

1 **Out of the blue: The evolution of horizontally polarized signals**  
2 **in *Haptosquilla* (Crustacea, Stomatopoda, Protosquillidae)**

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35 The polarization of light provides information that is used by many animals for a  
36 number of different visually guided behaviours. Several marine species, such as  
37 stomatopod crustaceans and cephalopod molluscs, communicate using visual  
38 signals that contain polarized information, content that is often part of a more  
39 complex multi-dimensional visual signal. In this work, we investigate the  
40 evolution of polarized signals in species of *Haptosquilla*, a widespread genus of  
41 stomatopod, as well as related protosquillids. We present evidence for a pre-

42 existing bias towards horizontally polarized signal content and demonstrate that  
43 the properties of the polarization vision system in these animals increase the  
44 signal-to-noise ratio of the signal. Combining these results with the increase in  
45 efficacy that polarization provides over intensity and hue in a shallow marine  
46 environment, we propose a joint framework for the evolution of the polarized  
47 form of these complex signals based on both efficacy-driven (proximate) and  
48 content-driven (ultimate) selection pressures.

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50

## 51 **INTRODUCTION**

52

53 Polarization sensitivity is a common visual specialization that has evolved in  
54 both terrestrial and aquatic animals, and is particularly prevalent in  
55 invertebrates (Wehner and Labhart, 2006). On land, many insects use the  
56 celestial polarization pattern for navigation (Wehner, 1976; Rossel and Wehner,  
57 1986; Labhart and Meyer, 1999; Dacke et al., 2003), while in the ocean, some  
58 crustaceans and cephalopod molluscs use polarization information to detect  
59 prey and possibly as a means of conspecific communication (Shashar et al., 1996;  
60 Cronin et al., 2003a; Chiou et al., 2007; Mäthger et al., 2009; Cronin et al., 2009;  
61 Chiou et al., 2011). In the context of communication, polarization often forms  
62 composite signals with other visual dimensions, such as hue and brightness  
63 (Cronin et al., 2003a; Cronin et al., 2009).

64 The term polarization is used to define several properties of light. The  
65 angle of polarization describes the predominant direction in which the electric  
66 field of the light oscillates, while the degree of polarization defines the extent to  
67 which waves oscillate at the same angle. Underwater, differential sensitivity to  
68 either angle or degree of polarization has several fundamental advantages over

69 other forms of visual information (Cronin et al., 2003a; Cronin et al., 2003b;  
70 Cronin et al., 2009; Shashar et al., 2011). For instance, in shallow, clear marine  
71 waters, the intensity and spectral composition of the down-welling light can vary  
72 dramatically, both as a function of the time of day, and because of environmental  
73 factors such as turbidity (Cronin et al., 2014). In such changing conditions, the  
74 polarization of light remains more constant than other visual dimensions over  
75 short ranges (Waterman, 1954; Cronin, 2001), which renders it a reliable  
76 provider of information (Shashar et al., 2011; Johnsen et al., 2011). Previous  
77 research in this field has focused on either the underlying retinal mechanisms of  
78 polarization sensitivity (for review see Horváth and Varjú, 2004; Roberts et al.,  
79 2011), or the optical mechanisms by which polarization and multi-component  
80 polarization/colour signals are produced (Chiou et al., 2005; Mäthger and  
81 Hanlon, 2006; Chiou et al., 2007; Mäthger et al., 2009; Cronin et al., 2009). In  
82 contrast, the evolutionary context of polarization signal content relative to the  
83 visual system of receivers is still very much unknown.

84         Stomatopod crustaceans are some of the best-studied species in terms of  
85 polarization vision. Electrophysiological studies have detailed the spatial  
86 variation of polarization sensitivity in the different photoreceptor classes in the  
87 eye (Kleinlogel and Marshall, 2006; Chiou et al., 2008). Optical measurements  
88 (Marshall et al., 1991; Chiou et al., 2008), optical modeling (Roberts et al., 2009)  
89 and molecular methods (Porter et al., 2009; Roberts et al., 2011) have provided  
90 additional information on the underlying mechanisms of polarization sensitivity.  
91 Optical techniques have also shown that many species of stomatopod produce  
92 visual signals that are either linearly or circularly polarized (Chiou et al., 2005;  
93 Chiou et al., 2008; Cronin et al., 2009). The stomatopod genus *Haptosquilla*

94 (family Protosquillidae) is known to use signals from the first maxillipeds for  
95 both sexual and agonistic communication (Dingle and Caldwell, 1969; Caldwell  
96 and Dingle, 1975; Chiou et al., 2011). A common feature of *Haptosquilla* first  
97 maxillipeds is the production of a conspicuous blue structural reflection (Chiou  
98 et al., 2005; Cronin et al., 2009). Fig. 1 illustrates the blue signal in four species:  
99 *Haptosquilla trispinosa*, *H. glyptocercus*, *H. stoliura* and *H. banggai*. In some  
100 species of the genus (e.g. *H. trispinosa*, *H. stoliura* and *H. banggai*), this reflection  
101 is also horizontally polarized (Chiou et al., 2005; Cronin et al., 2009).

