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Whole-body photoreceptor networks are independent of ‘lenses’ in brittle stars

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Abstract

Photoreception and vision are fundamental aspects of animal sensory biology and ecology, but important gaps remain in our understanding of these processes in many species. The colour-changing brittle star *Ophiocoma wendtii* is iconic in vision research, speculatively possessing a unique whole-body visual system that incorporates information from nerve bundles underlying thousands of crystalline 'microlenses'. The hypothesis that these form a sophisticated compound eye-like system regulated by chromatophore movement has been extensively reiterated, with consequent investigations into biomimetic optics and similar 'visual' structures in living and fossil taxa. However, no photoreceptors or visual behaviours have ever been identified. We present the first evidence of photoreceptor networks in three *Ophiocoma* species, both with and without microlenses and colour-changing behaviour. High-resolution microscopy, immunohistochemistry and synchrotron tomography demonstrate that putative photoreceptors cover the animals' oral, lateral, and aboral surfaces, but are absent at the hypothesised focal points of the microlenses. The structural optics of these crystal 'lenses' are an exaptation and do not fulfil any apparent visual role. This contradicts previous studies, yet the photoreceptor network in *Ophiocoma* appears even more widespread than previously anticipated, both taxonomically and anatomically.

Keywords: Extra-ocular photoreception, vision, ophiuroids, photoreceptors, sensory biology.

Background

The ability to sense light without eyes, extraocular photoreception (EOP), is being discovered across an increasingly diverse range of animal groups at an accelerating rate [1–4]. EOP generally confers behaviours such as circadian rhythms, phototaxis, reflexes, and colour change, but not spatial resolution [1,3,5]. Controversially, it has been proposed that some echinoderms may be able to consolidate extraocular information to facilitate image-forming

vision [6–9], placing them in a position of exceptional research interest [5]. Understanding the functional model and limits of integration in dispersed photoreceptor systems that may provide spatial resolution will have profound implications for neurobiology, visual evolution, and biomimetic design [1,10], but despite considerable research effort these remain elusive [5].

The brittle star *Ophiocoma wendtii* first attracted attention for its charismatic colour-changing behaviour and extreme sensitivity to illumination [11]. Animals undergo a striking transformation from black-brown during the day to beige-grey with dark bands at night, which can be artificially induced by changing their light environment, and strongly prefer shade to light exposure, including moonlight [11]. Morphological studies reported nerve bundles beneath expanded, highly regular calcite hemispheres on the dorsal arm plates (enlarged peripheral trabeculae, EPTs) [11,12]. The EPTs were speculatively interpreted as potential 'microlenses', proposed to focus light onto putative photoreceptors within or associated with the nerve bundles, with the passage of incoming light regulated by the activity of surrounding "pupillary" chromatophores [5,9,11–13]. This proposal remains unexplored and no photoreceptors have been identified to date; however, many subsequent studies interpreted new data in the context of this hypothesis being accepted. The architecture, distribution and optical properties of the arm plates in *Ophiocoma* are fundamental to the hypothesis that they focus light onto underlying photoreceptor elements [9,11,12], which has also contributed to interpretations of skeletal involvement in echinoid photoreception, yet the EPTs have only been presented in the literature from removed and chemically treated plates [9,12,14,15].

The repeated-unit nature and apparent optical sophistication of this system even led to the speculative suggestion of a compound eye-like function across the dorsal surface of the

58 animal, as has also been proposed in echinoids [7,16,17], facilitating its apparent ability to
59 detect shadows and navigate towards dark shelters from a distance [7,9]. The hypothesis that
60 the EPTs, chromatophores, and underlying nerves could form an advanced visual system has
61 been extensively reiterated by other authors [1,5,7,15,18–26], with resultant investigations
62 into biomimetic optics [9,10,19,20], and vision in both living [22,25] and fossil taxa [14,27].
63 However, there is no morphological or behavioural evidence to support this idea, and no
64 candidates for the necessary neural integration centres that might be required by such a
65 system (though the precise nature of such centres remain unclear) [28].

