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1 **Title**

2 Long-wavelength reflecting filters found in the larval retinas of one mantis shrimp family  
3 (Nannosquillidae)

4  
5 **Authors**

6 Kathryn D. Feller<sup>1,†,2,3\*</sup>, David Wilby<sup>3,4,†</sup>, Gianni Jacucci<sup>5</sup>, Silvia Vignolini<sup>5</sup>, Judith Mantell<sup>6</sup>,  
7 Trevor J. Wardill<sup>1,†,2</sup>, Thomas W. Cronin<sup>7</sup>, Nicholas W. Roberts<sup>3</sup>

8  
9 **Affiliations**

10 <sup>1</sup> Ecology, Evolution and Behavior Department, 1479 Gortner Avenue, University of  
11 Minnesota, St. Paul, Minnesota, 55108, USA

12 <sup>2</sup> Physiology Development and Neuroscience Department, Physiological Laboratories,  
13 Downing Street, University of Cambridge, Cambridge, CB2 3EG, UK.

14 <sup>3</sup> School of Biological Sciences, University of Bristol, Bristol, BS8 1TQ, UK.

15 <sup>4</sup> Department of Biology, Lund University, Lund, 223 62, Sweden.

16 <sup>5</sup> Department of Chemistry, University of Cambridge, Cambridge, CB2 1EW, UK.

17 <sup>6</sup> Wolfson Bioimaging Facility, University of Bristol, Bristol, UK, BS8 1TD, UK.

18 <sup>7</sup> Department of Biological Sciences, 1000 Hilltop Circle, Baltimore, University of  
19 Maryland Baltimore County, Maryland, 21250, USA.

20 \*Correspondence to: [kate.feller@gmail.com](mailto:kate.feller@gmail.com)

21 † present address

22  
23 **Summary**

24 Animals are known to exploit either transmissive coloured filters or reflectors for  
25 adaptive visual benefits. Here we describe a new category of biological optical filter that acts  
26 simultaneously as both a transmissive spectral filter and narrowband reflector. Discovered in the  
27 larval eyes of only one family of stomatopod crustaceans (Nannosquillidae), each crystalline  
28 structure bisects the photoreceptive rhabdom into two tiers and contains an ordered array of  
29 membrane-bound vesicles with sub-wavelength diameters of  $153 \pm 5$  nm. Axial illumination of  
30 these intrarhabdomal structures *in vivo* produces a narrow-band of yellow reflectance (mean peak  
31 reflectivity at  $572 \pm 18$  nm). While analogous visual structures are not known in nature, the  
32 optical performance of these intrarhabdomal structural reflectors is similar to synthetic devices  
33 used in the optical industry, such as band gap filters, laser mirrors, or fiber Bragg gratings. The  
34 interaction of these structural filters with wavelengths of light between 475 nm and 520 nm and  
35 the animal's visual ecology together suggest that these structures may help improve the detection  
36 of pelagic bioluminescence in shallow water at night.

37  
38 **Key Words:** invertebrate vision, stomatopod, larvae, photonic structure, bioluminescence

39  
40  
41 **INTRODUCTION**

42  
43 Optical filters are commonly used by animals to modify the spectral or overall sensitivity of their  
44 visual systems. Many species of both vertebrates and invertebrates use a reflecting structure  
45 (tapetum) behind their photoreceptors to increase photon capture and enhance vision in dim light

46 [1-4]. Others use coloured filters positioned lateral or distal to their photoreceptors to tune  
47 spectral sensitivity by transmitting specific wavelengths of light not absorbed or scattered by the  
48 filter [5-7]. We recently discovered an optical structure positioned within the photoreceptive  
49 rhabdoms in the eyes of mantis shrimp (stomatopod) larvae from a single family, the  
50 Nannosquillidae. These intrarhabdomal structural reflectors (ISRs) are specialized to  
51 simultaneously reflect and filter specific wavelengths of light into rhabdomeric tiers above and  
52 below the structure, respectively. The ISR appears to be a feature unique to the nannosquillid  
53 lineage since all presently examined nannosquillid larvae (six species) test positive for the  
54 structures. The ISR is similar to several synthetic devices such as band gap filters, laser mirrors,  
55 and (in particular) fiber Bragg gratings used in optical sensors for a wide range of industries. To  
56 our knowledge, the mantis shrimp larval ISR is the first example of a naturally occurring analog  
57 to these human-made devices.

58 Specialized optical structures are well known in adult stomatopod eyes [5, 8, 9] that contain  
59 visual adaptations such as UV and visible pigment based filters [10, 11], an unsurpassed  
60 diversity in colour and polarization sensitivity [5, 10, 12, 13], as well as unique structural  
61 reflectors used for visual communication [14]. Stomatopod larvae, by comparison, lack all of  
62 these adult features and are understood to have comparatively simple apposition compound eyes.  
63 Until this study, the larval stomatopod eye structure has been described as typical of most other  
64 planktonic crustacean larvae in the open-ocean habitats (epipelagic and mesopelagic) where they  
65 are found. Typical features of stomatopod larval visual ecology include compound eyes with a  
66 uniform array of ommatidia and a single photoreceptor type [15, 16]; morphological adaptations  
67 for hiding in open-water, such as highly transparent bodies and reflective eye camouflage [17];  
68 and diel vertical migrations, where animals reside at depths during the day and travel to the  
69 surface at night, similar to decapod larvae (pers. observ, [18]). Though the daytime depths and  
70 migratory behavior of stomatopod larvae have yet to be specifically measured, one study found  
71 that collections at 10-200 m daytime depth were sufficient to quantify larval abundance across a  
72 geographic region for the species (*Squilla mantis*, [19]).

73 Compound eyes evolved in crustacean larvae to mediate visually guided behaviors such  
74 as predation and anti-predation responses, as well as migration [16, 20]. The gross examination  
75 of stomatopod larval eyes from diverse taxa, however, reveals that a single family  
76 (Nannosquillidae) deviate from the typical eye structure by their possession of ISRs within the  
77 majority of their rhabdoms. In this study we characterize the anatomical and optical properties of  
78 nannosquillid larval ISR-containing retinas as well as consider how these structures may  
79 influence task specific behaviors in their dim light habitat.

80

## 81 RESULTS

82

83 **Histology:** In general, stomatopod larvae possess a pair of compound eyes composed of several  
84 hundred ommatidial units, each using transparent, apposition optics to focus light onto the  
85 photoreceptive rhabdom that is similar in structure to other crustacean larvae [21, 22]. The  
86 typical crustacean larval rhabdom is formed from a tightly packed column of visual pigment-  
87 expressing, inter-digitated microvilli projected from a ring of seven reticular cells (R1-7). Each  
88 rhabdom is optically isolated by screening pigments in the reticular cells. In many crustacean  
89 larvae, including stomatopods, reflective structures lie on the surface of the retina, between each  
90 rhabdom, to camouflage the dark eye in open water [17]. While the majority of stomatopod  
91 larval retinas adhere to this typical arrangement, the retinas of nannosquillid species contain a

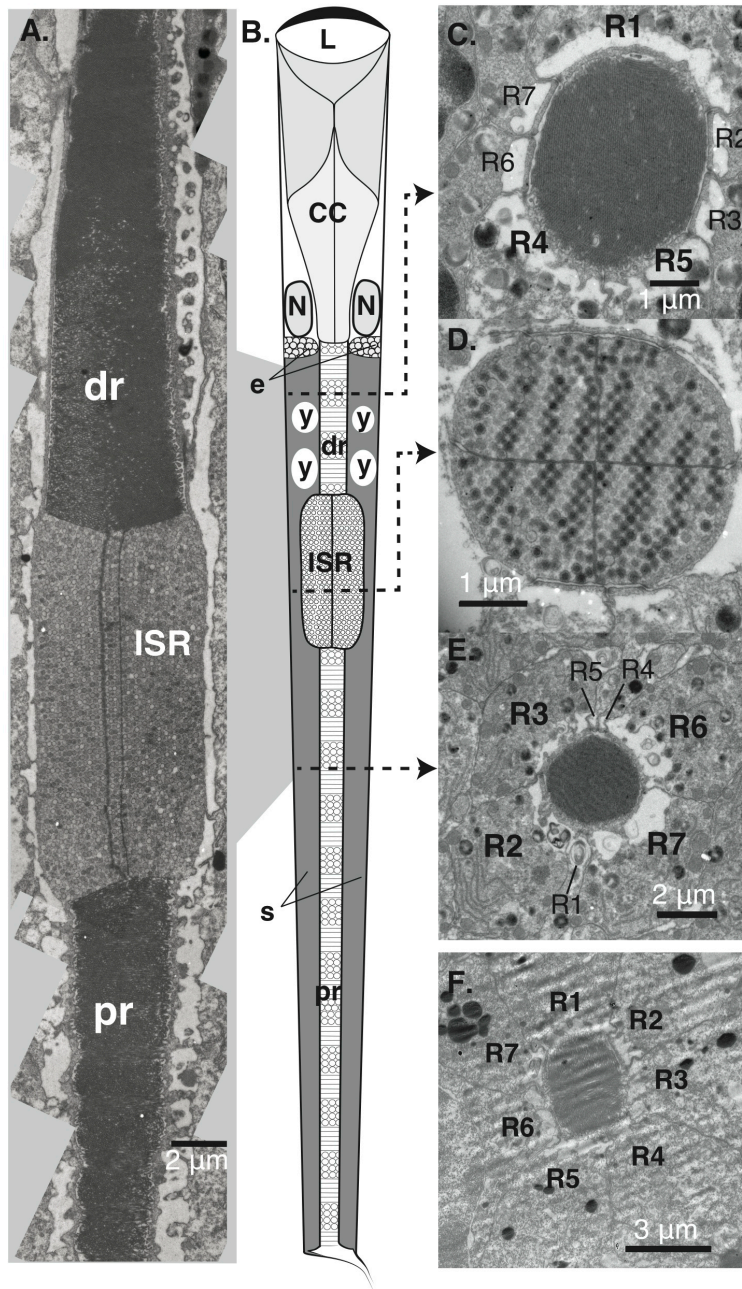
92 conspicuous addition: the ISR. The ISR is a 4-segmented, barrel-shaped structure that sits  
93 approximately one-third of the total length from the distal end of the rhabdom (Fig. 1). Electron  
94 and light microscopy reveal that distribution of ISR-containing ommatidia is not uniform across  
95 the eye but regionalized to the ventral and lateral regions (Fig. 2). A subset of 30-50 ommatidia  
96 in the dorsal region of the eye are devoid of ISR structures and instead possess the untiered  
97 photoreceptor structure typical of other crustacean larval retinas (Fig. 1F, 2D). Two-photon  
98 microscopy further established the three-dimensional distribution of tiered (ISR present) and  
99 untiered (ISR absent) ommatidia across the nannosquillid eye (Fig. 2A-B, Movie S1).