102 Here we explore the potential evolutionary pathways of polarization  
103 communication in protosquillid stomatopods. First, we use experiments to  
104 investigate whether the behavioural responses to different forms of polarization  
105 signal content are species specific. We do this by exploiting the animal's innate  
106 behavioural responses to polarized looming stimuli presented on modified LCD  
107 monitors. We compare four representative protosquillid species: *H. trispinosa*, *H.*  
108 *glyptocercus*, *Chorisquilla tweediei* and *C. hystrix*. Second, and in the context of the  
109 signal's polarization content, we measure the threshold at which *H. trispinosa* are  
110 no longer able to discriminate between two different angles of polarization.  
111 Finally, we construct a phylogeny of protosquillid species to consider the  
112 evolution of the polarization properties of maxilliped signals.

## 113 RESULTS

114

### 115 Responses to polarized stimuli

116 *H. trispinosa*, *H. glyptocercus* and *C. tweediei* all showed a significantly greater  
117 probability of response to the horizontally polarized stimulus compared with a  
118 vertically polarized stimulus. (*H. trispinosa*: Wilcoxon Test:  $Z=2.93$ ,  $d.f = 9$ ,  $p =$

119 0.002; Fig. 2A; *H. glyptocercus*:  $Z = 2.42$ , d.f = 9,  $p = 0.02$ ; Fig. 2B; *C. tweediei*:  $Z =$   
120  $2.77$ , d.f = 9,  $p = 0.004$ ; Fig. 2C). *C. hystrix* also appeared to be more responsive to  
121 horizontally polarized light (Fig. 2D), but the small sample size ( $n=5$ ) precluded  
122 statistical testing. There was no significant difference between *H. trispinosa*, *H.*  
123 *glyptocercus* and *C. tweediei* in their relative responses to the two stimuli  
124 (Kruskal-Wallis test:  $\chi^2 = 2.90$ , d.f. = 2,  $p = 0.24$ ).

125

### 126 **Level of discrimination between two angles of linearly polarized light**

127 *H. trispinosa* showed little or no response to stimuli when the difference between  
128 the polarization angles of the stimulus and background was between 31.4  
129 degrees and 20 degrees (Fig 3, Supplemental Table S1). At angles of 20 degrees  
130 or less, the animals rarely responded to the polarization stimulus; at values of  
131 31.4 degrees and above, they displayed a consistent statistically significant  
132 response to the stimulus.

133

### 134 **Presence of polarized signals**

135 The 1<sup>st</sup> maxilliped reflections from *H. trispinosa*, *H. glyptocercus*, *C. tweediei* and  
136 *C. hystrix* are presented in the microscope images displayed in Figs 4A–D. Both *H.*  
137 *trispinosa* (Fig. 4A) and *H. glyptocercus* (Fig. 4B) show blue reflections from the  
138 maxillipeds compared with very weak, spectrally broad reflections from the  
139 *Chorisquilla* species (Figs 4C, D). Of the blue *Haptosquilla* reflections, *H. trispinosa*  
140 are horizontally polarized (Fig. 4A) whereas the reflections from *H. glyptocercus*  
141 are unpolarized (Fig. 4B).

142 Visual analyses of other species of *Haptosquilla* showed that *H. stoliura*, *H.*  
143 *banggai*, *H. pulchella*, *H. nefanda* and *H. hamifera* all have blue-reflecting 1<sup>st</sup>

144 maxillipeds, but only the reflections from *H. stoliura*, *H. banggai*, *H. pulchella* and  
145 *H. nefanda* are horizontally polarized. Within the rest of the Protosquillidae, five  
146 further species have been analyzed (*C. excavata*, *C. hystrix*, *C. tweediei*,  
147 *Echinosquilla guerinii*, and *Protosquilla folini*) with none possessing blue or blue  
148 and horizontally polarized 1<sup>st</sup> maxillipeds. Outside of the Protosquillidae, six  
149 other stomatopod species from nine genera and four families have been  
150 inspected for 1<sup>st</sup> maxilliped signal types. Of these species, only *G. smithii* possess  
151 blue signals and no other species possess either blue or horizontally polarizing  
152 signals (Fig. 5).

153

#### 154 **Phylogenetic analyses**

155 Phylogenetic analyses of protosquillid relationships recapitulate previous  
156 studies (Barber & Boyce 2006; Porter et al., 2010) recovering the protosquillids  
157 (bootstrap percentages (BP) = 98), and in particular the genus *Haptosquilla* (BP  
158 = 89), as monophyletic (Fig. 5). Within the *Haptosquilla*, our phylogeny  
159 recovered two sub-groups of species that correspond to the two known types of  
160 1<sup>st</sup> maxilliped signaling, either blue and unpolarized or blue and polarizing.

161

#### 162 **DISCUSSION**

163 Our results provide the direct evidence that several species of stomatopod have  
164 an *inherent* (i.e. non-trained) behavioural response to a looming, linearly  
165 polarized stimulus. Moreover, all the protosquillid species tested displayed a  
166 greater probability of response to horizontally polarized stimuli compared with  
167 those that are vertically polarized. The measurements of the structural colour  
168 and polarization properties of the maxillipeds, in combination with the

169 comparative phylogenetic analyses, revealed that of these protosquillids, only  
170 the genus *Haptosquilla* displays the blue signals. Furthermore, it is only the sub-  
171 group of *Haptosquilla* including *H. trispinosa* that possesses the additional  
172 polarized signal dimension. In these species, the polarization of the signals is  
173 always orientated horizontally. Therefore, it is possible that the common  
174 behavioural predisposition towards horizontally polarized stimuli seen across  
175 the protosquillids could have biased the polarization content of 1<sup>st</sup> maxilliped  
176 signals to be horizontal in the *H. trispinosa* clade (Guilford and Dawkins, 1991;  
177 Endler and Basolo, 1998). A common question raised by the concept of sensory  
178 bias is why does the bias preexist? Whilst we can only speculate, the bias for a  
179 horizontal angle of polarization may come from the fact that this angle is most  
180 prevalent in reflections from objects and preferential sensitivity may have  
181 previously evolved to improve contrast discrimination (Temple, 2012).