66 Since the last morphological investigations of *O. wendtii*, numerous opsins – key components
67 of most photosensitive pigments – were identified in the genome of the sea urchin
68 *Strongylocentrotus purpuratus* [29]. This facilitated the discovery of the first opsin-
69 expressing cells in urchins, brittle stars and sea stars, using antibodies subsequently raised
70 against Sp-Op targets [16,26,30], as well as many more opsin sequences in other echinoderms
71 [25,26,31]. Brittle stars, like other echinoderms, possess both rhabdomeric (r-) and ciliary (c-)
72 visual opsins as well as multiple non-visual classes [25,26,32], but exhibit multiple
73 duplications of the rhabdomeric class (closest to Sp-Op4) [26]. These are considered non-
74 visual in most deuterostomes, but are strongly implicated in visual behaviour in both urchins
75 and sea stars [16,33], and sequencing of arm transcriptomes in two brittle stars demonstrated
76 detectable levels of expression of r-opsins similar to Sp-Op4, but not c-opsins, though these
77 were detected at low levels by immunolabelling against Sp-Op1 [25].

78 We established multiple lines of evidence to investigate the presence and location of
79 photoreceptors, determine their arrangement in relation to putative microlenses *in situ*, and
80 compare *Ophiocoma wendtii* with two ecologically co-occurring congeners, one lacking
81 EPTs and colour change behaviour [11]. Immunohistochemistry, scanning electron

microscopy (SEM), synchrotron tomography, and histology were supplemented with exploratory behavioural experiments (supplementary material) in order to finally locate putative photoreceptors and compare their distribution and structure across *Ophiocoma*.

Materials and methods

Specimens

Specimens of *Ophiocoma wendtii*, *O. echinata*, and *O. pumila* were collected from shallow reef rubble at Punta Hospital, Isla Solarte, Bocas del Toro, Panama (9°19'44.4"N, 82°12'21.6"W, 0–3 m), and housed in outdoor flow-through unfiltered seawater aquaria under a natural 12:12 hr light:dark cycle at the Smithsonian Tropical Research Institute, Bocas del Toro, Panama. Animals were photographed, measured, and identified by disc diameter and longest arm length, and allowed three days recovery between collection and experiments. Animals that autotomised arms during or following collection were excluded from trials. Specimens were collected under ARAP permit 2014-52b and exported under ARAP export permit 2015-2.

Synchrotron tomography

Arm segments were fixed in 4% glutaraldehyde in a sodium cacodylate buffer (0.1M, pH 7.4) in their daylight state and stored in sodium cacodylate buffer. Segments were rinsed in buffer and serially dehydrated in acetone before drying with hexamethyldisilazane (HMDS) and mounting on stubs.

Three samples from *Ophiocoma wendtii* (three arm segments), *Ophiocoma pumila* (two arm segments and a pair of arm spines), and *Ophiocoma echinata* (two arm segments and one arm spine) were studied with non-destructive synchrotron tomography. Synchrotron radiation X-ray tomographic microscopy was performed at the TOMCAT beamline (Swiss Light Source, Paul Scherrer Institut, Villigen, Switzerland). Samples were scanned using an X-ray energy

of 20 keV, 1501 projections, and an exposure time of 250 ms. This gave tomographic datasets with a voxel size of 1.75 µm (x, y and z), which were digitally reconstructed as three-dimensional virtual models (electronic supplementary material) using SPIERS [34] and AMIRA (FEI Visualization Science Group).

Histology and scanning electron microscopy

Whole specimens and excised arm segments from *Ophiocoma wendtii*, *O. echinata*, and *O. pumila* were fixed in glutaraldehyde as above and stored in sodium cacodylate buffer (pH 7.4). For histology, arm segments were post-fixed in 1% osmium tetroxide, decalcified in 2% ascorbic acid in 0.15 M sodium chloride solution for 72 hours [16] and dehydrated in an acetone series before embedding in Epon epoxy resin (Agar Scientific). Blocks were sectioned at 1 µm on a Leica RM2255 automated microtome with a diamond knife (HistoJumbo, 8 mm, DiATOME, Switzerland) and stained with Richardson's solution. Sections were photographed using an Olympus E-600 digital camera mounted on an Olympus BX41 microscope.

