus laevis* (the only sea snake previously tested for phototactic behaviour), three other
109 *Aipysurus* species, three *Hydrophis* species and one semi-aquatic species *Hydrelaps darwiniensis*
110 (Supplementary materials; Table S1; ESM File 1). Tail injuries were recorded for a total of 111
111 museum specimens from two phototactic species, *A. laevis* ($n = 39$) and *A. duboisii* ($n = 12$), and two
112 non-phototactic species *H. major* ($n = 45$) and *H. stokesii* ($n = 15$) (ESM File 2). The examined
113 specimens were chosen from the same collection locality (Gulf of Carpentaria, Queensland, Australia)
114 to minimise the effect of geographic variation in predation pressures; specimen information (snout-
115 vent length, weight, sex and age) was available from (Fry G, personal communication).

116 Experiments and euthanasia were conducted in accordance with the Animal Ethics
117 Committees of University of Adelaide (S-2015-119) and University of Florida (201502798) and
118 specimens were collected and transported in accordance with Department of Parks and Wildlife of
119 Western Australia licences to take fauna for scientific purposes (Permit #SF010002) and export fauna
120 interstate (Permit #EA007665), Department of Environment, Water and Natural Resources of South
121 Australia import permit (Permit #I12978) and from the Area de Conservación Arenal Tempisque
122 (ACT) del Sistema Nacional de Areas de Conservación (SINAC), Costa Rica (No. ACT-OR-DR-055-
123 17).

124 **Behavioural experiments**

125 *Experimental set up*

126 During experiments on *A. duboisii*, *A. laevis*, *A. tenuis* and *H. major* at the University of Adelaide, a
127 snake was transferred from the seawater holding tank (24–28°C, 450L volume, 35 ppm, 12 h:12 h day:

128 night) to a round, black plastic behavioural arena (60 cm diameter × 60 cm height, 50 L volume) filled
129 with seawater (24-28°C, 35 L volume, 13 cm depth) and covered by a mesh net. The arena was housed
130 in a dark room lit by a single florescent red globe positioned 1 m above the arena (Figure S7). A lid
131 was placed over the arena for 1-2 h to allow the snakes to adapt to the arena before initiating trials.
132 Trials were recorded with a camera (GoPro Hero3+, Go Pro Inc., USA; 29.97 fps; 1920 × 1080)
133 positioned above the behavioural arena. During experiments on *A. laevis*, *A. mosaicus*, *A. tenuis*, *H.*
134 *darwiniensis*, *H. major*, *H. stokesii* and *H. platurus* at field sites, snakes were transferred to a
135 rectangular, black plastic behavioural arena (66 cm length × 44 cm width × 23 cm height, 60 L
136 volume) filled with freshwater (29 L, 10 cm depth) and covered by a mesh net. A lid was placed over
137 the arena as described in experiments at the University of Adelaide, and trials were recorded directly
138 by the observer and (where possible) with a camera (GoPro Hero3+, Go Pro Inc., USA; 29.97 fps;
139 1920 × 1080) positioned above the behavioural arena.

140 *Light source*

141 The light stimulus was delivered to localised areas of skin (Figure 4B) using a hand-held flashlight
142 (UltraFire SH98 3-mode white light zooming, WhaFat Technological, Hong Kong) that incorporated a
143 light-emitting diodes (LEDs) bulb that emitted white light with a spectral range of 300-900 nm. To test
144 phototactic responses to different wavelengths of light, a hand-held flashlight (UltraFire 4-in-1 1-mode
145 light) with interchangeable coloured LED bulbs was used to emit four colours: violet, blue, green and
146 red of wavelengths of 393 nm, 457 nm, 514 nm and 623 nm, respectively. The flashlights were
147 powered by two 7.4 volts rechargeable batteries (Fenix ARB-L3, Fenixlight, USA) that were re-
148 charged after 6-12 trials to maintain a near-constant light output. At the start of each trial, the relative
149 flashlight irradiance was measured using a PM100 digital optical power meter (Thorlabs, USA) and
150 S210A UV-NIR thermal power head held 30 cm below the flashlight. Spectral and relative irradiance
151 measurements are in Supplementary Materials (Figure S1).

152 *Behavioural trials*

153 Trials commenced after the snake had been inactive for at least 2 min. Experiments consisted of two to
154 four sets of six trials, each trial being separated from the next by intervals of at least 1 h. Each snake
155 was subjected to a mean of 17 trials over the course of the experiment. White light was shone on the
156 dorsal surface of the tail skin ($T_{(s)}$) for duration of 5.3 s (\pm 1.30 s) at a distance of approximately 30cm.
157 To control for the possibility that snakes responded to scattered light reaching the eyes, or the sight or
158 sound of approaching experimenter, a white light was also shone on the dorsal surface of the mid-body
159 skin ($B_{(s)}$) (Figure 1; Figure S7). Presentation of light was alternated between $T_{(s)}$ and $B_{(s)}$, and the
160 order of presentation was reversed every set of six trials, for *A. duboisii* ($n = 1$), *A. laevis* ($n = 4$), *A.*
161 *mosaicus* ($n = 1$), *A. tenuis* ($n = 2$), *H. major* ($n = 1$) and *H. platurus* ($n = 2$). In separate experiments,
162 white light was presented only on $T_{(s)}$ in a single individual each of *A. laevis*, *A. tenuis*, *A. mosaicus*,
163 *Hydrelaps darwiniensis*, *H. major* and *H. stokesii* (Table S1). *Aipysurus tenuis* ($n = 2$) appeared to be
164 responsive to illumination of the body in addition to the tail, thus a new experiment was performed to
165 test for phototactic response to body illumination in this species using an individual from *A. laevis* (n
166 = 1) as a control. Experiments consisted of six sets of five trials in which white light was shone on the
167 dorsal surface of the body at four locations (Table S2); light was presented sequentially along the body
168 and the order of presentation was reversed between each trial set. A final experiment was conducted to
169 test for sensitivity to different colours of light (violet, blue, green and red) in *A. tenuis* ($n = 2$) and *A.*
170 *laevis* ($n = 1$).