182

183 *H. trispinosa* also displayed a threshold of between 21.4 and 30 degrees in their  
184 response to distinguishing between two angles of polarization. Such a coarse  
185 level of discrimination would improve the signal-to-noise ratio of a linearly  
186 polarized signal by effectively low-pass filtering any variation in the background.  
187 This threshold is an order of magnitude higher than measured in other species  
188 (fiddler crab, *Uca vomeris*, 3.2 degrees - How et al., 2012; cuttlefish, *Sepia*  
189 *plangon*, 1 degree - Temple et al., 2012) and is suggestive of tuning for high  
190 contrast signals compared with the current evidence that other crustacean and  
191 cephalopod polarization visual systems are used to resolve high levels of  
192 polarization detail.

193           The complex nature of stomatopod eye design (two hemispheres  
194 separated by a specialized midband) may place limitations on the amount of  
195 information that can be processed from the visual scene but in turn enhance the  
196 processing efficiency. Currently, it is thought that the two hemispheres are  
197 primarily involved in producing a two-dimensional representation of the visual  
198 scene, over which the midband is then scanned, rather like a line-scan sensor, to  
199 expand on the colour and polarization information (Land et al., 1990). The  
200 motion component of the LCD looming stimulus used in our experiment is  
201 therefore most likely to be stimulating responses in the stomatopod visual  
202 hemispheres, which elicit a visual saccade to the target, and presumably this  
203 would be followed by a subsequent visual scan of the target with the midband to  
204 fill in the remaining information. It is conceivable therefore, that much of the  
205 early visual information is simplified to speed up sensory processing (for an  
206 equivalent discussion for colour vision see Thoen et al., 2014). If so, the  
207 polarization discrimination responses we have measured specifically represent a  
208 property of the visual system in the dorsal and ventral hemispheres. However,  
209 the precise behavioural context should also not be ignored. It is quite possible  
210 that the measured discrimination threshold is specific to the task demanded of  
211 the animals. Further work is also still needed to investigate how the degree of  
212 polarization affects behavioural responses to such polarization signals.

213           Overall, our findings provide a framework for understanding the potential  
214 evolutionary pathway of the polarization properties of these maxilliped signals  
215 in stomatopods. Successful communication relies on information being sent  
216 through the environment in such a way that it will be received in its intended  
217 form, and be interpreted as to elicit a behavioural response in the intended



218 receiver (Parten and Marler, 2005). In this context, the selective pressures on  
219 signal evolution are both efficacy-driven and content-driven (Guilford and  
220 Dawkins, 1991; Hebets and Papaj, 2005). As described in the Introduction,  
221 polarization provides a reliable form of visual information, particularly in  
222 spectrally variable light environments, such as the conditions that these species  
223 of stomatopod inhabit. The increase in signal efficacy by the inclusion of this  
224 extra visual dimension is therefore fairly clear. The behavioural bias towards  
225 horizontal polarized light provides a further explanation for why the polarized  
226 content of the signals has evolved to be horizontally polarized. Together, the  
227 addition of polarization to the signal and nature of the bias suggest both the  
228 proximate and ultimate drivers respectively for the evolution of this complex  
229 signal.

230         Two questions for the future are: can manipulating the relative  
231 polarization contrast of the signal and the background influence the bias?  
232 Secondly, do the spectral and polarization dimensions act independently for  
233 purposes of information redundancy or do they combine in a functional way; for  
234 example, increasing the accuracy of receiver response as is described by an  
235 amplifier hypothesis of multi-component signals (Hasson, 1991; Candolin, 2003;  
236 Hebets and Papaj, 2005)? We suggest that future studies of combined  
237 polarization and colour signals in other animals should also carefully consider  
238 how these dual dimensions are viewed together by receiver visual systems  
239 under the correct environmental light conditions. Whilst it is not always easy to  
240 decompose complex signals and test the functions of individual components  
241 (Hebets and Papaj, 2005), the combined colour and polarization signals in

242 stomatopods represent an excellent behavioural system to investigate the  
243 function and evolution of signal complexity.

244

245

## 246 **MATERIALS AND METHODS**

### 247 **Animals**

248 To investigate the inherent ability of stomatopods to generate distinct behavioral  
249 responses to polarized stimuli, we collected 39 individuals of *H. trispinosa*, 10  
250 individuals of both *H. glyptocercus* and *C. tweediei* and five individuals of *C.*  
251 *hystrix* from off-shore reefs near Lizard Island, Great Barrier Reef, Australia in  
252 August 2011 (Queensland–GBRMPA permit G12/35042.1). Animals were  
253 maintained before testing in a natural seawater flow-through marine aquarium  
254 facility at the Lizard Island Research Station (24–25°C, natural daylight  
255 illumination, and fed pieces of frozen shrimp). All procedures were approved by  
256 the Animal Ethics Committees of the University of Queensland (AEC, permit #  
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258