For SEM, glutaraldehyde-fixed arm segments from *Ophiocoma wendtii* were washed in dilute cacodylate buffer, serially dehydrated in acetone, chemically dried overnight with HMDS, mounted on stubs and visualised on an FEI Quanta FEG scanning electron microscope at 15 kV.

Immunohistochemistry

Light-adapted arm segments from *Ophiocoma wendtii*, *O. echinata*, and *O. pumila* were tested for reactivity to sea urchin ciliary (Sp-Op1) and rhabdomeric (Sp-Op4) opsins [31]. Segments were fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS, pH 7.4) for 30 minutes at room temperature before washing in PBS and decalcifying in 2% ascorbic acid in 0.15 M sodium chloride solution for 72 hours [adapted from 15]. Samples were rinsed in

PBS and stored in 0.05% sodium azide in PBS. Tissue used for sectioning was rinsed in PBS for 20 minutes before embedding in 4% agarose gel. Thick sections (150 μm) were taken using a Leica VT 1200S vibratome. Arm segments and sections were washed in PBS and 0.1% Triton X (PBS-T) and blocked in PBST and 0.5% normal goat serum (NGS) for one hour before incubation with anti-acetylated tubulin (1:200) and either anti-Sp-Opsin4 or anti-Sp-Opsin1 (*Strongylocentrotus purpuratus*, 1:50) [16] overnight, all at room temperature. These antibodies bind to and exhibit high sequence similarity to discovered homologs in brittle stars [25,26]. Specimens were then washed in PBST and incubated with either Alexa Fluor 633 goat anti-mouse (1:500) or Alexa Fluor 488 goat anti-rabbit (1:500) for at least three hours at room temperature, rinsed with PBST and visualised on a Leica TCS SPE confocal laser scanning microscope. Images and image stacks were captured using Leica Application Suite Advanced Fluorescence v.2.6.3 and prepared in Fiji [35].

Results

Arm plate structure

High-resolution synchrotron tomography and SEM visualised expanded peripheral trabeculae (EPTs, putative microlenses) *in situ* without disrupting soft tissue. Regular, near-hemispherical EPTs, 30–40 μm in diameter, cover the dorsal (aboral) arm plates, but also the ventral (oral) arm plates and the dorsal and ventral margins of the lateral plates in *Ophiocoma wendtii* (Figure 1A,B,C), contrary to previous reports that they are restricted to the dorsal plates and dorsal margins of the lateral plates [9,12]. In cross-section, EPTs often appear to be at the distal face of an uninterrupted calcite core projecting through the plate (Figures 1D, S1, S4), leaving little or no room beneath the centre of the EPT for soft tissue. *In vivo*, the plates are covered by a fine dermal cuticle that is highly sensitive to chemical treatment [13]

153 (Figure 1A'). EPTs are interspersed by the projection of short ciliary tufts through the cuticle
154 (Figure 1A') that may represent receptors described as *Stäbchen* [36,37].

155 *Ophiocoma echinata* and *O. pumila* are sympatric with *O. wendtii* and were included in the
156 original study of colour change and light sensitivity in the latter [11]. Whereas *O. echinata*
157 exhibits a similar day-to-night colour change to *O. wendtii*, *O. pumila* does not [11], and the
158 EPTs found in both *O. wendtii* and *O. echinata* were apparently lacking in *O. pumila* [9,12].
159 However, synchrotron scans of *Ophiocoma echinata* and *O. pumila* showed similarities
160 between all three species. *Ophiocoma echinata* have slightly smaller (diameter 20–30 µm)
161 EPTs than *O. wendtii*, again present on the dorsal, ventral, and lateral arm plates and highly
162 regular in shape (Figures 2A–E and S2). The dorsal, ventral, and dorso-ventral margins of the
163 lateral arm plates in *O. pumila* also bear EPT-like structures, in contrast to previous findings
164 from chemically treated plates [9,12] (Figure 2F–J). These structures are smaller (diameter
165 20–25 µm), particularly on the ventral arm plates (diameter 15–20 µm), and more irregular
166 yet anatomically similar to the EPTs observed in the other two species (Figures 1, 2, and S1–
167 S3).