171 Consistent with previous behavioural testing (Zimmerman & Heatwole 1990), a response was
172 considered as ‘negative phototaxis’ if the part of the skin illuminated moved away from the light
173 within 10 s and no other part of the snake moved. Behavioural responses were converted to a
174 phototactic score to indicate whether a negative phototaxis occurred (Table 1) and mean response per
175 species was calculated as a percentage (%) of trials in which phototaxis was observed. Latency to
176 response was determined by viewing video footage of the phototactic response frame-by-frame (i.e. at
177 33.4 ms intervals) in GoPro Studio software v2.5.12 (CA, USA), and calculated as the difference
178 between the time at which the light stimulus was switched on and the time at which the first
179 phototactic movement of the snake occurred.

180 We mapped phototactic behaviour as a binary character (tail phototaxis absent or present) onto
181 an existing phylogeny for sea snakes (Sherratt et al. 2018). Using these data, the most parsimonious
182 interpretation of the origin of phototaxis in sea snake evolution was inferred by eye based on the
183 assumption that gains and losses of this trait are rare and equally likely.

184 **Transcriptome profiling**

185 *Tissue collection*

186 Because the cellular structures responsible for light-sensing in the tails of sea snakes are unknown, we
187 were unable to target specific locations in the skin for differential gene expression. Instead, we
188 sampled the whole skin tissue (dermis, epidermis, beta layer) from three regions (two tail and one
189 body) in two phototactic species and used whole transcriptome profiling to identify phototaxis genes.
190 Seven skin samples were taken for RNA-sequencing: the photoreceptive tail tip of two *A. laevis* and
191 one *A. tenuis*, putatively non-photoreceptive anterior ventral surface of the tail of a single *A. laevis* and
192 *A. tenuis*, and the variably photoreceptive dorsal surface of the hind-body a short distance anterior of
193 the vent of a single *A. laevis* and one *A. tenuis* (Figure 4B). In addition to the skin samples, we
194 assessed the tissue-specificity of expression of genes related to photoreception by also sampling four
195 non-skin tissues available from other projects: whole eye of *A. laevis*, and heart, testis and liver of the
196 olive-headed sea snake *H. major* (Table S3).

197 *Details of RNA extraction, sequencing, filtering and assembly*

198 Tissues were homogenised using mortar and pestle in lysis buffer and grinder with liquid nitrogen
199 before extracting total RNA (Roche Tissue RNA extraction kit). Library preparation and transcriptome
200 sequencing for six skin tissues was performed by the Queensland Brain Institute Centre for Brain
201 Genomics (QBI, Brisbane, Australia), for the eye by Beijing Genomics Institute (BGI, Shenzhen,
202 China), and for one skin, testis, heart and liver by Australian Genome Research Facility (AGRF,
203 Adelaide, Australia). Following RNA extraction (Roche Tissue RNA Extraction Kit) and quality
204 control, dual indexed TruSeq libraries were generated and sequenced on an Illumina HiSeq2000

205 machine (Illumina Inc., San Diego, CA) using V4 chemistry, producing 125 and 150 bp paired-end
206 sequencing reads.

207 The quality of the raw reads was assessed using FastQC v0.11.4 (Andrews 2010), QUAST
208 v4.5. (Quality Assessment Tool for Genome Assemblies; Gurevich et al. 2013), and using *ngsReports*
209 v.0.99 (Ward et al. 2018) package in R. v3.4.2 (R Core Team 2017). Adapter sequences and low-
210 quality reads were trimmed using AdapterRemoval v2.1.7 (Schubert et al. 2016) applying default
211 quality parameters and a minimum sequence length of 20 bp. To reconstruct transcriptomes, *de*
212 *novo* assembly was carried out the Trinity v2.5.1 pipeline (Grabherr et al. 2011; Haas et al. 2013) with
213 default settings and a minimum contig length of 200 bp. Following transcript assembly, protein-coding
214 regions were determined using TransDecoder v3.0.1. (Haas et al. 2013). Finally, assemblies were
215 assessed for completeness, both by assessing the RNA read representation of the assemblies by
216 aligning the trimmed reads back to their respective assemblies using Bowtie2 v2.2.9 (Langmead &
217 Salzberg 2012) and by examining the presence of full-length protein-coding genes in the assemblies
218 by searching against the SwissProt protein databases (The UniProt Consortium 2017) using BLAST+
219 (Camacho et al. 2009).

220 Unsupervised clustering of tissue samples was carried out using multi-dimensional scaling
221 plots in R using the *edgeR* package v3.20.1 (Robinson et al. 2009) and log counts per million, with
222 gene selection set to ‘pairwise’ for the top 500 genes. The intersections of expression levels among
223 tissue samples were explored using the *UpSetR* package v1.3.3 (Lex et al. 2016).

224 *Abundance estimates of genes*

225 Estimated transcript abundances were generated using Salmon v8.2 (Patro et al. 2017), a pseudo-
226 alignment program that quantifies gene expression without the need for direct genome alignments.
227 RNA reads were mapped to a pitviper (*Protobothrops mucrosquamatus*) transcriptome (Aird et al.
228 2017), which was the best-annotated and closely related transcriptome currently available, and
229 quantified reads were normalised using fragments per kilobase of transcript per million mapped reads
230 (FPKM). To compare transcript abundance of genes related to photoreception among tissue samples,

231 FPKM counts were filtered by reference sequence gene categories (O’Leary et al. 2016) for predicted
232 mRNA that are known to be involved in phototransduction and retinoid metabolism pathways of
233 squamate reptiles (Schott et al. 2017) (Table S4). FPKM counts (Table S5) for visual genes were then
234 log-transformed and a heatmap generated in R using the *heatmap* package v1.0.8 (Kolde 2012) (ESM
235 File 3).