100 Within each ISR is an ordered lattice of spheroid, membranous vesicles, each an average  
101 of  $153.3 \pm 5.6$  nm (mean  $\pm 1$  standard deviation; Fig S1A) in diameter. TEM and electron  
102 tomography reveal that the ordered arrangement of these vesicles is preserved across the  
103 membranes of the four primary segments (Fig. 1D) and in three dimensions (Movie S2). Each  
104 ISR measures an average of  $11.1 \pm 1.5$   $\mu$ m long by  $4.9 \pm 1.1$   $\mu$ m wide and is positioned directly  
105 in the optical pathway of light, bisecting the retinular cells (R1-7) of the rhabdom into a proximal  
106 (R2, R3, R6, & R7) and distal tier (R1, R4, & R5; Fig. 1). This cellular arrangement is similar to  
107 that of the tiered rhabdoms found in the ommatidia of rows one to four of the midband of some  
108 adult stomatopod eyes, including adult nannosquillids [12].

109 Diverse taxonomic sampling of stomatopod larvae for transmission electron microscopy  
110 (TEM) or two-photon microscopy revealed that ISRs may only occur in larvae from the family  
111 Nannosquillidae. At least four of the 13 genera described in the Nannosquillid family are  
112 represented by species in this study: *Pullosquilla thomassini*, *Pullosquilla litoralis*, *Alachosquilla*  
113 *vicina*, *Coronis scolopendra*, and two unknown nannosquillids (1 and 2) currently lacking adult  
114 DNA barcode reference sequences but nested within the nannosquillid clade (Fig. 3; Table S1).  
115 The absence of ISRs from other stomatopod lineages suggests that this visual adaptation is  
116 unique to nannosquillid larval ecology. Additionally, the presence of ISRs in the first and  
117 terminal stage larvae suggests that the function serves the animal throughout the entire pelagic  
118 phase of life.

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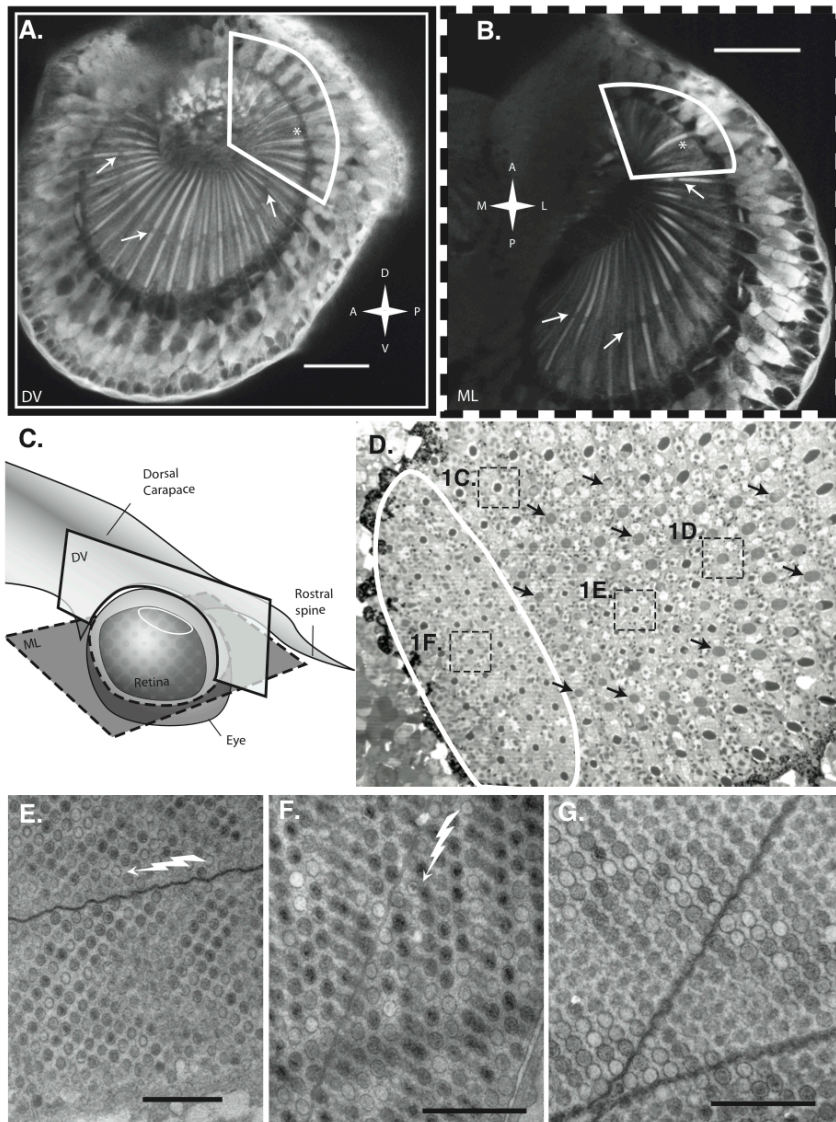
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**Fig. 1. Anatomy of stomatopod larval ommatidia containing intrarhabdomal structural reflectors (ISR) described by histology.** (A) Composite TEM of longitudinal section through a lateral ommatidium. Orthogonal microvilli in the distal (dr) and proximal (pr) rhabdoms flank the ISR. (B) Diagram of an ISR-containing ommatidium. (C) TEM cross-section through the distal tier of the rhabdom, formed by microvilli from retinular cells R1, R4, and R5 (nomenclature from [10]). Like adult tiered rhabdoms, extensions of the remaining retinular cells are visible and do not contribute microvilli to the rhabdom. (D) TEM cross-section through ISR. (E) TEM cross-section through the proximal rhabdomeric tier, formed by microvillar projections from retinular cells R2, R3, R6 and R7. (F) TEM cross-section through a non-ISR expressing photoreceptor in the dorsal region of the eye. Note the equal contribution of microvilli from retinular cells R1-7. Lens, L; crystalline cone, CC; Retinular cell nucleus, N; reflective eye camouflage, e; yellow long-pass screening pigments [23], y; dark, ommachrome lateral screening pigments, s.





136 **Fig. 2. Regional localization of ISRs across the retina and in the eyes of five different nanosquillid**  
 137 **species. (A-B)** Two-photon optical sections of *Coronis scolopendra* first stage larval eyes revealing a  
 138 small region of dorsal pointing ommatidia (within white line) lacking ISR structures (denoted by \*).  
 139 Arrows indicate the dark, less autofluorescent ISRs separating rhabdoms into two distinct tiers.  
 140 Compasses indicate anatomical directions: A, anterior, P, posterior, M, medial, L, lateral, D, dorsal, V,  
 141 ventral. Scale bars, 50  $\mu\text{m}$ . **(C)** Diagram of dorsal-ventral (DV) and medial-lateral (ML) sections through  
 142 eye in A (solid border) and B (dashed border), respectively. **(D)** Light micrograph of retina cross-section  
 143 from early stage larva, unknown nanosquillid species 1. Boxes depict regions imaged via TEM in Fig.  
 144 1C-F. White line denotes dorsal region of untiered ommatidia lacking ISR expression, similar to zone  
 145 identified in A-C. Arrows highlight subset of ISRs in the remainder of the eye. **(E)** TEM longitudinal  
 146 section of ISR in last stage larva, *Pullosquilla thomassini* and **(F)** mid stage *Alochosquilla vicina*. Jagged  
 147 arrows indicate incoming optical axis in longitudinal sections. **(G)** Oblique TEM section of ISR in early  
 148 stage, *Pullosquilla littoralis* larva. E-G scale bars, 1  $\mu\text{m}$ .  
 149

150 ***In vivo* Reflectance Spectroscopy:** The size and arrangement of vesicles within the ISR  
151 structures led us to hypothesize that certain wavelengths will be preferentially reflected when the  
152 structure is illuminated. To investigate how white light interacts with the ISR, we used a custom  
153 microscope system to illuminate, image, and measure reflectance spectra from the pseudopupils  
154 of larval ommatidia *in vivo* (Fig. S2). The pseudopupil is the dark spot that moves smoothly  
155 across the surface of a compound eye as it rotates, which is produced when the optical axes of a  
156 subset of ommatidia align with the optical viewing axis of the observer. Since the ISR lies in the  
157 optical axis, we predicted that a distinct reflection profile would be observed when light was  
158 imaged axially into ISR-containing rhabdoms.

159 Prior to this experiment, the phylogenetic distribution of species possessing ISR-  
160 containing retinas was not known. Therefore, we tested a diverse range of species available at  
161 our field site (Lizard Island, Australia) both to measure the interaction of light with the ISR as  
162 well as to examine the distribution of species that express these structures. The experiment was  
163 conducted blind to species identity by using wild-captured larvae that were only identified using  
164 DNA barcoding after light reflectance measurements were collected [24]. It was observed that  
165 some larvae reflect a band of yellow light (average peak of  $572 \pm 18$  nm) from the ventral and  
166 lateral ommatidia, but only when illuminated on-axis (Fig. 3; Fig. S2). While side-illumination  
167 was sufficient to visualize and measure the blue and green camouflage structures that lie over the  
168 pigmented retina [as in 17], only on-axis, epi-illumination could produce a yellow reflectance  
169 from the pseudopupil (Movie S3).