168 *Nerves and opsin reactivity*

169 Immunohistochemistry allowed us to specifically target nerve fibres and cells reactive to sea
170 urchin opsins, where photoreceptors have proved elusive using classical methods [12]. In all
171 three *Ophiocoma* spp., a branching nerve net covers the proximal faces of the arm plates,
172 extending laterally from the midline and emitting branching nerve bundles distally into the
173 plate (Figures 3A,B, 4A, S5A). These originate in the radial nerve cord at the oral side and a
174 smaller medial nerve at the aboral side (Figures 3B, 4A, S4, S5A). Crucially, the bundles
175 innervating the arm plates do not terminate at the proposed focal point of the EPTs according
176 to [9], instead projecting between them towards the outer surface of the arm (Figures 3B,D,

4C). Ovoid cells (soma approx. 10 µm) associated with these nerves surround the EPTs and react to r-opsin antibody Sp-Op4 (Figures 3A, 4D, S5B,C; see Figure S6 for controls). Cell bodies are located just above the midline of the EPTs, project towards the surface of the arm and bear rounded terminal expansions that react strongly to the r-opsin antibody (Figure 4D). These cells are notably absent at the putative focal point of the EPTs, where photoreceptors had been predicted [9,11,12]. They appear to lack specialised membrane structures and are reminiscent of the general receptors described in *Ophioderma longicauda* [38], though a short cilium is not always visible (e.g. Figure 4D); however, they do not resemble those reported in *Ophiura ophiura* [39], which are more akin to the *Stäbchen*. The opsin-reactive cells are regularly arranged over the aboral, lateral, and oral sides of the arm, as well as some at the surface of the spines, in *O. wendtii*, *O. echinata*, and *O. pumila*. They sometimes appear associated with ciliated cells potentially corresponding to those in *Ophionereis schayeri* [40]. Single and multiciliary tufts protrude between the EPTs (Figure 3).

There are also scattered Sp-Op1-reactive cells of similar size (Figure 4A), but these were less consistently observed and so are not further discussed here other than to highlight their presence. We also observed some reactivity to both opsins within the medial and lateral nerves and the radial nerve cord (Figures 3B, 4A and S5C), of which the latter has been reported to exhibit intrinsic photosensitivity and opsin expression [2,26,31].

Potential nerve connections between Sp-Op4-reactive cells, both laterally at the surface and in convergent innervating bundles (Figures 3A,B and S5C), could indicate integration or summation between them. However, we found no unusual or concentrated area of neuropil as might be expected for integrating visual information across such an expansive network.

Discussion

The putative photoreceptor system in *Ophiocoma wendtii*, *O. echinata* and *O. pumila* is extensive; our findings revealed a much larger network than previously posited, which is present across almost the complete body surface in all three species. The morphology, reactivity and arrangement of Sp-Op4-reactive cells support their candidacy as photoreceptors; past work indicates that r-opsins homologous to Sp-Op4 are involved in brittle star photoreception, and that they are likely expressed at higher levels than c-opsin homologs to Sp-Op1 [25,26], in line with our findings. Critically, the nerve bundles proposed to act as photoreceptors project past the EPTs towards the opsin-reactive cells. Contrary to expectations, these putative photoreceptors appear to be entirely independent of the EPTs; their anatomical configuration relative to the EPTs demonstrates no support for an optical role as 'microlenses' (Figures 3A,B and 4D).

The three *Ophiocoma* species possess vast networks of putative dermal photoreceptors covering their dorsal, ventral, and lateral arm plates. This is a considerable expansion on the system hypothesised to exist beneath the EPTs [9,12], both anatomically and taxonomically, and may represent one of the largest dispersed photoreceptor systems described to date, thanks to the ability to monitor expression of molecular markers. These findings complement proposed dermal photoreceptor networks in other echinoderms, most notably sea urchins [7,41], but turn the tables on previous theories about *Ophiocoma wendtii* [9,11]. We anticipate that future researchers will find similarly large extraocular systems in other taxa.