236 *Verifying the presence of genes related to visual and non-visual photoreceptors*

237 Many vertebrate visual genes are part of large gene families that have high sequence similarity but
238 include genes with non-visual functions (Porter et al. 2011). To verify the sequence identity of
239 quantified transcripts with putative visual functions, we assessed the phylogenetic position of
240 assembled sequences within maximum likelihood trees of visual genes from representative vertebrate
241 groups (*Python molurus*, *P. mucrosquamatus*, *Thamnophis sirtalis*, *Pogona vitticeps*, *Gekko*
242 *japonicus*, *Anolis carolinensis*, *Homo sapiens*). Transcripts nested within clades of vertebrate visual
243 genes were considered to be verified visual genes. Conversely, if transcripts were recovered inside a
244 clade of related genes with non-visual functions, these were considered to be erroneously mapped
245 reads and indicated as such on the FPKM heatmap. Briefly, putative visual transcripts were located by
246 custom nucleotide BLAST searches (Altschul et al. 1990) of assembled tissue transcriptomes (ESM
247 File 4) with visual genes from representative vertebrate groups: squamates (*P. molarus*, *P. vitticeps*, *T.*
248 *sirtalis*, *G. japonicus*), birds (*Gallus gallus*) and mammals (*Homo sapiens*), obtained from GenBank
249 (Coordinators 2016). Significant nucleotide BLAST search hits (E-value < 1e-02; bit score > 200)
250 were extracted from transcriptomes and aligned with representative vertebrate visual genes in
251 Geneious v9.1.8 (Kearse et al. 2012) using a MUSCLE translation alignment v3.4 (Edgar 2004).
252 Aligned sequences were checked for ambiguities and a maximum likelihood tree for each gene was
253 built using RAxML v7.2.8 (Stamatakis 2006). We used an unpartitioned GTR GAMMA substitution
254 model and the “rapid bootstrapping and search for best-scoring ML tree” algorithm with 1000
255 replicates. Trees were rooted by con-familial genes or, if tree was for a single gene only, a mammal
256 gene sequence.

257 **Quantifying tail injuries**

258 Tail condition was recorded in museum specimens of sea snake (Figure S8). To evaluate the
259 prevalence of tail injuries among the sampled species we used a hurdle model to examine 1) the
260 presence of tail damage, and conditional upon damage occurring, 2) the number of tail injuries. The
261 presence of damage was modelled assuming a binomial variance and logit link, and the count of tail
262 injuries component of the model assumed a truncated Poisson variance and log link. We included the
263 interaction between snout-vent length (cm) and species in our models because older (typically larger)
264 snakes are expected to have more tail injuries and this relationship may differ between species. Snout-
265 vent length (svl) was mean-centred for analysis. Other explanatory variables (sex, weight) were
266 assessed using likelihood ratio tests. The likelihood of tail damage seen in non-phototactic species (*H.*
267 *major* and *H. stokesii*) was compared to that observed in phototactic species (*A. laevis* and *A. duboisii*)
268 using planned contrasts (Torsten et al. 2008). These analyses were conducted in R using additional
269 packages *multcomp* v1.4.8 (Bretz & Westfall 2014) and *countreg* v0.2 (Zeileis et al. 2008).

270 **Results**

271 **Evidence for dermal phototaxis in eight species and evolutionary origin in sea snakes**

272 Our behavioural tests provided evidence of negative tail phototaxis in all individuals of *Aipysurus*
273 *laevis*, *A. tenuis* and *A. duboisii* (tail withdrawals in response to white LEDs, 100%, 87% and 70% of
274 trials, respectively), but not in any of *A. mosaicus* (5%), *Hydrophis major* (3.2%), *Hydrelaps*
275 *darwiniensis*, *Hydrophis stokesii* or *H. platurus* (Figure 1A). Consistent with previous observations in
276 *A. laevis* (Zimmerman & Heatwole 1990), tail phototaxis in individuals of *A. laevis*, *A. duboisii* and *A.*
277 *tenuis* was a stereotyped movement of the tail towards the centre of body mass and away from the
278 light stimulus (Table 1; ESM File 5). Tail latencies recorded for the three phototactic species showed
279 that tails moved within 7 seconds (s) of illumination. Mean response times were 2.1 s for *A. tenuis*, 2.5
280 s for *A. duboisii* and 3.4 s for *A. laevis* (Figure 1B), and the shortest tail latencies recorded for each
281 species were 0.25 s for *A. tenuis*, 0.55 s for *A. laevis* and 1.05 s for *A. duboisii* (Figure 1B). To control

282 for the effects of the experimenter and to test for phototactic responses to scattered light reaching the
283 eyes, trials of tail response were alternated with trials of white light shone on the mid-body (instead of
284 tail). This was done for 11 individuals of six species, and yielded no phototactic responses to mid-
285 body illumination in *A. duboisii*, *A. mosaicus*, *H. major* and *H. platurus*, and low response rates in *A.*
286 *tenuis* (9% of 11 trials, $n = 2$) and *A. laevis* (2.8% of 36 trials, $n = 5$) (Figure 1A).

287 To test preliminary observations of phototactic responses to hind-body illumination in *A.*
288 *tenuis*, a separate experiment was performed on *A. tenuis* ($n = 2$) and *A. laevis* ($n = 1$). Here, phototaxis
289 was also recorded in response to white LED light shone on four dorsal regions along the body axis.
290 *Aipysurus tenuis* showed a stereotyped withdrawal (movement of the illuminated part of the body
291 towards the centre of body mass and away from the light stimulus; ESM File 6) in response to
292 illumination of the dorsal skin on the hind-body (pre-vent, 66.7% of 12 trials), posterior mid-body
293 (16.7% of 12 trials), anterior mid-body (16.7% of 12 trials) and neck region (22.1% of 13 trials), but at
294 a comparatively lower rate compared to tail illumination (100% of 12 trials; ESM File 6). In contrast,
295 no phototactic responses were recorded in any of the four regions of body skin in *A. laevis*.

296 For phototactic species *A. tenuis* and *A. laevis*, we compared latencies of responses to tail
297 illumination from four different wavelengths of light produced by LEDs having approximately equal
298 intensities. The results suggest peak sensitivities of tail photoreceptors between 457 and 514 nm
299 (Figure 2); but this pilot experiment did not allow us to generate full response curves for spectral
300 sensitivity or latency of tail movement because only four wavelengths were tested. Relative irradiance
301 measurements for the white, violet, green, blue and red light are shown in Figure S1.