### 259 **Relationship between behavioural responses and polarization stimulus**

#### 260 **content**

261 Individual stomatopods were placed in a 30 x 15 x 15 cm tank containing local  
262 beach sand. Each individual was placed inside an 8 mm diameter clear tube and  
263 restrained using a small amount of fishing line (Land et al., 1990; Cronin et al.,  
264 1991). The animal was positioned such that the eyes were forward of the front  
265 end of the tube (Fig. 6A). Directly above the animal was a video camera (Canon  
266 Legria FS20) that recorded its response to the presentation of the stimuli. On the

267 outside of the tank, and in front of the animal, was an LCD screen (Viglen LC552;  
268 1280 x 1024 spatial resolution at 60 Hz); the eyes were at a distance of  
269 approximately 12 cm from the screen. By removing the front polarizer from the  
270 LCD screen and addressing the LCD with a grayscale value of either 0 (black) or  
271 255 (white), the local output polarization could be controlled as vertical (V  
272 stimulus) or horizontal (H stimulus) respectively (Pignatelli et al., 2011). The  
273 stimuli expanded to cover 22.5° of the visual field angle in 1 s (taking into  
274 account refraction at the air / glass / water boundaries). The simple electro-  
275 optic control of the polarization of the light permitted not only dynamic control  
276 of the polarization, but most importantly an inherent zero luminance and  
277 chromatic contrast between the background and the looming stimulus. To check  
278 the polarization properties of the LCD, accurate broadband Stokes parameter  
279 measurements (Fig. 6B) were made using Glan-Thompson polarizers and a ¼  
280 wave Fresnel-rhomb (Edmund Optics, York, UK), which permitted the  
281 computation of the polarization ellipse of each of the stimuli for any wavelength  
282 (Fig. 6C).

283 All animals received a balanced pseudo-randomized presentation of 10 H  
284 stimuli and 10 V stimuli, against a perpendicularly linearly polarized  
285 background. No more than three instances of the same stimulus were presented  
286 in a row. We randomly varied the time between successive stimuli, from 20 to  
287 120 s, to minimize any effect of habituation. To determine whether the animal  
288 responded to the two stimulus types, we monitored the optokinetic response of  
289 the focal animal. We defined a positive response to the stimulus as a saccadic eye  
290 movement, in which one or both eyestalks were rapidly brought together (see  
291 Fig. 7 for an example). No such saccadic eye movements were observed in a 5 s

292 period before the onset of the stimulus or from 3 s after its presentation. Animals  
293 were scored by their number of responses out of the 10 presentations giving a  
294 probability of response to each stimulus type.

295

### 296 **Discrimination threshold between two angles of linearly polarized light**

297 A similar method was used to measure the polarization angular contrast  
298 sensitivity of *H. trispinosa*. Individual unrestrained animals were housed in a 20 x  
299 20 x 30 cm aquarium partition in burrows positioned approximately 12 cm from  
300 the front wall. A different polarization LCD monitor (HP L1906; see How et al.,  
301 2012 for calibration details) to that described above, but with very similar  
302 properties, was positioned against the front wall. A looming circle stimulus  
303 expanded to cover 27° of the visual field angle in 1 s (taking into account  
304 refraction at the air / glass / water boundaries). The greyscale values addressed  
305 to the monitor were set to 0 (black) for the background and ranged between 0  
306 and 255 for the stimulus, resulting in a stimulus that varied in the angle of  
307 polarization against a horizontally polarized background, with no corresponding  
308 changes in hue or light intensity. Stomatopod eye movements in response to the  
309 stimulus were recorded using a digital video camera (Sony HDR-SR11, Tokyo,  
310 Japan) mounted on the top edge of the front aquarium wall. Stimuli were  
311 generated automatically using MATLAB (r2011, Mathworks, Natick, MA, USA)  
312 and the whole experiment was conducted without experimenter intervention.  
313 Video recordings were synchronized to the stimulus by means of an audio signal  
314 conveyed by audio cable directly from the computer to the microphone port of  
315 the camera. Measures of saccadic eye movements were made in a 5 s period both  
316 before and after the stimulus presentation. Two independent groups (n=15 and

317 14 animals) were tested using two sets of stimuli (angles of 0, 0.5, 1, 5, 7, 9, 11  
318 degrees and of 20, 31, 56, 70, 74 degrees respectively). The stimulus order was  
319 fully randomized and the interval between stimuli was randomized between 20 s  
320 and 60 s.

321

### 322 **Polarization analysis of the maxilliped signals**

323 Images of the maxillipeds of *H. trispinosa*, *H. glyptocercus*, *C. tweediei* and *C.*  
324 *hystrix* were taken through a Leitz compound microscope (Leica Microsystems,  
325 Wetzler, Germany) using a 10x objective and Canon G9 digital camera (Canon,  
326 Melville, USA) mounted using a photo tube extension on the trinocular head.  
327 Spectral reflection data of the same four species were measured using an Ocean  
328 Optics halogen HL-2000 light source (Ocean Optics, Dunedin, USA) mount at the  
329 back focal plane of the eyepiece and illuminating the maxillipeds normally. The  
330 reflected light was collected at the back focal plane of the second eyepiece using  
331 a 1 mm diameter optic fibre connected to a QE65000 spectrometer (Ocean  
332 Optics, Dunedin, USA). Linear horizontal and vertical polarization filters were  
333 placed in the path of the reflected light inside the microscope to collect each  
334 respective polarized reflectance spectrum. Over several preceding years, the  
335 colour and polarizing nature of the 1<sup>st</sup> maxillipeds from 17 other representative  
336 species of stomatopods across the superfamily Gonodactyloidea have been  
337 assessed visually by viewing the maxillipeds through a rotatable linear  
338 polarizer.