The optical involvement of the EPTs in a photoreceptor system is problematic for several reasons. The EPTs are present on the oral (ventral) and lateral surfaces (Figures 1 and 2) as well as the dorsal arm plates. The lateral plates would be a complex surface for integrated photoreception, let alone vision, and the oral surfaces would be largely redundant; although some brittle stars expose the ventral arm during feeding, *Ophiocoma* does not [42]. Second,

the sheer number of EPTs is enormous; we found an average of 510 EPTs per dorsal arm plate in *Ophiocoma wendtii*, with around 75 plates per arm (mean length 112 mm). Rough calculations indicate that an average-sized individual would possess over 300,000 EPTs. However, they apparently lack any further organisation of the photoreceptors into discrete units, as seen in other distributed visual systems [18,43,44], or a processing centre beyond the radial nerve cords, providing no indication of potential integration mechanisms for such an enormous network. Additionally, the acceptance angle of each receptor between the EPTs would be too large to enable high resolution. Indeed, *Ophiocoma wendtii* exhibits limited visual behaviour according to preliminary tests herein (Figure S7). As a third, independent, argument against an optical role for the EPTs, the cuticle, chromatophores, and other biological material also occlude their rounded shape and surface *in vivo* and may interfere with the passage of light (Figure 1). Expanded chromatophores cover the EPTs completely, with no aperture to indicate pupillary function [5,11,12] (Figure 3). Conversely, contracted chromatophores appear to lie beneath as well as between the EPTs [see 12], further shielding peripheral nerve elements from incoming light in dark-adapted animals.

Finally, and most importantly, the presence of photoreceptive elements is primarily detected in between and not beneath the EPTs. No opsin-reactive cells were observed at the reported focal point beneath the EPTs, and the nerve bundles that were implicated as primary photoreceptors [12] not only lack reactivity to the tested opsins, but project past the EPTs towards the plate surface. Visual photoreceptors in other taxa are not universally located at the optical focal point [43,45], but these opsin-reactive cells are within the dermal layer and apparently far from any potential optical effect of the EPTs; their projection and expansion above the EPTs also negate channelling or light-gathering roles. An identical pattern of anti-Sp-Op4 reactivity is present in *O. pumila*, which lacks highly regular EPTs and colour change

(Figure 3). The optical properties of the EPTs may be an exaptation relevant to materials science [9,10], but they do not appear to perform any optical role in *Ophiocoma*.

Although our findings contest the interpretation of the EPTs as microlenses in *Ophiocoma*, they are still compatible with the electrophysiological studies of Cobb and Hendler [13]. They demonstrated increasing photosensitivity correlating with increasing loss of arm tissue, bleaching EPTs and dermal tissue, including chromatophores, until the nerve bundles beneath each EPT were affected. They argued that this demonstrated these nerve bundles are the primary photoreceptors. However, their findings that the receptors were located beneath the epidermis, regulated in their sensitivity by chromatophores, and became more sensitive with the removal of overlying tissue, are also compatible with the data presented here. The authors acknowledge that other unrecognised cell types could be responsible; given the resemblance of the r-opsin-reactive cells to generalised dermal receptors, it appears that they were indeed overlooked.

Of course, we too cannot eliminate the possibility that additional cells at the base of the EPTs were not detected in this (or any other) study, and echinoderms [46] including brittle stars [26] demonstrate high opsin diversity. Identifying a complete suite of opsin candidates in *Ophiocoma* will help detect other opsin-expressing (or cryptochrome-expressing [47]) tissues underlying the EPTs, if present, although transcriptomic studies in other brittle stars support a key role for Sp-Op4 homologs [25,26]. In addition, functions besides photoreception have now been described for several r-opsins in some arthropods and vertebrates [48]. However, the Sp-Op4-reactive cells we interpret as photoreceptor candidates conform to previous descriptions of receptor morphology and r-opsin expression in other ophiuroids, are positioned within the EPT-chromatophore layer in line with Hendler and Cobb [13], are highly numerous, and represent the only candidates identified in any study in over 30 years.