302 Based on the results of our behavioural tests, and an expectation that evolutionary gains and
303 losses of phototaxis are rare and equally likely, the most parsimonious inference is that this sensory
304 modality evolved in the ancestor of a clade of six *Aipysurus* species: *A. laevis*, *A. fuscus*, *A. tenuis*, *A.*
305 *duboisii*, *A. foliosquama* and *A. apraefrontalis* (Figure 3). An alternative scenario under which
306 phototaxis evolved in the common ancestor of all *Aipysurus* and was lost on the lineage leading to *A.*
307 *mosaicus* involves one additional step. Hence, pending future studies of additional *A. mosaicus*
308 individuals and key taxa such as *Emydocephalus* and *Ephalophis-Parahydrophis* (indicated by

309 asterisks on Figure 3), we tentatively infer a single origin of phototaxis within *Aipysurus*, and an
310 absence of this trait in all other sea snakes (i.e. 90% of species, including the ~50 *Hydrophis* species).

311 **Expression of genes related to visual and non-visual photoreceptors**

312 Assembled transcriptomes for sea snake eye, heart, liver, testis and seven skin tissues were profiled for
313 genes relating to visual and non-visual photoreceptors (Table S3; S4). Five vertebrate
314 phototransduction genes were not detected in the eye transcriptome (*sws2*, *rho2*, *grk1*, *gnat1*, *gucy2F*,
315 *pde6a*, *pde6h*). This is consistent with previous genomic and transcriptomic studies that suggest these
316 genes are missing in snake genomes, hence they were not profiled in the remaining tissue
317 transcriptomes. Summary statistics for sequencing, assembly and transcript completeness are given in
318 Table 2, Table 3 and Supplementary Results (Figure S2). Multidimensional scaling plots and overall
319 expression profiles for tissue transcriptomes are also given in Supplementary Results (Figure S3; S4;
320 S5).

321 *Opsins*

322 Three genes encoding for visual opsins (*opn1sw*, *rho1*, *opn1lw*) were detected in *A. laevis* eye, and a
323 single visual opsin (*opn1sw*) was detected in *H. major* testis (Figure 4). Genes for two non-visual
324 opsins were also expressed: *xenopus-like* melanopsin (*opn4x*) was detected in *A. laevis* eye, *H. major*
325 testis and two skin transcriptomes each from *A. laevis* and *A. tenuis*. Neuropsin (*opn5*) was expressed
326 in *A. laevis* eye, *H. major* testis and a single skin transcriptome from *A. laevis* (Figure 4).

327 *Phototransduction*

328 A total of 24 genes related to phototransduction in visual photoreceptors of vertebrates (i.e. ciliary
329 genes) were detected in the *A. laevis* eye, 17 in *H. major* testis, nine in *H. major* heart, seven in *H.*
330 *major* liver and 13 across *Aipysurus* skin tissues (Figure 4). There was no discernible co-expression
331 pattern between putatively photoreceptive skin and variably or non-photoreceptive skin (Figure 4).
332 Phototransduction genes detected in the majority (four or more) of skin tissue samples were *arrb2*,
333 *gna11*, *guca1b*, *cdc-like*, *cdc-likeb1*, *cdc-likeb3*, *pde6d*, and *pde6g* (Figure 4). Genes *grk7-like* and

334 *grk5* were detected in a single skin transcriptome each from *A. laevis* and *A. tenuis*, and *sag* in a single
335 skin transcriptome from *A. tenuis* (Figure 4). Phototransduction genes *grk5*, *guca1a/c*, *pdcc*, *gnat2*, *sag*,
336 and *rcvrn* were detected in the skin using FPKM levels, but gene tree analyses indicated that these are
337 most likely homologous with *grk5-like*, *guca1b*, *pdcc-like2/3*, *gnai2/3*, *arrestin C-like*, and *hippocalcin-*
338 *like (hpcl-like)*, respectively (ESM File 7). The following 11 phototransduction genes were not
339 detected in skin transcriptomes: *cnga3*, *cngb1*, *cngb3*, *gnat2*, *guca1a*, *guca1c*, *gucy2d-like*, *pdcc*, *pdcc-*
340 *like2*, *pde6b-like*, *pde6c* and *slc24a2* (Figure 4; ESM File 7). Genes related to phototransduction in
341 non-visual photoreceptors (e.g. intrinsically-photoreceptive retinal ganglion cells; ipRGCs) and
342 invertebrate visual photoreceptors (i.e. rhabdomeric genes), *gna11*, *plcb1*, *plcb3*, *plcb4*, were also
343 profiled and found to be widely expressed across all organs including skin (data not shown).

344 *Retinoid regeneration*

345 A total of 13 genes related to retinoid regeneration were detected in the *A. laevis* eye, eight in *H.*
346 *major* testis, nine in *H. major* heart, seven in *H. major* liver, and eight across *Aipysurus* skin tissues
347 (Figure 4). There was no discernible co-expression pattern between putatively photoreceptive skin and
348 variably or non-photoreceptive skin (Figure 4). Genes detected in the majority (more than four) skin
349 tissues were *lrat*, *rdh8-like*, *rdh10*, *rdh11-like*, *rdh12-like*, *rdh14*, and *rpe65*, and the gene *rgs9bp* was
350 detected in two skin tissues from *A. laevis* (Figure 4). The identity of some retinoid regeneration genes
351 that were detected in the skin tissues using FPKM levels (*rdh5* and *rgs9*) could not be verified by
352 custom nucleotide BLAST searches and phylogenetic analysis (ESM File 7). The following retinoid
353 regeneration genes were not detected in skin transcriptomes: *abca4*, *rbp3*, *rdh5*, *rgr* and *rgs9*.

354 **The relationship between tail damage and phototactic ability**

355 Phototactic species *A. laevis* and *A. duboisii* had slightly higher proportions of damaged tails (67% and
356 58 %, respectively) compared to non-phototactic species *H. major* (47%) and *H. stokesii* (40%) from
357 the same geographic location (Gulf of Carpentaria, Australia) (Table S6). We predicted that
358 phototactic ability (i.e. species) would explain differences in the likelihood of tail damage occurring.
359 However, there was no effect of species on likelihood of tail damage ($\chi^2 = 2.5$, $P = 0.47$; Table S7).