170 DNA barcode identification after the experiment revealed that yellow pseudopupil  
171 reflectance was only found in species of the Nannosquillidae family (Fig. 3H). These species  
172 represent three of the six species shown to contain ISRs histologically: *Pullosquilla thomassini*,  
173 *Alachosquilla vicina*, and unknown species 1 (Fig. 3H). Stochastic sampling and post-hoc  
174 identification precluded reflectance data collection from the remaining species. TEM was used to  
175 verify the presence of ISR structures in post-reflectance-measured specimens (Fig. 2D-F),  
176 suggesting that ISR structures were the source of observed yellow reflectance. This conclusion  
177 was bolstered by coincidence between ISR anatomical regionalization and the pattern of yellow-  
178 reflectance observed in only lateral and ventral ommatidia (Fig. 2A-D, Fig 3A-F, Movie S1).  
179 Neither yellow reflectances, nor ISR structures were found in the dorsal-most ommatidia.

180 Of the three nannosquillid species measured for *in vivo* pseudopupil reflectance, different  
181 spectral shapes with similar peaks were observed from each species (Fig. S2). Variation in  
182 spectral shape and/or reflectivity (seen in Fig S2B-E) may be attributed to subtle differences  
183 among species in the material and anatomical assembly of the ISRs (Fig. S1); slight alignment  
184 deviations of the sample probe with the optical axis; imaging through multiple layers of dioptics  
185 and rhabdomic material; and/or different sizes of the eyes and ommatidia aperture among  
186 individuals of different species or the same species at different stages. Several ISR reflectances  
187 contain short-wavelength components we attribute as contamination from the surrounding, blue-  
188 reflective material (eyeshine) that lies on the surface of the retina between ommatidia (Fig S2B,  
189 [17]). ISR-containing ommatidia correspond to the lateral and ventral areas of the eye that  
190 express this blue eyeshine (Movie S1; Fig. 3A). No contamination arose from the green eyeshine  
191 around dorsal ommatidia since these lack ISRs. At present, a pure reflectance from the ISR alone  
192 cannot be obtained until methods are devised to adequately remove the crystal from within the  
193 center of the eye.

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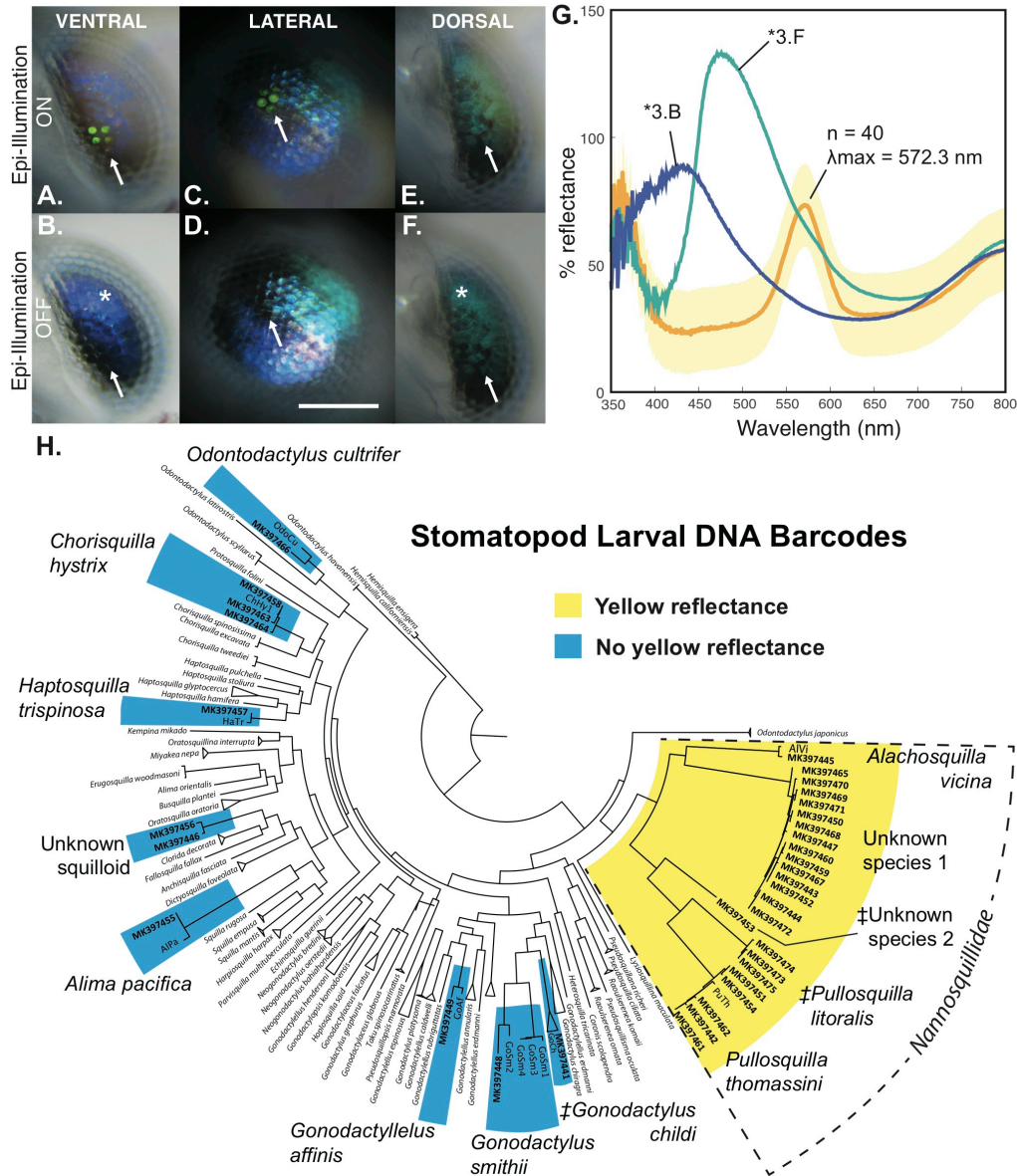
195 **Photonic Modelling:** To understand the spectral reflectance from the ISR (Fig. 3G), we  
196 developed a semi-analytical optical model. This mathematical model uses a combination of  
197 Bragg's law [25] with finite-distance time-domain numerical simulations. By using these  
198 methods, we were able to account for the findings of the *in vivo* reflectance experiment and test  
199 for any morphological disorder of the ISR potentially induced by TEM. The amount of disorder  
200 and the periodicity of the vesicles was determined by calculating the structure factor estimated  
201 from TEM micrographs (S.I.). The structure factor of the vesicles within the ISR is related to a  
202 face-centered cubic geometry of the vesicles with an estimated lattice constant of  $392 \pm 7$  nm  
203 (mean  $\pm 1$  standard deviation; Fig. 4A), which takes into account any structural deformations  
204 from TEM embedding and slicing of the tissue.

205 Since the materials contained within the ISR vesicles are not currently known, two  
206 different vesicle refractive indices ( $n_v$ ) were tested in the model: a high-refractive index material,  
207 pteridine ( $n_v$  1.70) and a low-refractive index material, lipid ( $n_v$  1.48; further details in S.I.).  
208 Pteridine was selected as the high-refractive index material due to the similarity in appearance  
209 between our TEM results with membrane-bound pteridine granules and those found in other  
210 crustacean photonic structures [26-28]. Our model resulted in agreement with the mean  
211 experimental reflectivity spectrum for both pteridine and lipid vesicles with a realistic  
212 histological expansion factor ( $\psi$ ) of 5% and 8% respectively (further details in Supplemental  
213 Information, Fig S2F, Fig 4B). This is further evidence to suggest that the observed yellow  
214 reflectance measured from the larval pseudopupil is produced by the ISR. The residual difference  
215 between our predicted and observed reflectivity can be attributed to several factors including  
216 potential histological distortion of ISR dimensions (i.e.  $\psi$ ); unknown dispersion relation for the  
217 refractive indices of vesicle and matrix materials; and additional disorder in the orientation and  
218 periodicity of the 3D lattice across large volumes outside the range of TEM tomography (Fig  
219 S2F-J).

220 Though we were able to collect sufficient data to describe the reflection properties of the  
221 ISR, we were unable to measure how light is transmitted through the structure. The location of  
222 the ISR within the rhabdom poses a logistical problem for collecting light transmission data.  
223 Frozen, fresh, or fixed sectioning methods all distort the vesicle lattice, affecting spectral  
224 transmission. Though transmission data could not be collected, gross dissection and visual  
225 inspection of fixed retinas revealed that ISR structures lack colorful pigments (Movie S4)  
226 suggesting that any transmitted wavelengths are likely the difference between the incident light  
227 and the reflection or any scattering from the photonic structure, rather than absorption by  
228 photostable pigments.

229





230  
 231 **Fig. 3** *In vivo* illumination of the larval eye pseudopupil with on-axis incident light reveals a sharp, yellow  
 232 reflectance from ISR-containing ommatidia in nannosquillid species only. (A-F) Illumination of a single  
 233 nannosquillid eye (Unknown species 1) oriented in three different directions with on-axis (epi) incident illumination  
 234 on and off. The only condition varied between each pair of ventral, lateral, or dorsal images is the state of the epi-  
 235 illuminated light (on or off; as in Movie S3). White arrows indicate pseudopupil. Side-illumination was not used  
 236 during spectral measurements. Scale bar = 150  $\mu$ m for all images. (G) Yellow line, mean reflectance spectra of  
 237 axially illuminated pseudopupil measurements from 40 individuals of Unknown species 1 (plotted in Fig S2B).  
 238 Yellow shading, standard deviation of all reflectance spectra used to calculate mean pseudopupil reflectance. Blue  
 239 and green lines, mean eye camouflage reflectances from regions denoted by \* in B (n=4) and F (n=2). Mean blue  
 240 and green reflectances were measured from the individual photographed in A-F. (H) Maximum likelihood tree of  
 241 cytochrome oxidase I DNA barcodes from adult references and larval sequences. Sequences highlighted in yellow  
 242 indicate larvae in which a yellow pseudopupil reflectance was measured. Sequences highlighted in blue represent  
 243 larval eyes that did not produce yellow reflectances in epi-illumination experiment. ‡ indicate barcoded species  
 244 where reflectance was not measured, but ISR absence or presence was determined by histology only.