339

340

### 341 **Phylogenetic analyses**

342 To investigate the potential evolutionary pathway of color and polarization  
343 signals within the genus *Haptosquilla*, DNA sequences from both nuclear and  
344 mitochondrial genes for all available species were either obtained from GenBank  
345 or provided by P. Barber (Barber and Boyce, 2006), or were sequenced following  
346 the methods of Porter et al., (2010) (Supplemental Table S2). Additional  
347 representative stomatopod species from within the same family  
348 (Protosquillidae) and superfamily (Gonodactyloidea) were included to provide  
349 increased resolution and stability at deeper nodes within the phylogeny and to  
350 use as outgroups. We used a concatenated matrix consisting of nucleotide  
351 sequences from the cytochrome oxidase I (COI) and 16S mitochondrial genes,  
352 and the 18S and 28S nuclear rDNA genes, although the number of sequences  
353 available varied across species (see Supplemental Table S2 for full description of  
354 data sources and gene representation).

355 Nucleotide sequences of the 16S, 18S, and 28S genes were aligned using  
356 the E-INS-I strategy in MAFFT v6.0.0 (<http://mafft.cbrc.jp/alignment/server/>)  
357 (Kato et al., 2002; Kato et al., 2005). The COI sequences were inspected for  
358 evidence of pseudogenes (e.g. stop codons, indels not continuous with codons)  
359 and then manually aligned using the translated amino acid sequences. The four  
360 gene regions were then concatenated and the combined dataset was used to  
361 reconstruct a phylogeny using Randomized Accelerated Maximum Likelihood  
362 (RAxML) v.7.2.7 with rapid bootstrapping as implemented on the  
363 Cyberinfrastructure for Phylogenetic Research (CIPRES) Portal v.2.0 (Stamatakis  
364 2006; Stamatakis et al., 2008; Miller et al., 2009). Three partitions were  
365 designated for the RAxML analysis: (1) COI codon positions 1 and 2; (2) COI  
366 codon position 3; and (3) all of the ribosomal genes (16S, 18S, and 28S). All

367 partitions were analyzed with the GTR+gamma model, as this was the best-  
368 fitting model available in RAxML, according to the results of jModelTest v0.1.1  
369 (Guindon and Gascuel 2003; Posada 2008).

370

### 371 **Statistical analysis**

372 All statistical analyses were conducted in R 3.0.2 (R Foundation for Statistical  
373 Computing). Response probabilities to either horizontally or vertically polarized  
374 looming stimuli were analysed using Wilcoxon Signed-Rank tests and differences  
375 between species were calculated using a Kruskal-Wallis rank sum test. The  
376 individual saccadic responses of *H. trispinosa* to different angular e-vector  
377 contrasts were analysed using a McNemar's test.

378

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## 558 **Figure Captions**

559 Figure 1. **Illustrative examples, shown by arrows, of the conspicuous**  
560 **maxilliped signals.** (A) *H. trispinosa*, (B) *H. glyptocercus*, (C) *H. stoliura* and (D)  
561 *H. banggai*.

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563 Figure 2. **Paired plots of the probability of response of each individual to the**  
564 **vertically and horizontally polarized stimuli.** Numbers of points (open

565 circles) at each probability represent the number of individuals that responded  
566 with that probability. (A) *H. trispinosa*, (B) *H. glyptocercus*, (C) *C. tweediei*, and  
567 (D) *C. hystrix*.

568

569 Figure 3. Responses of *H. trispinosa* (black dots) to differences between the angles of  
570 polarization of the stimulus and the background (x-axis). The response data are fitted  
571 with a hyperbolic tangent (dashed line). The background level of false positive  
572 responses are represented for each stimulus type (white dots) and as an overall mean  
573 (dotted line). McNemar's test was used to determine which response values differed  
574 from the level of false positives (\* =  $p < 0.05$ ).

575

576 Figure 4. **Microscope images of the maxillipeds.** *H. trispinosa* (A), *H.*  
577 *glyptocercus* (B), *C. tweediei* (C) and *C. hystrix* (D). Accompanying each plot are  
578 the reflection spectra from the area denoted by the circle in each image. In the  
579 spectral plots, open circles represent the horizontally polarized reflectivity and  
580 open triangles represent the vertically polarized reflectivity. V and H in (A)  
581 denote the vertical and horizontal directions respectively relative to the axes of  
582 the maxillipeds.

583

584 Figure 5. **A maximum likelihood phylogeny of protosquillid species**  
585 **relationships, rooted using representative species from the**  
586 **Gonodactyloidea.** Branch support values represent bootstrap percentages.  
587 Nodes representing the genus *Haptosquilla* and the family Protosquillidae are  
588 indicated by 'H' and 'P', respectively. Where known, the presence or absence of  
589 blue signals and polarizing signals on the 1<sup>st</sup> maxillipeds has been mapped onto

590 the phylogeny. Species names in bold indicate those animals measured in this  
591 experiment, all of which have a bias to horizontally polarized stimuli, illustrating  
592 the occurrence across the two main genera of the Protosquillidae.

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594 Figure 6. **Schematic diagram of the experimental apparatus.** (A) The tank  
595 setup in front of the LCD screen. (B) An example measure of the normalized  
596 Stokes parameters (P0–3) of the horizontally polarized stimulus as a function of  
597 wavelength. (C) An example of the vertical and horizontal polarization ellipses at  
598 560 nm.