360 There was a positive relationship between body length (measured from the snout to the vent: svl) and
361 the probability of tail damage; 10cm increases in svl nearly doubled the likelihood of tail damage
362 (1.97-fold increase, 95% confidence interval 1.26-3.27; $\chi^2 = 10.9$, $P = 0.001$; Table S7; Figure S6).
363 This relationship was consistent across all the species sampled (i.e., no species *svl interaction; $\chi^2 =$
364 1.4, $P = 0.69$). We therefore found no evidence for our *a priori* hypothesis of differences in the
365 likelihood of tail damage between non-phototactic (*H. major* and *H. stokesii*) and phototactic species
366 (*A. laevis* and *A. duboisii*; Table S8). Furthermore, conditional on damage occurring, there was no
367 evidence for differences in the number of injuries between species ($\chi^2 = 5.4$, $P = 0.14$) or associated
368 with size (i.e., svl; $\chi^2 = 0.05$, $P = 0.9$; Table S7).

369 **Discussion**

370 Our study presents substantial new data on a novel sensory trait that is underexplored in vertebrates.
371 The difficulties inherent in collecting and housing live sea snakes meant that we were unable to
372 extensively replicate behavioural experiments. However, our tests of 17 individuals of eight species
373 yielded highly consistent results with low variability both within individuals and among individuals
374 within species. These results confirm the phototactic ability of the only previously studied species of
375 reptile (the olive sea snake, *Aipysurus laevis*) and reveal this trait for the first time in *A. duboisii* and
376 *A. tenuis* (Figure 1; ESM File 5). We recorded phototactic responses of the hind-body in *A. tenuis* that
377 have not previously been reported and may be linked to the elongate body form of this species (thus
378 increased distance between the hind-body and cephalic sensory organs). All other species tested
379 showed little or no response to light on the body skin, which suggests that photoreceptive regions are
380 primarily located in the tail skin.

381 We found that snakes were most responsive to blue and green light, and least responsive to violet
382 and red. Considering the narrow bandwidth and the approximately balanced in light output (at least, in
383 energy terms) of the coloured LEDs, we suggest that dermal photoreceptors have spectral sensitivities
384 between 457 and 514 nm (the peaks of the blue and green LEDs). Such a spectral location is consistent
385 with the spectral sensitivities of other dermal photoreceptors such as chromatophores in cephalopods

386 (470–480 nm; Ramirez and Oakley 2015) as well as that of our candidate non-visual opsins (e.g.
387 melanopsin; Díaz et al. 2016; Bertolesi and McFarlane 2018). However, our pilot experiment lacks the
388 necessary spectral resolution that would allow us to distinguish melanopsin-based photoreception,
389 with a peak sensitivity typically around 480nm, from that of rhodopsins (with peak sensitivities
390 generally around 500 nm). Latencies recorded for sea snake tails were comparable to hagfish and
391 lampreys, i.e. between one and six seconds (Newth & Ross 1954; Steven 1955).

392 Based on an expectation that losses and gains of phototaxis are rare, we offer a preliminary
393 hypothesis that this sensory modality originated in the ancestor of a clade of six *Aipysurus* species
394 (Figure 3). To better resolve the origin of phototaxis, future studies will be needed to increase
395 individual sampling (particularly of putatively non-phototactic *Aipysurus*, i.e. the *A. mosaicus* species
396 complex), and target key lineages such as *Emydocephalus* and *Ephalophis-Parahydrophis*.
397 Nevertheless, the absence of phototactic responses in six individuals from four species that are widely
398 distributed in the large *Hydrelaps-Hydrophis* clade suggests that most of the > 60 known sea snake
399 species lack phototactic tails, prompting the question of why only some sea snakes have evolved (or
400 retained) this sensory behaviour.

401 Numerous species traits must influence vulnerability to predation and/or the locomotory costs of
402 tail damage, including diel and spatial activity patterns, preferred habitat and depth, and size of body
403 and tail. *Aipysurus* species have smaller geographic ranges and stronger patterns of mitochondrial
404 geographic structure compared to *Hydrophis* (Nitschke et al. 2018); these observations indicate lower
405 dispersal propensities in *Aipysurus*, which might result in slower swimming speeds and thus stronger
406 selection for strategies for crypsis such as tail phototaxis. However, there is no particular trait, or
407 combination of traits, that solely characterizes the species shown (or inferred) in our study to have
408 phototactic tails. All sea snakes have paddle-shaped tails used for locomotion, and all species are
409 active foragers that rest at times during the day, often under coral or rocky overhangs (Rasmussen et
410 al. 2011). Furthermore, we found no difference in the likelihood of tail damage or in the total number
411 of injuries sustained by phototactic *A. laevis* and *A. duboisii* compared to the non-phototactic *H.*

412 *stokesii* and *H. major*, which suggests that there is no intrinsically higher (or lower) vulnerability to
413 predation in the phototactic populations sampled. The *Aipysurus-Emydocephalus* and *Hydrophis*
414 clades, however, show notable differences in their adaptations to marine habits, including use of
415 different tissues to seal the mouth and different vertebral processes to support their tail paddles
416 (Sanders et al. 2012). Hence, a recent origin of tail phototaxis in just the *Aipysurus-Emydocephalus*
417 clade might be best explained by historical contingency, rather than an absence of similar selection
418 pressures in other sea snakes.

419 **Candidate genes underlying dermal phototaxis**

420 The conspicuous absence of classical visual photoreceptor structures in the skin of phototactic sea
421 snakes, lampreys, hagfish and aquatic amphibians poses a significant challenge to research on
422 vertebrate dermal photoreception. Based on our expectation that tail phototaxis could be mediated by
423 independent or novel genetic pathways, we decided to screen whole skin transcriptomes for genes
424 related to visual and non-visual photoreceptors. This approach yielded genes of interest in the eye of
425 sea snakes and low expression abundance and variably non-specific patterns of expression across
426 tissue types (Figure 4; Table S5). Below we discuss a putative role for these candidate genes in a non-
427 visual photoreceptor pathway in sea snake skin.