245

## 246 DISCUSSION

247 The discovery of ISR structures raises three major questions. First, what source of light in  
248 the nannosquillid larval habitat interacts with the ISRs? Stomatopod larvae encounter few  
249 sources of light in this range of the visible spectrum since, like many other larval crustaceans,  
250 they live in dim, blue water by day (10-200 m depth) and come to surface only at night (1 m  
251 depth). Though the broad irradiance spectrum of moonlight is brighter during periods around the  
252 full moon, nannosquillid larvae only rise to the surface between the new and the quarter phases  
253 of the moon (pers. observ; pers comm. RL Caldwell) when the total irradiance is 1/100 to a 1/10  
254 that of full moonlight [29]. Furthermore, since moon rise occurs 30 and 60 minutes later each  
255 day, the moon may not rise for large portions of a given night when it is full. The only other  
256 source of long-wavelength light in this dim-light habitat is bioluminescence (BL). BL has  
257 evolved multiple times in diverse pelagic taxa, resulting in a range of peak emission wavelengths  
258 that vary with depth. The majority of bioluminescent species live in the deep-sea and emit  
259 narrow spectra that peak between 460 nm and 500 nm [30]. And while there are less than half as  
260 many bioluminescent species characterized from shallow water (where mantis shrimp larvae  
261 reside), the emission spectra of these species are red-shifted, with peaks between 475 nm and  
262 520 nm [30]. Such emission spectra often contain more long-wavelength radiation than the  
263 surrounding space light, which may provide a salient signal in the close range of nannosquillid  
264 larvae, which measure 2 - 12 mm in body length.

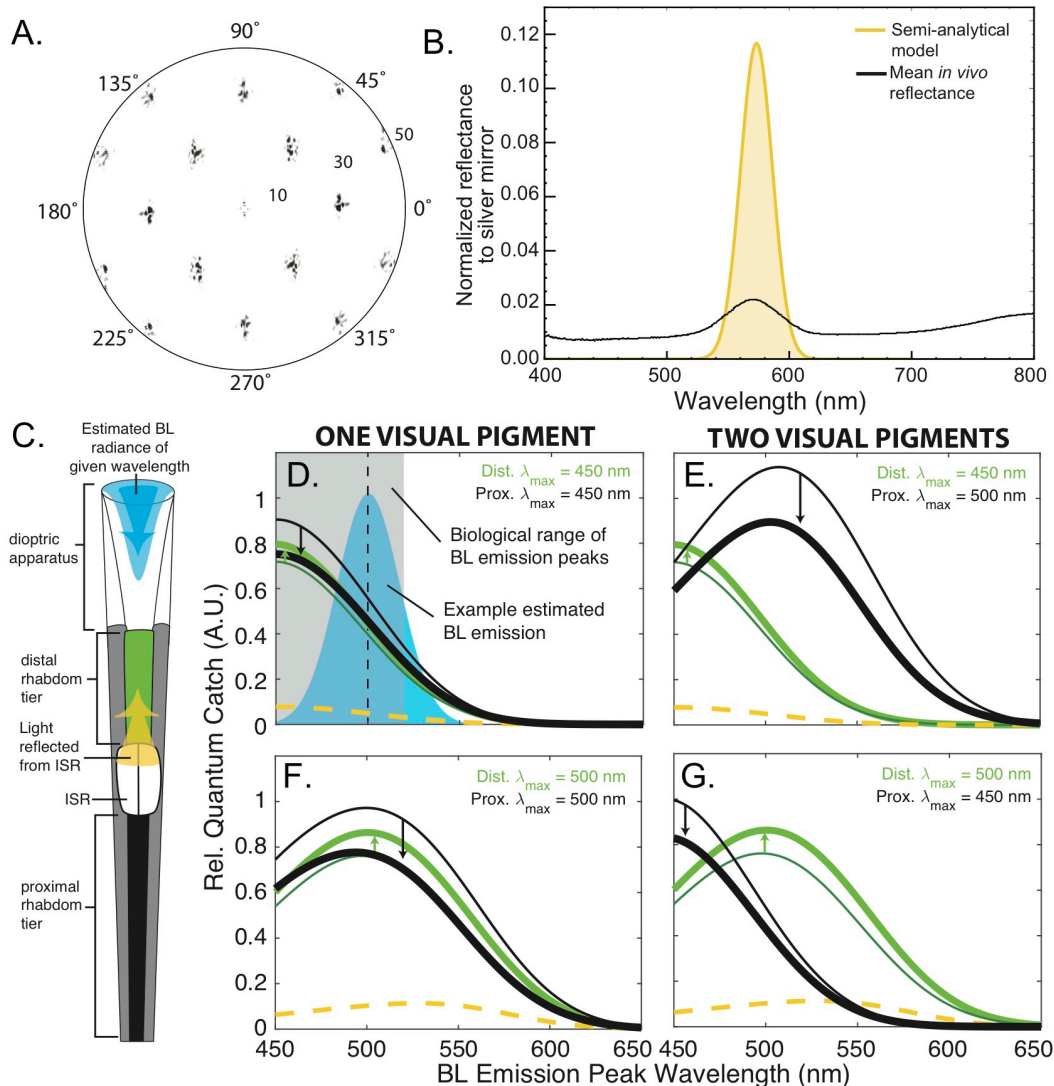
265 In such a visual environment, could the ISR even improve contrast detection of BL? One  
266 known mechanism for improving BL contrast detection is the use of yellow, transmissive filters  
267 in the lenses of many deep-sea animals. In these species, a greater proportion of the longer  
268 wavelengths of BL emission spectra are able to reach photoreceptors, which is proposed to  
269 improve the contrast of a BL target against the shorter-wavelength dominant background of the  
270 deep sea (for example [31, 32]). The ISR does not, however, operate in the same way as it is not  
271 a short-wavelength absorbing, long-pass filter. Rather, it is a long-wavelength reflector that also  
272 removes a narrow band of wavelengths from being incident on the proximal tier of  
273 photoreceptors. Since many of the variables surrounding nannosquillid ecology and visual  
274 physiology have yet to be described, we developed a visual model to assess the potential function  
275 of the ISR. This model tests the minimal possible effect of ISRs on nannosquillid sensitivity  
276 when viewing BL light sources of different peak wavelengths. The model estimates the relative  
277 proportion of photons captured (quantal catch, QC) by the two rhabdomeric tiers from an  
278 incoming light source both with and without the 50% reflective and filtering effect of the ISR  
279 (full details in Supplementary Materials, [based on 33]). Different combinations of the two  
280 known nannosquillid visual pigments were used in each tier of the rhabdom (450 nm and 500 nm  
281  $\lambda_{\max}$ , Fig. S3A; [15]).

282 All visual pigment scenarios of the model predict an increase in QC in the proximal tier,  
283 with the maximum increase of 11% occurring when the visual pigment  $\lambda_{\max}$  is 500  
284 nm. Moreover, this maximum gain in photon capture occurs when the BL emissions peak is  
285 between 475 nm and 520 nm, matching the range of BL in the shallow pelagic habitat (Fig 4;  
286 [30]). The proximal tier also receives a decrease in QC, regardless of visual pigment expression.  
287 The most interesting scenario occurs when the distal and proximal tiers have a  $\lambda_{\max}$  of 500 nm  
288 and 450 nm respectively as the ISR acts to enhance the difference between the number of  
289 photons absorbed by each tier (Fig 4F). Opponent processing of this amplified difference  
290 between tier outputs would create a novel mechanism for increasing the contrast of any such

291 shallow water BL against the background. Overall, these calculations demonstrate that the ISR  
292 has the potential to alter the relative photoreceptor absorbance by simultaneously reflecting a  
293 narrow waveband of light and filtering specific wavelengths from the transmitted component. To  
294 experimentally test this hypothesis, future research must characterize the transmission of the ISR,  
295 the chemical nature of the ISR materials and the optical properties of the overlying structures  
296 (lens & crystalline cone), as well as assess the spectral sensitivity of each rhabdomeric tier in a  
297 nannosquillid retina, and radiometrically quantify the larval light environment.

298 Finally, which ecological pressures might have led ISRs to become localized in specific  
299 regions of the eye? In the lateral and ventral regions of the eye, ISRs would be beneficial for  
300 imaging point sources of bioluminescent light horizontally and below [22] the animal. The ISR  
301 may have evolved in those positions in the eye as a selective advantage for gaining more visual  
302 information about a specific food resources and aiding predation. For these animals, the dorsal  
303 regions of their eye are unlikely to be involved in targeting food and therefore correlating with  
304 the lack of the ISRs. In comparison to other species of stomatopod larvae, nannosquillid larvae  
305 are much smaller and faster swimmers (pers. observations). Future evaluation of nannosquillid  
306 larval behaviour may reveal that they use different predation strategies relative to other, often  
307 sympatric species of mantis shrimp larvae.

308 Stomatopods, at all life history stages, continue to provide a compelling system for  
309 understanding visual adaptation in the ocean. We have described a new type of visual structure  
310 that functions concurrently as both a transmissive and reflective colour filter in stomatopod  
311 larval eyes. Looking forward, the homology between these ventral and lateral larval  
312 nannosquillid ISR ommatidia and tiered adult stomatopod rhabdoms may provide insight into the  
313 developmental mechanisms that led to the rise of the elaborate colour vision system found in  
314 many adult stomatopod eyes and the evolution and development structural optics in animals.



315  
 316 **Fig. 4. Results of two optical models (A)** Two-dimensional structure factor unveiling the FCC packing of the  
 317 vesicles in the ISR, measured from TEMs of unknown nannosquillid 1 retina. **(B)** Comparison between the optical  
 318 response of the ISR predicted by our semi-analytical model for lipidic vesicles and the experimental data, yellow  
 319 and black curve, respectively. The model indicates an exact correspondence between the predicted wavelength of  
 320 maximum reflection and the observed wavelength-selective response. Both calculated and measured mean spectral  
 321 reflectance were mathematically normalized to a silver mirror. **(C)** Diagram representing different components of  
 322 QC model to BL light sources modeled with and without experimental ISR reflectance. Colors correspond to traces  
 323 in D-G **(D-G)** Relative proportion of photons captured from different BL wavelength emission spectra. Calculations  
 324 for distal and proximal tier contain different combinations of visual pigments with peak absorbances ( $\lambda_{max}$ ) of 450  
 325 nm and 500 nm (combinations indicated top right of each panel). Left two panels (D & F) contain one visual  
 326 pigment; Right two panels (E & G) contain two different visual pigments. Green traces, QC calculated for distal  
 327 (Dist.) tier rhabdom; black traces, QC calculated for proximal (Prox.) tier rhabdom. Thin line, QC calculated for  
 328 ommatidium without ISR; thick line, QC calculated for ommatidium with ISR; yellow dashed line, amount of light  
 329 reflected from ISR that is absorbed by the receptor. Example BL emission spectrum with peak emission at 500 nm  
 330 in blue; intersection of black dashed line with green curves represents QC calculated to the 500 nm peaking BL  
 331 spectrum. Grey shading denotes range of biologically relevant estimated BL emission spectra in the shallow pelagic  
 332 environment reviewed in [30].