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600 Figure 7. **Measurements of the behavioural saccadic response of the**  
601 **stomatopods.** (A) Time sequences of images from a video recording illustrating  
602 the typical saccadic eye movement response in *H. trispinosa* to a looming  
603 polarized contrast stimulus (horizontally polarized on a vertically polarized  
604 background). Each image is a single frame, approximately 0.2 s apart; the first  
605 two images show the eyes before the stimulus, the 3rd image shows the eye  
606 position 0.1 s after the stimulus onset, and the final image shows the eye position  
607 approx. 0.3 s after the stimulus onset. (B) The measured change in the angular  
608 separation of the eye stalks as a function of the onset of the looming polarized  
609 contrast stimulus. The numbers and filled points correspond to the numbered  
610 frames displayed in (A). The red line indicates the stimulus diameter as a  
611 function of time.

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630 **Figures**

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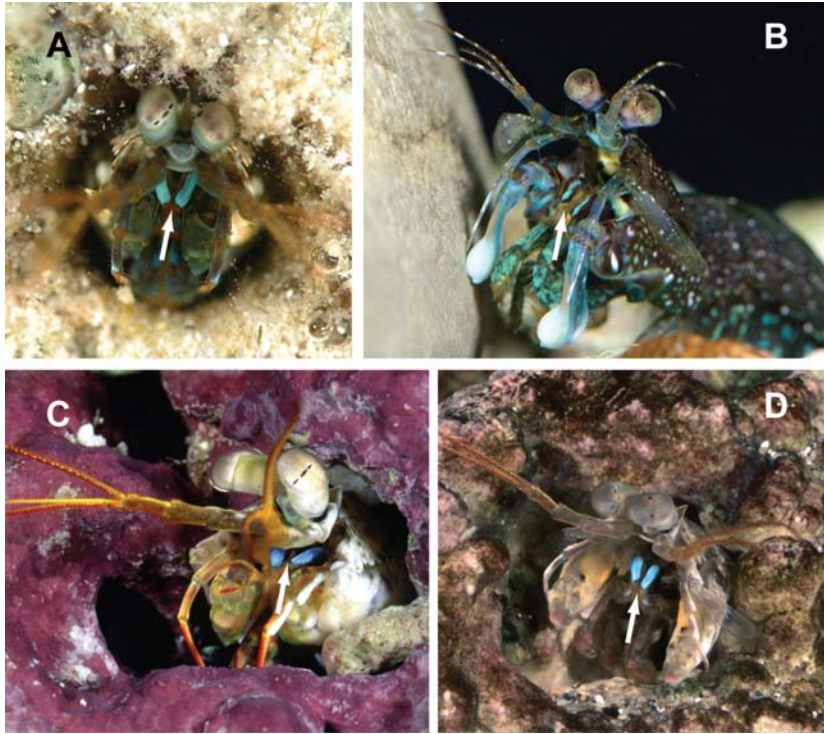
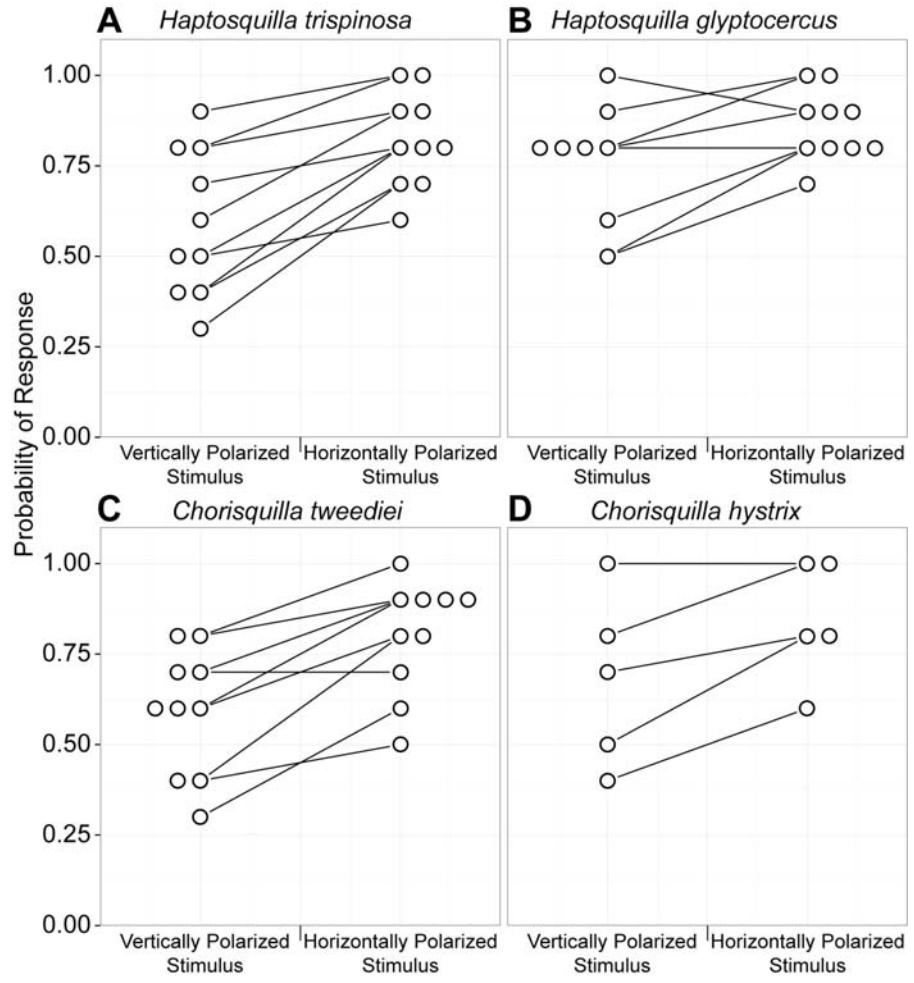