428 Light detection pathways begin with light-absorbing pigments such as the visual opsins that
429 are expressed in the classical retinal photoreceptors, rods and cones, and are also implicated in dermal
430 photoreception in cephalopods (Kingston & Cronin 2016; Ramirez & Oakley 2015), teleosts
431 (Schweikert et al. 2018; Chen et al. 2013) and gekkonid lizards (Fulgione et al. 2014). Absorption of
432 light by opsins initiates a complex phototransduction cascade in which the chromophore retinaldehyde
433 (vitamin A) bound with the opsin must photoisomerize from a *cis* to an *all-trans* conformation. In
434 visual opsin systems, photoisomerization then activates phosphodiesterase-6 (PDE6) through coupling
435 with a heterotrimer G protein ‘transducin’ (GNAT), producing a hyperpolarising current by the
436 opening of cyclic-nucleotide gated channels (CNG) in the photoreceptor membrane (Figure 5A).

437 As expected from transcriptomic and genomic studies of vision in snakes, several
438 phototransduction genes (*grk1*, *gnat1*, *gucy2F*, *pde6a*, *pde6h*) and two visual opsin genes (*sws2* and
439 *rho2*) were absent in the *A. laevis* eye transcriptome (Bhattacharyya et al. 2017; Davies et al. 2009;
440 Hart et al. 2012; Hauzman et al. 2017; Schott et al. 2015; Schott et al. 2017; Simões et al. 2016). All
441 three of the visual opsins found in snakes (*opn1lw*, *opn1sw* and *rho1*) were detected in the *A. laevis*
442 eye, but none were detected in the skin of *A. laevis* or *A. tenuis* (Figure 4). Consistent with the absence
443 of a visual opsin to absorb light, only a few vertebrate phototransduction genes were present in the
444 skin, and together these genes form an incomplete phototransduction cascade for image-forming
445 vision (Figure 5A). Importantly, we did not detect transcripts for GNAT (*gnat2*), PDE6 rod-specific
446 units (*pde6b*) and regulator of G-protein signalling 9 (*rgs9*). However, 13 visual phototransduction
447 genes were found to be expressed in the skin of sea snakes (Figure 4), providing to a shortlist of genes
448 that might be involved in independent, non-visual photoreceptor pathways (Figure 5).

449 We detected in the skin two candidate light-absorbing pigments for initiating tail phototaxis in
450 sea snakes: ‘xenopus-like’ melanopsin (*opn4x*) and neuropsin (*opn5*) (Figure 5C) are vertebrate genes
451 associated with a range of non-visual protein functions and patterns of tissue-specific expression.
452 Neuropsin is present in the brain and skin of vertebrates, and is thought to play a role in retinal
453 photoentrainment, changes of skin colour in fish (Buhr et al. 2015; Schweikert et al. 2018) and dermal
454 phototaxis in *Xenopus* tadpoles (Currie et al. 2016). The ‘mammal-like’ class of melanopsin (*opn4m*)
455 is present in the ipRGCs of the eye (Provencio & Warthen 2012; Bellingham et al. 2006) and some
456 cranial nerves (Matynia et al. 2016), and has a range of photosensory functions including
457 photoentrainment of molecular clocks, local pupil light reflex, DNA repair and melatonin synthesis
458 (reviewed in Peirson et al. 2009; Bertolesi and McFarlane 2018). The role of *opn4x* is understudied
459 but it is expressed in a wide range of tissues including the brain and eye of fish, amphibians, reptiles,
460 turtles and birds (reviewed in Davies et al. 2014). Because *opn4x* is expressed in dermal melanophores
461 of fish and amphibians (Oshima 2001; Bertolesi & McFarlane 2018; Provencio et al. 1998) and
462 neuromasts of the lateral line system in *Xenopus* tadpoles (Baker et al. 2015) it is a good candidate

463 pigment for non-visual light detection pathways in non-mammalian vertebrates such as sea snakes
464 (Kelley & Davies 2016).

465 The pathways interacting with *opn4x* are incompletely known, but the gene is similar in DNA
466 sequence and function to opsins that use phototransduction pathways of invertebrate photoreceptors
467 (i.e. rhabdomic) (Graham et al. 2008; Isoldi et al. 2005; Díaz et al. 2016). Following
468 photoisomerization, melanopsin is thought to activate a G-protein Gq/11 (GNAQ / GNA11) and
469 phospholipase C (PLC) second messenger cascade, producing depolarizing currents by the activation
470 of TRP-like channels (TRP) (Díaz et al. 2016). We detected genes that encode the primary proteins in
471 the putative melanopsin pathway, GNAQ (*gnaq*) and PLC beta (*plcb1*, *plcb3*, *plcb4*), across a range of
472 sea snake tissues including skin (data not shown), suggesting that some type of Gq rhabdomic
473 signalling pathway is possible for melanopsin-based dermal photoreception in sea snakes. However,
474 these genes are also integral to a range of cellular pathways, so further molecular studies are needed to
475 confirm their role in tail phototaxis. If *opn4x* is indeed responsible for mediating phototaxis in sea
476 snakes, previous studies of *opn4x* expression (Baker et al., 2015; Davies et al., 2014; Provencio et al.,
477 1998) would suggest three candidate cell types that may be associated with dermal photoreceptors: 1)
478 dermal melanophores involved in colour change, 2) dermal mechanoreceptors, and 3) peripheral nerve
479 endings in the epidermis that may or may not be associated with dermal mechanoreceptors. Given that
480 dermal phototaxis is not linked to colour change in sea snakes, we suggest that future studies are most
481 likely to find photoreceptive structures in either peripheral nerve endings in the epidermis or dermal
482 mechanoreceptors, or a combination of both.