272 We propose it is highly likely that they are responsible for photosensitivity and corresponding
273 behaviours in *Ophiocoma*.

274 Concerning visual ability, and especially the compound eye model suggested by several
275 authors, we cannot support it based on our findings. *Ophiocoma wendtii* certainly exhibits
276 high sensitivity to light [11] and strong shade-seeking responses (Supplementary material,
277 Figure S7). Our preliminary behavioural experiments showed that *Ophiocoma wendtii* could
278 be capable of basic image formation, as indicated by its ability to detect large, high-contrast
279 targets (Figure S7). However, response to targets of 35–57° is coarse even in comparison to
280 other echinoderms, including urchins using a dermal photoreceptor system where skeletal
281 structures have also been implicated in spatial resolution [7,41]. The detection and location of
282 large, dark, high-contrast targets from short distances also do not necessarily equate to spatial
283 resolution rather than phototaxis (owing to lower overall light intensity in the region of the
284 target), so we hesitate to unequivocally support visual capability. It is not yet clear precisely
285 how the abilities of *O. echinata* and *O. pumila* compare to *O. wendtii* beyond their lesser
286 sensitivity [11]; in light of their relatively distant phylogenetic positions in the genus [49],
287 further comparisons will be of great interest in the context of wider photosensitivity in the
288 taxon. A compound eye requires that each repeated optical unit represents, or scales to, a unit
289 of resolution, a pixel. We find no evidence that the EPTs act as lenses in ommatidium-like
290 optical units, so the photoreceptors could theoretically represent these themselves. If it acts as
291 a compound eye *sensu stricto*, the vast photoreceptor network in *Ophiocoma* should confer
292 fine resolution [50], but this is not supported by behavioural data (Figure S6).

293 Local signal integration and spatial summation could explain high sensitivity and low spatial
294 resolution (if any; Figure S7) in *O. wendtii* [51]. However, the innervation networks do not
295 show any organisational structure that would presumably be a prerequisite for complex signal
296 integration in a compound-type eye, and synapses are known to be relatively rare in

297 ophiuroid nervous systems [28]. Photoresponsive behaviours may instead function through
298 reflex activity within arms or arm segments. Thus, even basic directional light/dark
299 perception could guide non-visual phototactic shelter-seeking behaviour in complex
300 environments with high light intensity and low turbidity [52].

301 **Conclusions**

302 The correlation between increasing responsiveness, EPT distribution, and colour change
303 formerly contributed a key piece of indirect evidence that EPTs are integral to photoreception
304 [9,12]. The joint absence of EPTs and colour change in *Ophiocoma pumila* was interpreted as
305 evidence for the involvement of the EPTs in light sensing [9,11], but it may still indicate their
306 function. Colour change in *Ophiocoma* depends on the expansion and retraction of
307 chromatophores over and around the EPTs [11]. Chromatophore activity is likely to be
308 autonomous and does not appear to be associated with nervous or muscular accessories [12].
309 We therefore propose that the large, regular EPTs found on the arm plates in *O. wendtii* and
310 *O. echinata* could be a structural adaptation relating to chromatophore activity. By
311 maximising separation of chromatophores in their contracted state, the distinction between
312 contracted and expanded states is amplified, producing a more dramatic colour change. The
313 chromatophore activity likely affects photoreceptor sensitivity by altering the amount of
314 screening pigment surrounding them, in line with increased sensitivity in dark-adapted arms
315 [13], but not by controlling the amount of light reaching the EPTs. Thus, the EPTs may have
316 an accessory role in photoreception, through their potential role in colour change, but there is
317 no optical focussing. This is dramatically at odds with the published literature and the popular
318 status of *O. wendtii* as an advanced visual species [5,9].