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**Author contributions:** KDF made the initial discovery and performed experiments. KDF, TWC, NWR designed the experiments and analyzed the data and wrote and edited the manuscript. TEM by KDF and JM; photonic modelling by GJ & SV; BL modeling by DW; and 2-photon microscopy prep by KDF and imaging by TJW. Figures created by KDF with additions by DW, GJ, and revisions by co-authors.

**Competing interests:** Authors declare there are no competing interests.

## SUPPLEMENTAL MATERIALS

### Materials and Methods

**Animals & DNA Barcode Identification:** All animals were captured at night while wading in shallow water (1-2 m depth) with dip nets and underwater lights at Lizard Island Research Station (August 2015; Queensland, Australia). Stomatopod larvae were sorted from the total plankton catch and stored in cups of seawater until completion of in vivo measurements. Identification was accomplished using previously published methods for DNA barcoding [24]. In brief, telson tissue was fixed in absolute ethanol and transported to the University of Bristol where total DNA was isolated (DNA XS, Machery-Nagel) and DNA amplicons of the cytochrome oxidase 1 mitochondrial gene were generated. Amplicons were sequenced (Eurofins Genomics, UK) and aligned to a database of manually curated stomatopod reference sequences (Geneious Pro 5.5.6) to generate a maximum likelihood phylogenetic tree (PHYML, [34]). Positive identification of a sample to a reference was given if the sequences exhibited either reciprocal monophyly or less than 3% sequence divergence. Several larval specimens were able to be identified to family (Nannosquillidae) due to their nesting within references of this taxonomic unit. These species were thus referred to as “unknown species 1” and “unknown species 2” since a reference barcode does not currently exist to the species level for this group of samples (Fig 3H). These two different species of Unknown nannosquillid represent one that is unique to this study and one that corresponds to a nannosquillid larval species found in a previous study of larval photoreceptors (KM982429, [15]). The same assignments were also

379 done for two larval sequences nested within the Squilloid superfamily, referred to as “unknown  
380 squilloids.” A complete list of sample sequences and their Genbank accession numbers is  
381 provided in Supplemental Table S1. One sequenced larval species, *Pullosquilla litoralis*, lacks a  
382 published adult reference CO1 sequence and was identified via direct collection from hatched  
383 clutches collected at the University of California Berkeley Gump Station (Moorea, French  
384 Polynesia). *Coronis scolopendra* larvae were hatched from morphologically identified and  
385 isolated adults, therefore they did not necessitate the use of DNA barcoding methods for  
386 identification.

387

388 ***In vivo* Reflectance Measurements:** In the field, a Leitz Dialux 22 compound microscope  
389 (Wetzlar, Germany) was modified to fit two custom machined optical fiber adaptors that were  
390 mounted in place of the eye pieces. An optical fiber (1000 um UV-Vis, Ocean Optics) was  
391 attached to each adaptor and connected to either a white light source (tungsten LS-1, Ocean  
392 Optics) or a spectrometer (QE6500, Ocean Optics) connected to a computer (MacBook Pro,  
393 Apple) running SpectraSuite software (Ocean Optics). The z-plane of each optical fiber adaptor  
394 was adjustable so that the focal point of each optical fiber could be fixed through the objective.  
395 This was accomplished by connecting the light source to one adaptor and focusing the beam onto  
396 a white standard while looking down the eye piece, then doing the same procedure in the reverse  
397 position for the second optical fiber adaptor. Once both optical fibers were focused, all imaging  
398 took place through the digital camera mount (Canon D-series). Live larvae were fixed to sticks  
399 with cyanoacrylate adhesive and mounted in a calibrated angle holder. The mounted larva was  
400 then immersed in a tray of seawater and imaged via a submerged 10× microscope objective (Carl  
401 Zeiss 4820813, West Germany) protected with clear plastic film (Fig. S2A). A goose-neck  
402 laboratory lamp was used to illuminate the specimen from the side for focusing purposes but was  
403 turned off for all spectral measurements. A Spectralon diffuse white standard (Labsphere; North  
404 Sutton, NH, USA) was affixed near the larva to generate a reference reflectance measurement  
405 from the epi-illumination light source through the microscope. Dark (0% reflectance)  
406 measurements were collected by shunting the light to the camera viewing mount. In this way, all  
407 internal reflections through the microscope system were removed from the reflectance  
408 measurement. Once dark and white standards were generated, the pseudopupil of the larval  
409 compound eye was brought into focus and spectral reflectances recorded. Off-optical axis, epi-  
410 illumination measurements of regions of the eye outside the pseudopupil produced similar  
411 reflectance spectra from the eye camouflage as were previously recorded using 45° side-  
412 illumination [17], though reflectivity was greater in the epi-illumination system than when  
413 measured using side-illumination (i.e. Fig. 3G vs. [17]). Epi-illuminated reflectances of the  
414 pseudopupil often contained contributions from the off-axis reflective camouflage due to the size  
415 of the illumination spot and reflectance sample area exceeding the size of the pseudopupil, which  
416 contained four to six ommatidia. On-axis pseudopupil and off-axis reflectance measurements  
417 were collected from the ventral, lateral and dorsal region of each eye. Photographs were also  
418 collected of each specimen in on- and off-illumination conditions by setting a partial shunt  
419 between the camera and the eyepiece connected to the light source (Fig. 3; Movie S3). Spectra  
420 were plotted and analysed using Matlab2015b to generate total ISR reflectance averages as well  
421 as an average pseudopupil reflectances from each ISR-expressing species measured (Fig. S2B-E)

422

423 **Electron & Two-Photon Microscopy:** Eyes were dissected from live animals in the field  
424 immediately post reflectance measurements and fixed with 2.5% glutaraldehyde in PEMS buffer

425 [0.05 M PIPES, 0.05 M PIPES 2K, 10 mM EGTA, 0.5 mM MgCl<sub>2</sub>, and 3.8% sucrose, pH 7.0;  
426 based on [35] for 60 min with intermediate gentle agitation. Remaining tissue was fixed in  
427 absolute ethanol for DNA barcoding identification. Eye tissue was then washed 3 x 10 min in  
428 PEMS buffer and stored in fresh buffer at 4°C for transport to University of Maryland Baltimore  
429 County (UMBC) Porter Imaging Facility or University of Bristol Wolfson Imaging Facility. The  
430 remaining body tissue was fixed in absolute ethanol for DNA barcoding for identification post  
431 hoc. Eyes were postfixed for 60 min in 1.0% osmium tetroxide in water, washed 3 x 10 min in  
432 megapure water, then gently agitated in a second postfix of 2% uranyl acetate aqueous solution  
433 for 60 min. Samples were dehydrated in an ethanol series before being transferred to propylene  
434 oxide for slow infiltration with Epon resin (Taab). Each infiltration step occurred for a minimum  
435 of three hours followed by a final embedding in 100% resin overnight. Tissue was then  
436 transferred to fresh resin and polymerized 7-8 hours at 70 °C. Ultrathin sections (70-80 nm  
437 thickness) were cut using a diamond knife, and imaged via a Zeiss 10 CA transmission electron  
438 microscope at 60 kV (UMBC) or a FEI Tecnai 12 120kV TEM (Bristol).

439

440 TEM micrographs were analysed using ImageJ Fiji software [36] to measure dimensions of ISR  
441 structures, vesicles, and rhabdoms. Since vesicles were often observed as ellipsoid in TEM  
442 micrographs, the average diameter ( $d$ ) of each ISR vesicle was determined by measuring the area  
443 ( $A$ ) of an ellipse drawn along the membrane of the the two-dimensional image of each vesicle  
444 and using the equation

445

446

$$d = 2 \sqrt{\frac{A}{\pi}}$$

447

448 Two unknown nanosquillid species 1 specimens were used for conducting TEM tomography  
449 experiment (LI15001 and LI15008). Serial sections of 300 nm thickness, were cut from Epon  
450 embedded tissue and mounted on open-slot copper grids coated with Formvar film. Prior to  
451 visualization, 15 nm gold fiducials (Aurion, NL) were applied to each side of the grid. The  
452 sample was then mounted in a Fischione tomography sample holder and the images recorded on  
453 an FEI 4k x 4k Eagle camera using FEI Tomography acquisition software in an FEI Tecnai 20,  
454 fitted with a LaB6 filament and operated at 200kV. Tilt series were then reconstructed using  
455 IMOD software (Boulder, Colorado) and segmented using AMIRA software (FEI Visualization  
456 Sciences Group, Bordeaux).

457

458 An individual from unknown nanosquillid species 1 (LI15030) and a first stage *Coronis*  
459 *scolopendra* larva hatched from captive adults in the laboratory (Marine Biological Laboratory,  
460 Woods Hole, MA) were fixed, dehydrated, and cleared for two-photon microscopy using  
461 previously published methods [37]. Imaging of eye structures was completed using an Olympus  
462 XLSLPLN25XGMP objective, exciting autofluorescence using a Newport Spectra-Physics  
463 InSight® DS+™ laser at 810 nm, and generating average images (from 32 frames) with a  
464 resonant scanner as part of a Bruker (Prairie Technologies) Ultima IV *In Vivo* Microscope using  
465 GFP and RFP detection channels. The voxel resolution was 0.2 μm in X, Y and Z. Images were  
466 compiled and animated using Vaa3D [38] and Fiji software.

467

468 **Photonic Model:** To understand the origin of the wavelength-selective response observed from  
469 the pseudopupil we developed a semi-analytical model. Our model combines an analytical

470 approach (Bragg's law [25], and numerical simulations (Finite distance time domain, FDTD) to  
471 describe the optical properties of the ISR.