483 The phototransduction cascade is completed with the regeneration of *all-trans*-retinaldehyde
484 to supply new *cis*-retinaldehyde to the opsin, which involves retinal pigment epithelium 65 Da
485 (RPE65), lecithin retinol acyltransferase (LRAT) and various retinol dehydrogenase (RDH) proteins
486 (Figure 5B). We detected seven genes involved in retinal regeneration that were widely expressed
487 across *Aipysurus* skin and *Hydrophis* tissues (Figure 5B), including *rpe65*, the expression of which is
488 generally thought to be restricted to the retinal pigment epithelium and cone photoreceptors of the eye
489 (Wright et al. 2015). *Rpe65*, in conjunction with *lrat* (also expressed in the tail skin), has a key role in

490 isomerization of the opsin chromophore (Wright et al. 2015; Saari 2012). Although the *opn4m* has an
491 intrinsically photoisomerizing (i.e. bistable) function, light stability in *opn4x* is variably monostable or
492 bistable depending on the isoform and/or taxon (Díaz et al. 2016; Tu et al. 2006). Significantly,
493 associated retinal regeneration proteins of the eye, *rlbp1* and *rgr*, are absent from *Aipysurus* skin
494 tissues. Although the operation and interaction of *opn4x* and/or *opn5* with *rpe65* and *lrat* (and other
495 retinal regeneration genes) within the skin is not entirely clear, a role in the regeneration of opsin
496 chromophore in dermal photoreceptors would seem likely.

497 **Conclusions**

498 Our sea snake skin transcriptomes yielded non-visual opsins (and an absence of visual opsins), in
499 addition to several phototransduction and retinal cycle genes, providing preliminary evidence that tail
500 phototaxis may be mediated by genes related to non-visual photoreceptors that do not involve image-
501 forming vision but rather provide information on overall light levels in the environment. Although
502 future studies are needed to confirm a functional role of our candidate genes in mediating tail
503 phototaxis, and uncover the precise location of photoreceptive structures in sea snake skin, these
504 findings highlight the utility of gene expression profiling as a first step in identifying the molecular
505 mechanisms underlying sensory evolution. Dermal phototaxis may be more prevalent in vertebrates
506 than currently recognised. We suggest that it is likely to be particularly important for aquatic or
507 burrowing taxa with elongate bodies and/or tails that are anatomically remote from the concentration
508 of sensory organs on their heads. Transcriptome profiling studies in other reptiles (including putatively
509 non-phototactic sea snakes) should target skin to identify patterns of taxon- and tissue-specific
510 expression of genes related to visual and non-visual photoreceptors.

511

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525 **Data accessibility:** Supplementary materials, results, tables and figures attached separately. Raw
526 RNA-reads are available at NCBI Sequence Read Archive (SUB4931382). Electronic supplementary
527 data are available at Figshare (DOI: 10.25909/5c1ade89814b0) including:

- 528 • **ESM File 1:** Beh-exp.xlsx containing raw and summary data for behavioural experiments of
529 17 individuals from eight species of sea snake.
- 530 • **ESM File 2:** Tail-beh-injuries.xlsx containing raw data of tail damage in museum specimens
531 of sea snakes.
- 532 • **ESM File 3:** FPKMheatmap.zip containing R script for generating MDS plot & FPKM
533 heatmap, TPM matrix for each sea snake transcriptome created using Salmon
- 534 • **ESM File 4:** TranscriptomeAssemblies-PhototacticTails-skin-heart-liver-eye-testis.zip
535 containing transcriptomes for sea snake tissues assembled using Trinity pipeline.
- 536 • **ESM File 5:** Video containing examples of tail phototaxis in sea snakes
- 537 • **ESM File 6:** Video containing examples of body phototaxis in sea snakes.
- 538 • **ESM File 7:** RAXML-gene-trees.zip containing maximum likelihood gene trees in fasta and
539 nexus format shows relationship among putative phototaxis sea snake transcripts and
540 phototaxis genes from representative vertebrate lineages.

541 **Author contributions:** J.M.C.-R. and K.L.S conceived of the study. J.M.C.-R. and K.L.S conducted
542 field work and behavioural experiments. J.M.C.-R., K.L.S, B.F.S. and D.J.G. collected tissues. A.L.,
543 J.B. and J.G.S. generated summary statistics for mRNA reads. J.G.S. assembled transcriptomes. J.B.
544 conducted read quantification. J.M.C.-R. and A.L. conducted gene profiling with input from B.F.S.,
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547 manuscript was written by J.M.C.-R. and K.L.S. with significant input from all co-authors. The
548 authors declare no competing interests.

549

550 **Tables**

551 **Table 1:** Categories of behavioural responses to light on the skin. Phototactic scores were negative
552 phototaxis = 1 and no phototaxis = 0.

<i>Category</i>	<i>Description of behaviour</i>	<i>Phototactic score</i>
CW	Tail withdraws completely out of light within 10 s and no other part of snake moves	1
W	Tail withdraws away from light within 10 s and no other part of snake moves	1
TL	Tail tilts from dorsal plane to sagittal plane within 10 s and no other part of snake moves	0
TJ	Sudden movement of tail only	0
BJ	Sudden movement of body only	0
B	Body undulates as in swimming movement	0
HT	Head moves to location of tail, tail may or may not withdraw	0
BW	Body withdraws away from light within 10 s and no other part of snake moves	1
NR	No response, body and tail do not change position	0

553

554 **Table 2.** Statistical summary of sequencing.

Species	ID	Phototactic region	Paired end raw reads	% Removed	Trimmed reads	Reads mapped	% Alignment
<i>Aipysurus laevis</i>	KLS0459	Eye	41,960,313	0	41,960,313	37,485,594	89.3
		Skin photoreceptive tail tip (dorsal)	30,343,697	37.9	18,839,010	18,377,480	97.6

	KLS0656	Skin photoreceptive tail tip (dorsal)	16,098,369	43.6	9,082,453	8,060,311	88.7
		Skin non-photoreceptive tail (anterior)	17,696,227	28.8	12,601,077	11,943,910	94.8
		Skin non-photoreceptive body near vent (dorsal)	27,845,728	36.8	17,588,152	16,791,799	95.5
<i>Aipysurus tenuis</i>	KLS0654	Skin photoreceptive tail tip (dorsal)	20,397,990	52.0	9,798,407	8,841,047	90.2
		Skin non-photoreceptive tail (anterior)	31,707,833	32.2	21,486,415	20,745,575	96.6
		Skin photoreceptive body near vent (dorsal)	16,828,408	32.1	11,428,795	10,375,249	90.8
<i>Hydrophis major</i>	KLS0460	Heart	31,189,072	41.0	18,392,676	17,928,884	97.5
		Liver	31,187,724	39.8	18,782,654	18,265,069	97.2
		Testis	27,457,192	40.4	16,371,706	15,700,688	95.9

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Table 3. Summary statistics of Trinity assemblies.