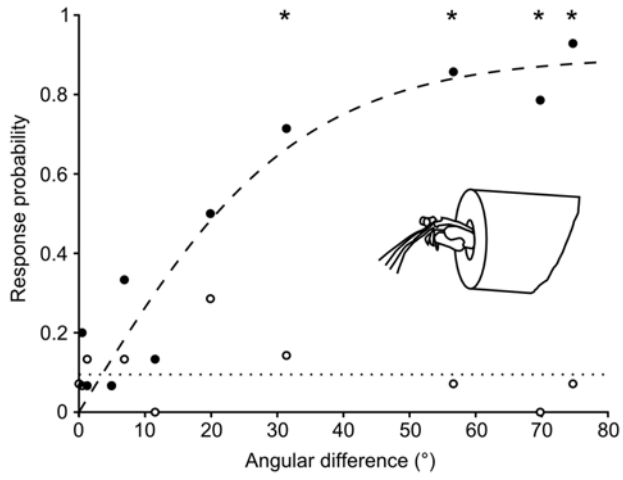
FIGURE 1

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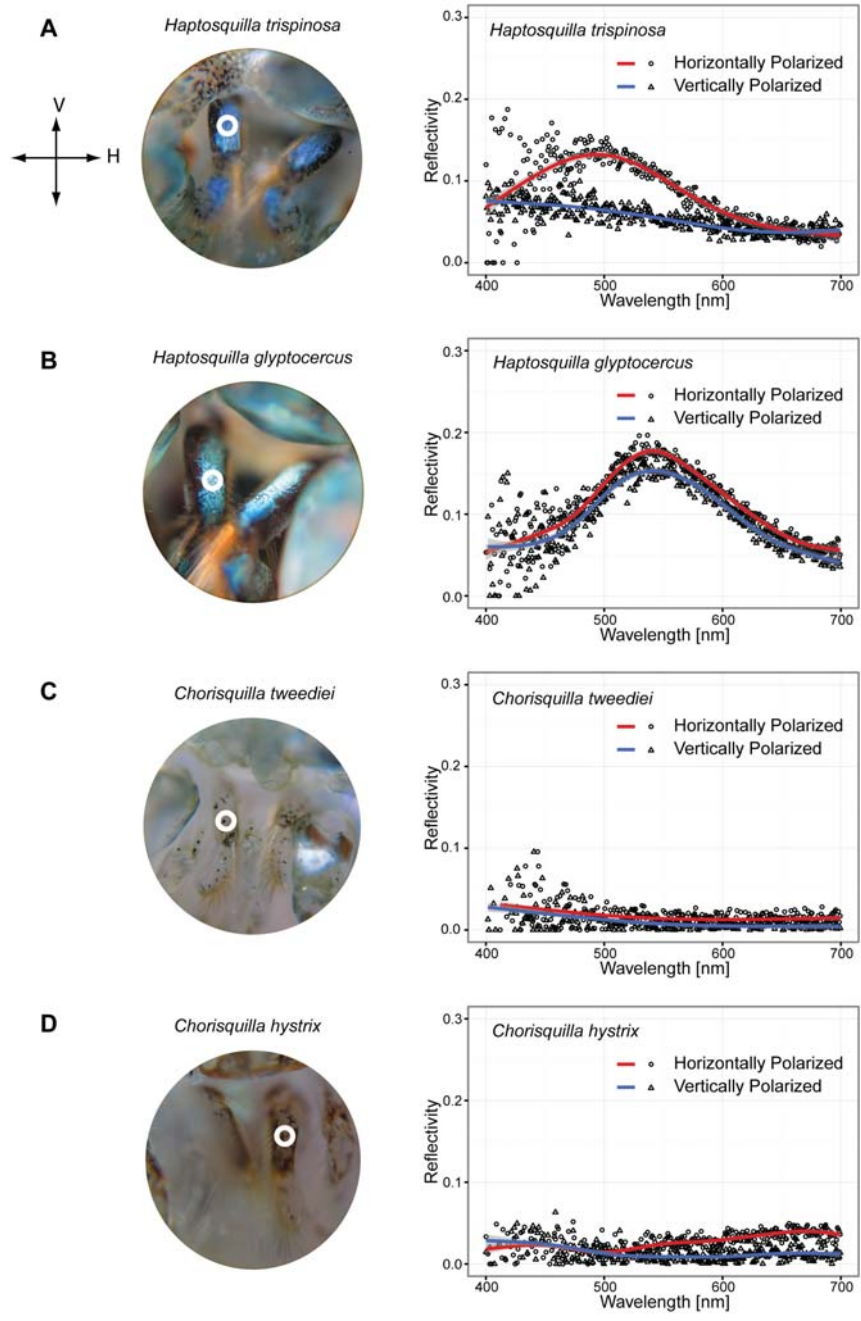
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FIGURE 2