472  
473 First, to estimate the structural parameters of the ISR we measured its structure factor starting  
474 from TEM images. All TEM and reflectance data used in the model are from unknown  
475 nannosquillid species 1, which provided the most complete set of data. The structure factor  
476 (S(q)) is formally defined as

477

$$S(q) = \frac{1}{N} \left\langle \sum_{i,j=1}^N e^{-iq \cdot (r_i - r_j)} \right\rangle,$$

478

479 where q is the wave vector, N the total number of particles,  $r_{i,j}$  is the position of the vesicles  
480 labeled i and j, and the summation equation  $\langle \dots \rangle$  denotes ensemble average. The structure factor  
481 allowed us to quantify the positional disorder of the ISR (Fig. 4A). From the structural factor we  
482 then integrated S(q) along the direction connecting to vesicles to estimated the lattice constant  
483 ( $\alpha$ ), which was determined to be  $392 \pm 7$  nm.

484

485 Our analytical approach used Bragg's law to predict wavelength position ( $\lambda$ ) of the optical  
486 response of the periodic crystal to incident light

487

$$\lambda = a' * n_{eff},$$

488

489 where  $n_{eff}$  is the effective refractive index, defined by  $n_{eff} = \sqrt{\phi * n_v^2 + (1 - \phi) * n_m^2}$  with  
490 the refractive index of the vesicles ( $n_v$ ) and the refractive index of the surrounding matrix ( $n_m$ ).  
491 The constant  $\phi$  is the filling fraction of the FCC crystal (i.e. the fraction of the crystal structure  
492 occupied by the vesicles) which is defined as  $\phi = \frac{\frac{16}{3}\pi * (r')^3}{(a')^3}$ . The variables a and r are the size of  
493 the crystal cell and the radius of the vesicles, respectively, adjusted such that  $a' = a * \psi$  and  
494  $r' = r * \psi$ .

495

496 It was necessary to consider a range of refractive index values for potential biological materials  
497 since the chemical identity of the membrane vesicles or their contents are not presently known.  
498 Fig S2G depicts the predicted optical response of the ISR with two different values for vesicle  
499 refractive index ( $n_v$ ), 1.48 and 1.70, which model lipid and pteridine-based systems, respectively.  
500 Pteridine was selected as a candidate vesicle material (alternate to lipid) due to its presence in  
501 other crustacean photonic systems [26-28]. Changing  $n_v$  from 1.48 to 1.70 increases the effective  
502 refractive index of the system from 1.35 to 1.39, resulting in both a red-shift of the peak position  
503 and an increase in reflectivity. A slight decrease in the shrinkage constant ( $\psi$ ) from 8% to 5%  
504 was applied to offset the red-shift in the higher  $n_v$  model so as to align both modeled and  
505 experimental reflectance spectra to the same peak for spectral shape comparison (Fig S2G). The  
506 variable  $\psi$  is a fitting parameter that accounts for artificial compression during the sample  
507 preparation for the TEM imaging [39]. In these experiments  $\psi$  was equal to 1.08 or 1.05, which  
508 translates to an estimation of 8% or 5% compression, or shrinkage, of the tissue, respectively.

509



510 Though Bragg's law is powerful tool for analytical analysis of the interaction between light and a  
511 material, it is not sufficient for determining intensity or spectral width of the reflection. For this  
512 reason we also performed numerical FDTD simulations of the optical response of the ISR using  
513 the commercial software LUMERICAL 8.18 (Lumerical 4 Solutions Inc., Vancouver, BC,  
514 Canada). These calculations incorporate values of vesicle periodicity (or distribution within the  
515 ISR), vesicle size, and the dimensions of the entire ISR structure. Using this approach, we also  
516 took into account the finite numerical aperture (NA) of the objective used during the *in vivo*  
517 reflectance experiments (NA=0.22), which strongly affects the intensity and width of the  
518 response from a periodic system (see Fig. S2H).

519  
520 To incorporate positional disorder and particle size distribution of vesicles within the ISR  
521 structure, we then combined the information generated from the FDTD simulations on predicted  
522 reflectance spectral shape with the predicted peak locations determined from Bragg's Law (Fig.  
523 S2I-J). To do this, we calculated the peak position for each value of  $a$  and  $r$  allowed by their  
524 distributions, with mean and standard deviation of  $392 \pm 7$  nm and  $82 \pm 5$  nm, respectively.  
525 Then, we weighted the numerical line shape with the probability of each value of  $a$  and  $r$ , such as  
526 the value of the associated normalized probability density function. The resulting line shapes  
527 (grey lines in Fig. S2I-J) were then fitted with a Gaussian curve that conserves the area of the  
528 starting numerical line shape. An unexpected finding in our calculations was that numerical  
529 aperture has a larger impact on the calculated optical response than the vesicle size distribution  
530 in the structure (Fig. S2H & I). In contrast to this finding, when we account for positional  
531 disorder in the calculations, the result is a broader and less intense reflection peak (Fig. S2J).

532  
533 Experimental reflectance spectra data collected from live larvae (Fig. 3G) used a white diffuser  
534 to generate white light reference standards. These experimental data thus needed to be  
535 normalized against the total incident radiation (silver mirror) to allow for direct comparison with  
536 the theoretical line shape. To do this, we calculated the fraction of light reflected by a white  
537 diffuser with a numerical aperture (NA) equivalent to the experimental condition (NA=0.22)  
538 using Lambert's Law

539  
540 
$$R(\theta_e) = \frac{\int_0^{\theta_e} \cos(\theta) * \sin(\theta) d\theta}{\int_0^{90} \cos(\theta) * \sin(\theta) d\theta}.$$

541  
542 Where  $\theta_e$  is the angle of a cone of reflectance off the standard white diffuser, and  $R(\theta_e)$  is the  
543 fraction of light reflected by a standard white diffuser within the defined cone. For a NA of 0.22,  
544  $\theta_e$  was  $9.8^\circ$  and  $R(\theta_e)$  was 0.03. The experimental data were then multiplied by  $R(\theta_e)$  to obtain  
545 values of reflectance referenced to the total incident radiation.

546  
547 Even after normalizing the data and accounting for the measured disorder, there was a substantial  
548 difference in the intensity between the experimental data and our model (Fig. 3B), which may  
549 arise as the result of several possibilities that are not mutually exclusive. First, due to distortion  
550 of the tissue during histological preparation, there may be error in the measured size of the ISR  
551 itself. Increasing the thickness of the ISR produces an increase in modeled reflectance (Fig.  
552 S2G). It may be that *in vivo* the ISRs are much shorter in length than was observed in  
553 histological sections, producing a less intense reflection (Fig. S2G).

554

555 A second reason for reflectivity differences between the observed and predicted data is related to  
 556 the true level of disorder in the orientation and periodicity of the 3D lattice of the structure. This  
 557 information was neither accessible by two-dimensional image data, such as TEM, nor the  
 558 limited thickness able to be sampled using TEM-tomography. Thus, the true values of disorder  
 559 among the 3D lattice are currently unknown. The third, final reason for differences in the  
 560 calculated and measured reflectances is that we do not know the true NA of the light returning  
 561 from the ISR. In the measured reflection, a fraction of the incident light is absorbed into the  
 562 rhabdom as well as refracted through the animal's light focusing units (crystalline cones and  
 563 lens). Since our calculations reveal that NA has a substantial impact on the calculated reflectance  
 564 (Fig S2H), this may be a major source of disparity between observed and predicted spectra.

565  
 566 **Quantal Catch Modeling:** Mathematical model scripts were compiled in Matlab2017b and are  
 567 provide as supplemental, annotated documents (visualModelfinal.m; a1SSHtemplate.m). In the  
 568 visual model, the change in light flux,  $I$ , as a function of distance from the start of the rhabdom,  
 569  $z$ , was calculated by

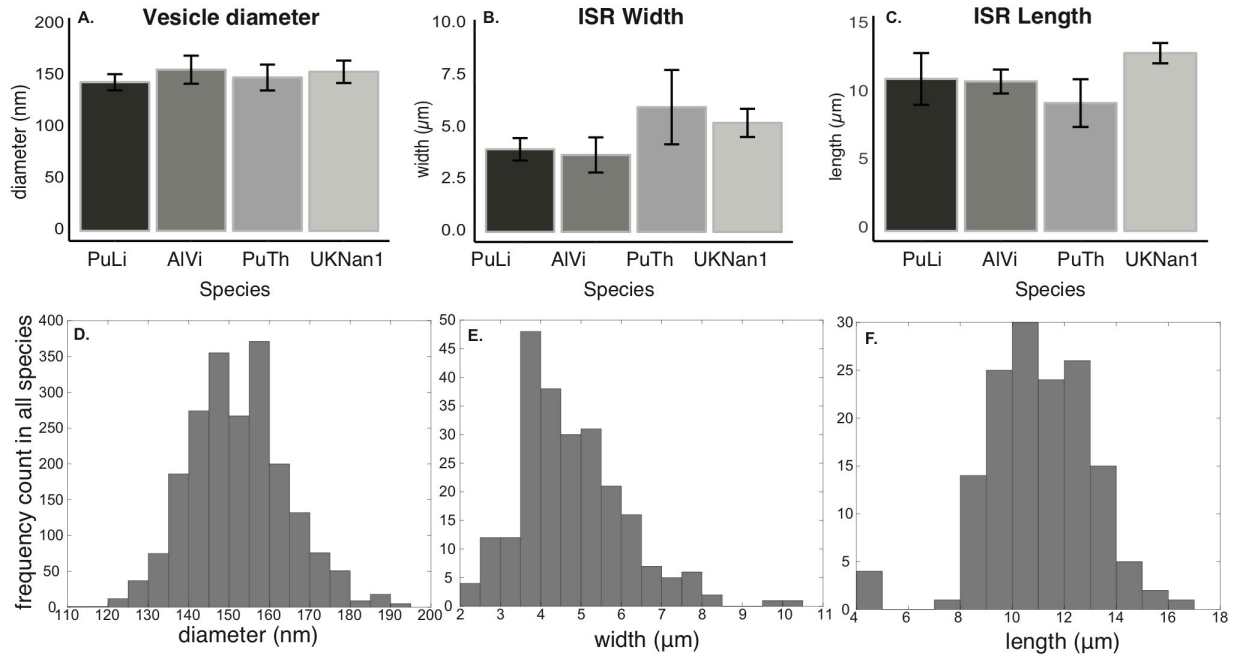
$$570 \frac{dI}{dz} = -\{\eta(\lambda)f(z)\kappa_v(z)\alpha_v(\lambda) + [1 - \eta(\lambda)]\kappa_s(z)\alpha_s(\lambda)\}I(z, \lambda),$$