<i>Specimen</i>			<i>Contigs</i> <i>No. assembled</i>	<i>Length</i> <i>Total length (bp)</i> <i>Longest contig (bp)</i> <i>N50 contig length (bp)</i>			<i>Contigs >= 1000 bp</i>		<i>Contigs >= 5000 bp</i>		<i>Contigs >= 10000 bp</i>		<i>GC %</i>
							<i>No.</i>	<i>%</i>	<i>No.</i>	<i>%</i>	<i>No.</i>	<i>%</i>	
<i>Spp.</i>	<i>ID</i>	<i>Tissue</i>											
A.	KLS												
<i>laevis</i>	0459	Eye	230,892	80,614,082	18,092	2,806	24,256	10.5	2,721	1.2	130	0.06	42.3
		Skin photoreceptive tail tip (dorsal)	130,893	70,608,010	21,767	2,164	24,074	18.4	1,163	0.9	21	0.02	42.7
	KLS	Skin photoreceptive tail tip (dorsal)	49,779	5,155,121	8,160	814	1,223	2.5	11	0.0	0	0.00	43.6
		Skin non-photoreceptive tail (anterior)	90,353	23,528,511	10,860	1,121	7,786	8.6	50	0.1	2	0.00	44.7
		Skin non-photoreceptive body near vent (dorsal)	159,456	63,237,696	14,787	1,784	21,986	13.8	708	0.4	20	0.01	44.4
A.	KLS	Skin photoreceptive tail tip (dorsal)	76,189	7,419,783	8,560	780	1,643	2.2	4	0.0	0	0.00	42.7
<i>tenuis</i>	0654	Skin non-photoreceptive tail (anterior)	118,552	44,671,944	10,895	1,478	16,550	14.0	144	0.1	4	0.00	44.4
		Skin photoreceptive body near vent (dorsal)	92,552	14,741,471	10,723	892	4,120	4.5	21	0.0	2	0.00	44.4
H.	KLS												
<i>major</i>	0460	Heart	124,657	68,064,584	51,061	1,995	23,763	19.1	820	0.7	18	0.01	42.9
		Liver	125,968	49,361,792	19,451	1,595	18,496	14.7	178	0.1	8	0.01	42.8
		Testis	200,649	92,040,799	11,282	1,743	32,250	16.1	784	0.4	5	0.00	43.1

560

561 **Figures**

562 **Figure 1.** Negative phototaxis in response to white LED light on the dorsal surface of skin in
563 sea snakes. ‘Negative phototaxis’ was recorded if the illuminated region moved away from
564 the light within 10 s and no other part of the snake moved. A) Response (%) to light on tail
565 skin and body skin in eight species; asterisks indicate species in which light was shone on the
566 tail skin only. B) Tail latency in the phototactic species; box plots represent median (middle
567 solid horizontal line), mean (black dots) and range (dotted line) of latencies across a mean of
568 6 trials per individual.

569 **Figure 2.** Negative tail phototaxis in response to four coloured LED lights, violet (392 nm),
570 blue (347 nm), green (514 nm) and red (623 nm), in three captive individuals from two
571 species, *Aipysurus laevis* (n = 1) and *A. tenuis* (n = 2), across a mean of 6 trials per individual.
572 ‘Negative phototaxis’ was recorded if the illuminated region moved away from the light
573 within 20 s and no other part of the snake moved.

574 **Figure 3.** Phylogenetic tree of sea snakes showing distribution of tail phototaxis: red branches
575 represent species that showed phototactic responses to localised white light on the tail but not
576 the mid-body, blue branches represent species that were unresponsive to localised white light
577 on both the tail and mid-body, and untested species are shown as grey branches. Based on
578 these currently available data (17 individuals of 8 species), the most parsimonious inference is
579 that tail phototaxis evolved in the ancestor of a clade of six *Aipysurus* species (the node
580 marked with a red dot). The only previously studied species, *Aipysurus laevis*, is indicated by
581 red asterisk. Tree modified from Sherratt et al., (2018); legend is in millions of years ago
582 (MYA); image of *Aipysurus tenuis* shows regions that were tested for phototactic responses,
583 taken with permission from Mirtschin, Rasmussen, & Weinstein (2017).

584 **Figure 4.** Gene profiling of tissue transcriptomes from *Aipysurus laevis*, *A. tenuis* and
585 *Hydrophis major* sea snakes. A) Heatmaps show normalised expression levels of genes for
586 visual pigments (opsins), phototransduction cascades related to visual photoreceptors and
587 retinoid regeneration. RNA reads were quantified by pseudoalignment to a pitviper
588 (*Protobothrops mucrosquamatus*) transcriptome; fragments per kilobase of transcript per
589 million mapped reads (FPKM) were log-transformed for visualising in heatmap; strikethrough
590 cells indicate transcripts whose visual function could not be verified by nucleotide BLAST
591 searching and phylogenetic analysis (ESM File 7). B) Schematic diagram of tail showing
592 where skin tissues were collected from phototactic species (*A. laevis* and *A. tenuis*). Putative
593 dermal light sensitivity is indicated by red circle (photoreceptive), green triangle (non-
594 photoreceptive) and blue square (photoreceptive in *A. tenuis* only). Diagram modified from
595 Zimmerman and Heatwole (1990).

596 **Figure 5.** Visual and non-visual phototransduction pathways; highlighted genes are expressed
597 in the sea snake skin tissue. A) Vertebrate phototransduction pathways specific to rod
598 photoreceptors (black circles), cone photoreceptors (red circles) and both rods and cones (blue
599 circles). Genes absent in snake genomes are also indicated (dashed line) and the visual genes
600 present in eye but absent in skin transcriptomes are faded. B) The retinoid regeneration
601 pathway (green circles). C) Non-visual opsins found in both putative phototactic and non-
602 phototactic sea snake skin (shaded purple). Diagrams modified from Fu (2015); Invergo,
603 Montanucci, Laayouni, & Bertranpetit (2013); Saari (2012).

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