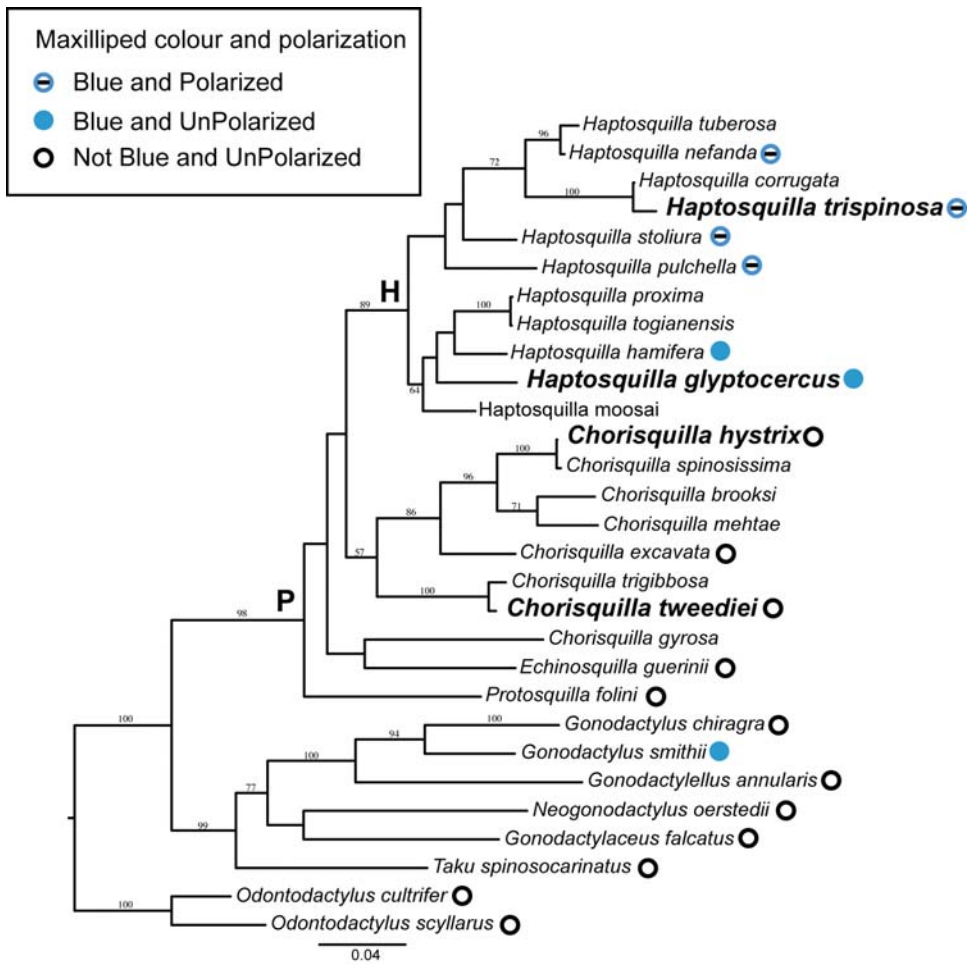


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FIGURE 3

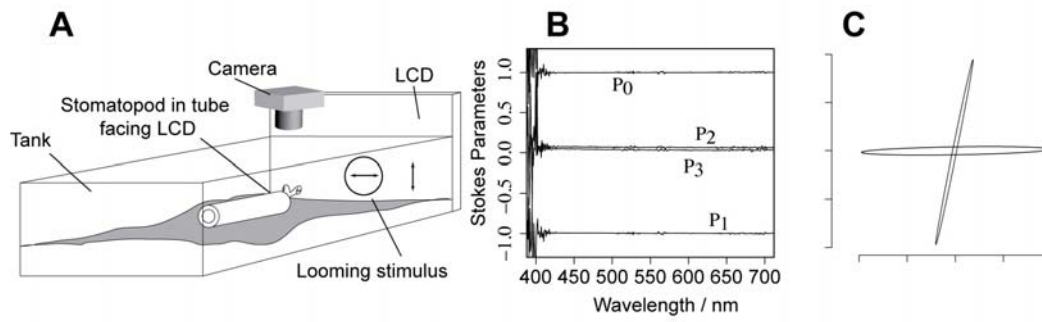


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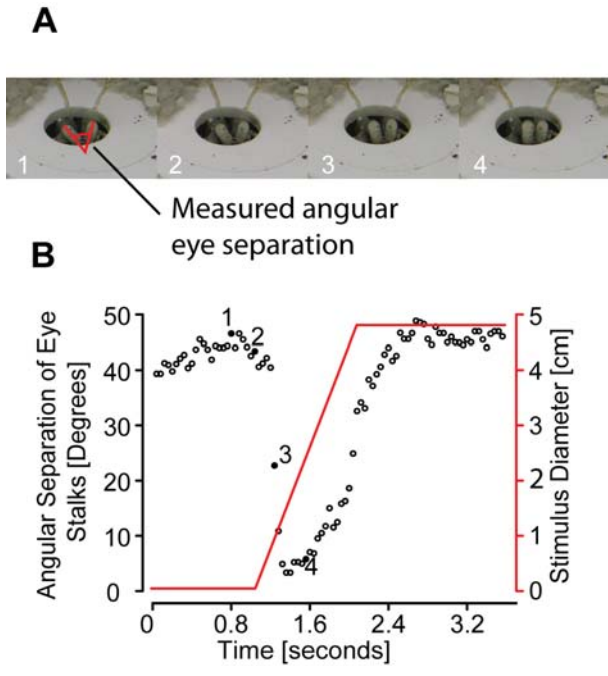
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FIGURE 5



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FIGURE 6



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FIGURE 7