571  
 572 from Arikawa et al. (1999, [33]). Where  $\eta$  is the fraction of the light flux which is inside the  
 573 rhabdom boundary as a function of wavelength,  $\lambda$ ;  $f$  is the fraction of the rhabdom cross-section  
 574 taken up by a single photoreceptor (in this case  $f = 1$ );  $\kappa_v$  and  $\kappa_s$  are the peak absorption  
 575 coefficients of the visual pigment and screening pigments respectively; and  $\alpha_v(\lambda)$  and  $\alpha_s(\lambda)$   
 576 are the normalized absorption spectra.  $\alpha_v(\lambda)$  was modelled using a visual pigment absorption  
 577 template1 [40]. The fraction of light within the rhabdom therefore was approximated by:  
 578  $\eta(V) = a - be^{-cV}$  with  $a=0.96$ ,  $b=2.82$ ,  $c=1.27$  and the waveguide parameter,  $V = \pi d(n_1^2 -$   
 579  $n_2^2)^{1/2} / \lambda$ ; the rhabdom internal refractive index,  $n_1=1.36$  and the surrounding refractive index  
 580  $n_2=1.34$ . Further model parameters for distal rhabdom length (34  $\mu\text{m}$ ), proximal rhabdom length  
 581 (72  $\mu\text{m}$ ), distal rhabdom diameter (4.5  $\mu\text{m}$ ), proximal rhabdom diameter (3.0  $\mu\text{m}$ ), and  
 582 experimental ISR reflectance curve (peak 572 nm) were derived from mean values calculated in  
 583 this study.

584  
 585  
 586 To test the impact of the ISR on light detection, we used a mathematical model of light  
 587 absorption in rhabdomeric photoreceptors surrounded by screening pigments [33]. Previously,  
 588 yellow, long-pass lateral screening pigments were described in the distal retinas of nannosquillid  
 589 larvae (Fig. 1B, [23]). Since the transmission spectrum of these pigments is similar to the  
 590 reflectance spectrum of the ISR, we incorporated these data into our model of light absorption in  
 591 the photoreceptors (for transmission spectrum see [23]). The light source used to pass along the  
 592 photoreceptor in this model was an estimated as a bioluminescent (BL) radiance emission  
 593 spectrum defined by a Gaussian curve with a full-width at half-maximum of 66 nm (Fig. S3C).  
 594 This estimated BL was determined by examining the spectral width of common bioluminescent  
 595 emission spectra in the literature [41, 42]. Since the specific bioluminescent target viewed by  
 596 nannosquillid retinas is not known, we calculated the quantal catch (QC) for each estimated BL  
 597 emission spectrum with a peak between 450 nm and 650 nm. Waveguide properties of the  
 598 rhabdoms are reported in Fig S3D.

599

600 The light absorbed by the visual pigment and lateral screening pigments was calculated per unit  
601 length of the rhabdom along with the resultant attenuation of the light. Light reaching the ISR  
602 was either reflected back along the distal tier, using the normalized experimental reflection  
603 spectrum,  $R(\lambda)$ , calculated for a conservative 50% reflectivity (Fig S3A; Fig. 4) or transmitted  
604 through to the proximal tier, with transmission spectrum  $=1 - R(\lambda)$ , since the absorption  
605 spectrum of the IPC was assumed to be nil. The light absorbed by the visual pigment was  
606 integrated along the length of the photoreceptor and taken as a measure of relative QC. QC was  
607 calculated as a function of the peak wavelength of the target Gaussian light source (each  
608 estimated BL). Two visual pigments have been measured from different species of nannosquillid  
609 larvae, with peak absorption wavelengths of 450 nm and 500nm [15]. Calculations were thus  
610 performed using all potential combinations of opsin templates for these two visual pigments in  
611 the proximal and distal rhabdomeric tiers (Fig. S3A).  
612



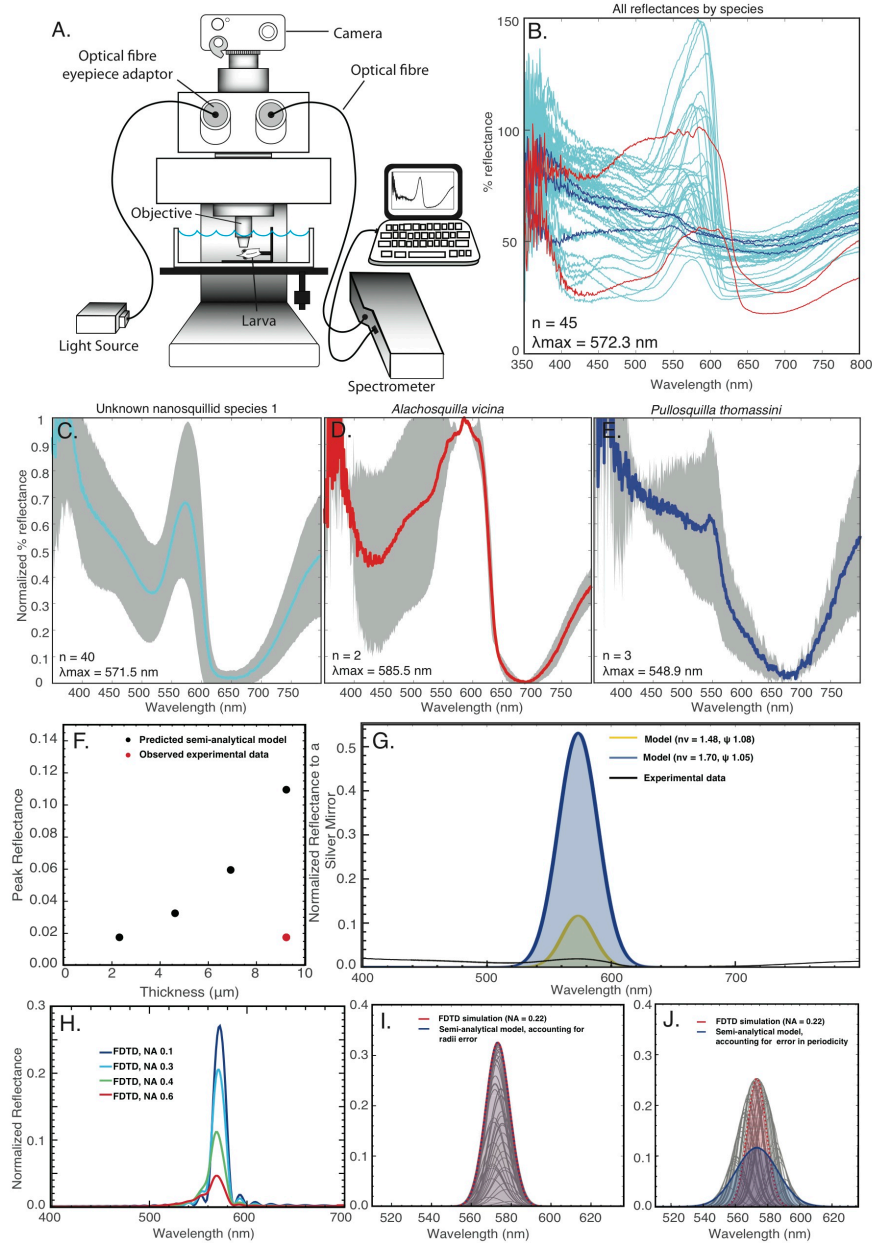
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615 **Fig. S1. Dimensions of intrarhabdomal photonic structure (ISR) features of different individuals from four**  
 616 **different species measured from TEM micrographs. (A)** ISR vesicle diameter measurements. The mean vesicle  
 617 diameters ( $\pm$  standard deviation) are similar between the four individuals of each different species. The overall mean  
 618 diameter across all individuals is  $153.3 \pm 5.6$  nm ( $n_{AIVi} = 314$ ;  $n_{PuLi} = 628$ ;  $n_{PuTh} = 630$ ;  $n_{UKNan1} = 498$ ). The number  
 619 of independent samples precludes any statistical comparison between species. **(B)** The width of ISRs. Mean ( $\pm$   
 620 standard deviation) ISR diameters are similar between the four individuals of each different species. The overall  
 621 mean width of the ISRs is  $4.9 \pm 1.1$  μm ( $n_{AIVi} = 46$ ;  $n_{PuLi} = 85$ ;  $n_{PuTh} = 43$ ;  $n_{UKNan1} = 60$ ). **(C)** The length of ISRs.  
 622 Mean ( $\pm$  standard deviation) ISR lengths are also similar between the four individuals of each different species. The  
 623 overall mean length of the ISRs is  $11.1 \pm 1.5$  μm ( $n_{AIVi} = 28$ ;  $n_{PuLi} = 23$ ;  $n_{PuTh} = 53$ ;  $n_{UKNan1} = 43$ ). **(A-C)**  
 624 Abbreviations for each species, AIVi, *Alochosquilla vicina*; PuLi, *Pullosquilla litoralis*; PuTh, *Pullosquilla*  
 625 *thomassini*; UKNan1, unknown nannosquillid species 1. **(D-F)** Histograms showing the combined distribution of  
 626 vesicle diameter, ISR diameter, and ISR length from all species.

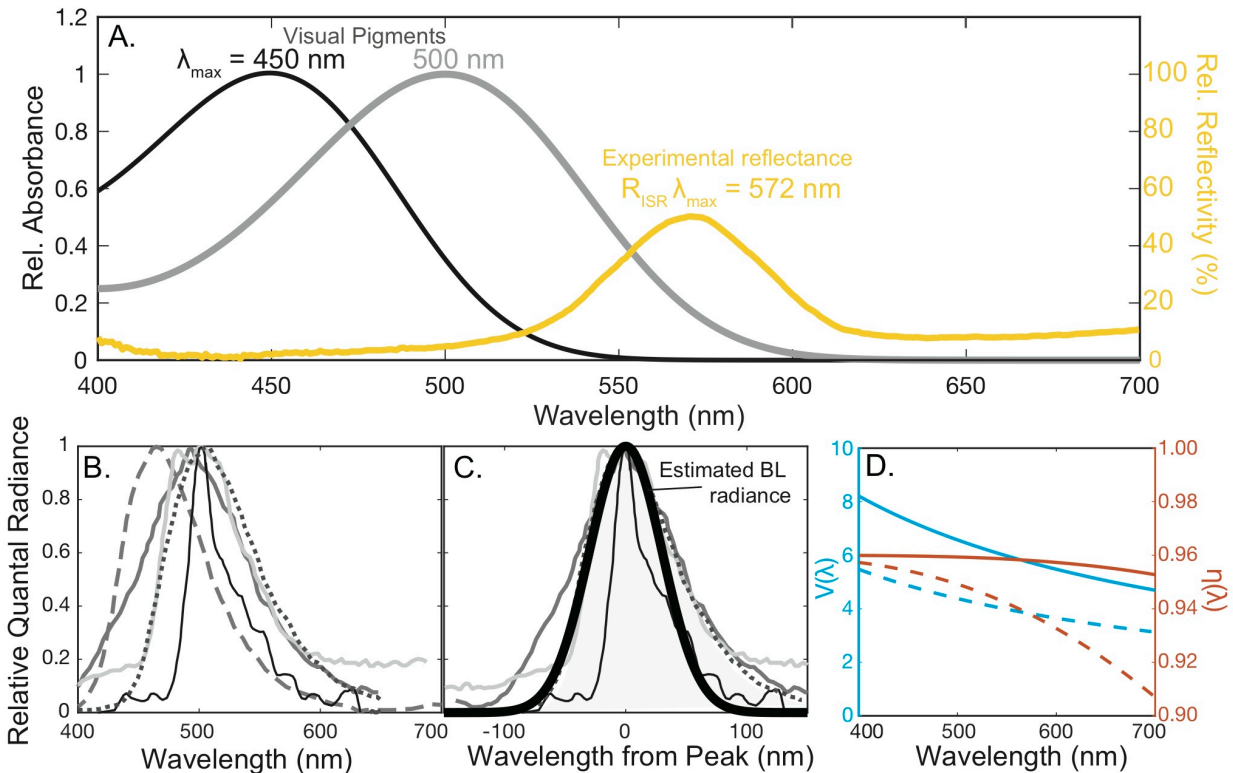
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629  
630 **Fig. S2. Measured and modeled light reflected from the ISR** (A) diagram of epi-reflectance microscope set-up. Objective has numerical aperture of 0.22, which is relevant to reflectance model in H. (B) All reflectances measured  
631 from illuminated pseudopupil of nanosquillid larvae. Variation in reflectivity are likely the result of difference in  
632 ommatidial size, objective alignment with pseudopuil, contributions from the blue reflective camouflage that  
633 surrounds each ommatidium, as well as variables evaluated in F-J. Trace colors correspond to species in in C-E. (C)  
634 Average pseudopupil reflectance from unknown nanosquillid species 1 (D) *Alachosquilla vicina* and (E)  
635 *Pullosquilla thomassini*.  $n$  values and average wavelength of peak reflectance ( $\lambda_{\max}$ ) reported in the bottom left  
636 corner of each figure. (F) Peak intensity calculated as a function of the thickness of the ISR. To retrieve a peak  
637 reflectance comparable to that of the experimental data it is necessary to reduce the thickness of the ISR. Thus, the  
638 difference in observed and predicted thickness may related to distortion of the tissue during histological preparation.  
639 (G) Results of modeled reflectance using two different refractive index values ( $n_v$ ) and associated expansion factors  
640 ( $\psi$ ) versus experimental reflectance (black trace). (H) Simulated reflectance as a function of the numerical aperture  
641 (NA). Note how reflectivity changes as a function of numerical aperture of the objective used to collect the  
642 reflected light. (I) Comparison of semi-analytical curves for different values of vesicles' radii (J) and ISR  
643 periodicity.



646

647

**Fig. S3. QC model spectra, waveguide properties, and results calculated using Gaussian model of ISR**

**reflectance.** (A) 450 nm and 500 nm peak absorbing opsin templates used to describe two possible visual pigment

absorptions in the distal tier. These absorption values were selected based on nannosquillid visual pigment data from

the literature [15, 23]. Thin, dark yellow curve, Gaussian model of ISR reflectance used to calculate QC in (E-H).

Thick, light yellow curve, average reflectance measurements reported in this paper. Both modeled and measured

reflectance calculated for 50% reflectivity (B) Sample spectra of bioluminescent sources as a function of wavelength

digitized from the literature: dark grey long dash line – Ostracod, *Vargula hilgendorf* [41]; dark grey solid line –

Siphonophore, *Bargmannia elongata* [42]; light grey solid line – Midshipman, *Porichthys motatus* [41]; thin black

line – Medusa, *Clytia hemisphaericum* [42]; dark grey short dash line – Ctenophore, *mertensiid* [42]. (C) The same

sample spectra from (B) plotted relative to peak wavelength. Thick black curve shows a 66 nm FWHM Gaussian

used in the quantum catch model to approximate different bioluminescent emission spectra. (D) The waveguide

parameter  $V$  (shown in blue), for the distal (solid) and proximal (dashed) rhabdom tiers. For the parameters used

here, the rhabdom is multi-mode ( $V > 2.405$ ) for all visible wavelengths. The fraction of light inside the rhabdom,

(shown in red) is between 0.9 and 0.96 across the spectrum for both the distal (solid) and proximal (dashed)

rhabdom tiers.

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667

668 **Table S1. Larval stomatopod specimens used in TEM, 2-photon, and *in vivo* reflectance experiments.** Most  
669 larvae were captured from the wild and identified post hoc by DNA barcoding, with the exception of *Coronis*  
670 *scolopendra*, whose larvae were hatched from adults captive in the laboratory. Pseudopupil reflectances were  
671 observed in all nannosquillid larvae tested, with the exception of one *P. thomassini* postlarva, likely due to the  
672 degeneration of the larval retina after metamorphosis.

<i>Species</i>	Larval stage	Genbank #	TEM	2Photon	Reflectance
<i>Alima pacifica</i>	last stage	MK397440	x		
<i>Gonodactylellus affinis</i> (Feller & Cronin 2016)	mid stage	KM982428.1	x		
Unknown nannosquillid 2 (Feller & Cronin 2016)	early stage	KM982429			
<i>Gonodactylus childi</i>	early stage	MK397441	x		
<i>Pullosquilla thomassini</i>	last stage	MK397442	x		
Unknown nannosquillid 1	mid stage	MK397443	x		+
Unknown nannosquillid 1	mid stage	MK397444	x		+
<i>Alachosquilla vicina</i>	early stage	MK397445	x		+
Unknown squilloid	early stage	MK397446			-
Unknown nannosquillid 1	mid stage	MK397447			+
<i>Gonodactylus smithii</i>	mid stage	MK397448			-
<i>Gonodactylellus affinis</i>	mid stage	MK397449			-
Unknown nannosquillid 1	early stage	MK397450	x		+
<i>Pullosquilla thomassini</i>	early stage	MK397451			+
Unknown nannosquillid 1	mid stage	MK397452			+
Unknown nannosquillid 2	mid stage	MK397453			+
<i>Pullosquilla thomassini</i>	post larva	MK397454	x		-
<i>Alima pacifica</i>	last stage	MK397455			-
Unknown squilloid	mid stage	MK397456			-

<i>Haptosquilla trispinosa</i>	mid stage	MK397457		-
<i>Chorisquilla hystrix</i>	mid stage	MK397458		-
<i>Unknown nannosquillid 1</i>	mid stage	MK397459		+
<i>Unknown nannosquillid 1</i>	early stage	MK397460		+
<i>Pullosquilla thomassini</i>	last stage	MK397461		+
<i>Pullosquilla thomassini</i>	last stage	MK397462		+
<i>Chorisquilla hystrix</i>	mid stage	MK397463		-
<i>Chorisquilla hystrix</i>	mid stage	MK397464		-
<i>Unknown nannosquillid 1</i>	mid stage	MK397465		+
<i>Odontodactylus cultrifer</i>	mid stage	MK397466		-
<i>Unknown nannosquillid 1</i>	early stage	MK397467	x	+
<i>Unknown nannosquillid 1</i>	mid stage	MK397468		+
<i>Unknown nannosquillid 1</i>	mid stage	MK397469		+
<i>Unknown nannosquillid 1</i>	early stage	MK397470		+
<i>Unknown nannosquillid 1</i>	mid stage	MK397471		+
<i>Unknown nannosquillid 1</i>	early stage	MK397472		+
<i>Pullosquilla litoralis</i>	early stage	MK397473	x	
<i>Pullosquilla litoralis</i>	early stage	MK397474	x	
<i>Pullosquilla litoralis</i>	early stage	MK397475		
<i>Coronis scolopendra</i>	first stage	hatch ID		x

673

674



675 **Movie S1:** Z-stack of the eye of *Coronis scolopendra* first larval stage, measured using two-  
676 photon microscopy using autofluorescence of the fixed tissue. ISR structures are visible as  
677 slightly darkened sections of the rhabdoms, which appear bright. Untiered, non-ISR expressing  
678 ommatidia are visible in the dorsal region of the eye.

679

680 **Movie S2:** Three-dimensional arrangement of vesicles (yellow) within the ISRs, segmented from  
681 TEM tomography data. This order is preserved across the membranes of the four primary ISR  
682 cells (pink). Arrow indicates the direction of incoming, on-axis light.

683

684 **Movie S3:** Yellow reflection is produced from on-axis illumination of ISR containing  
685 ommatidia. This video was captured during the *in vivo* reflectance measurement experiments and  
686 demonstrates how the pseudopupil moves as the animal rotates the eye in response to changing  
687 illuminations under the objective.

688

689 **Movie S4:** Light microscopy of fixed, disassociated retinas reveal that the ISRs do not contain  
690 colorful, photostable pigments, which is unlike adult mantis shrimp intrarhabdomal filters. Scale  
691 bar, 10  $\mu\text{m}$ .

692

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