319 Our findings also caution against interpretations of complex photoreceptor systems from
320 skeletal evidence alone in living and fossil echinoderms [14,22,27]. For example, some

asteroids with visual optic cushions also have EPTs [22,33,53]; these skeletal structures that have optical properties (in the physical sense) are likely irrelevant to the organism's sensory biology. We propose that the placement, concentration, and connectivity of dermal photoreceptors confer high photosensitivity across the body, resulting in sensitive directional extraocular photoreception and not vision *per se* in *Ophiocoma wendtii*. This more accurate model, without requiring focussing lenses, marks a significant advance in understanding the capabilities of extraocular photoreception.

Competing interests

The authors have no competing interests.

Author contributions

LSR designed the study, collected animals, performed histology, SEM, and behavioural experiments, and analysed the data, assisted and supervised by JDS. LSR and EUL performed immunohistochemistry and interpreted results. IAR scanned specimens at the synchrotron, and LSR and IAR processed scan data. LSR and JDS wrote the manuscript, and all authors contributed editorial input and gave their approval for submission.

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Supplementary material is available online as files S1–S3, Figures S4–S8, and Table S9.

Data availability

All data are available on Dryad.

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Figure 1. Expanded peripheral trabeculae (EPTs), skeletal structures in *Ophiocoma wendtii*. Synchrotron X-ray tomography of arm segments. Hemispherical calcite structures previously characterised as lenses (dashed outlines) on the dorsal (A, A'), lateral (B) and, to a lesser extent, ventral (C) arm plates. *In vivo*, arm plates are covered by the cuticle, which obscures the regular form of the EPTs, and is interspersed by ciliary projections (arrowhead) (A'). In cross section (D), the continuous nature of the EPTs with the rest of the stereom is visible, particularly in the lateral regions (arrowhead). See supplementary materials (S1) for reconstructed model.

Figure 2. Calcite elements on the arm plates in *Ophiocoma echinata* and *O. pumila* visualised by synchrotron X-ray tomography. *Ophiocoma echinata* (**A–E**) is covered with very regular, hemispherical EPTs on the dorsal arm plates (**A, B, C**), ventral arm plates (**D**), and the dorsal and ventral regions of the lateral (**A, E**) arm plates. The EPTs are surrounded by pigmented chromatophores giving a dark colour (**B**). *Ophiocoma pumila* (**F–J**) lacks chromatophores and appears much paler (**G**). The skeletal elements are less regular than the EPTs observed in *O. wendtii* (Figure 1) and *O. echinata* (**A–E**), but EPT-like hemispheres are present across the dorsal arm plates (**F, H**), margins of the lateral arm plates (**I**), and ventral arm plates (**J**) . See supplementary materials (S2–3) for reconstructed models. Scale bars: **A**, **F**, 250 µm; **B**, **G**, 500 µm; **C–E**, **H–J**, 25 µm.

Figure 3. Opsin-reactive cells are arranged between the EPTs in *Ophiocoma wendtii*. **A**, **A'**: Cells reactive to a sea urchin rhabdomeric opsin (Sp-Op4, red) and acetylated tubulin (green) are arranged around the distal part of the EPTs (dashed outlines) on the dorsal arm plate (DAP). Dorsal view of arm plate, with stack reaching slightly beneath plate surface. **B**, **C**, **D**: Stacked images of transverse sections through the DAP show the distal projection of nerves between EPTs towards the surface of the arm (**B**, **D**, arrowheads), originating from an

underlying lateral nerve (**B**) and terminating in multiciliary bundles at the surface (**C**). Proximal side of the plate is at the bottom of the image. Note that images in both planes show no opsin-reactive cells present at the focal point of the EPTs as predicted by [9]. Chr, chromatophore; EPT, expanded peripheral trabecula; ner, nerve.

Figure 4. **An expansive system of opsin-reactive cells and "lens"-like skeletal structures is also present in *Ophiocoma pumila*. A**, Horizontal section through dorsal arm plate (DAP, dashed outline) in *O. pumila* demonstrates the same innervation as *O. wendtii,* with a median nerve and paired, branching nerves (acetylated tubulin, green) extending laterally. Reactivity to the c-opsin Sp-Op1 is visible inconsistently across the plate surface and within the median nerve. Dorsal view. **B**, Surface of DAP reconstructed from synchrotron scan, with EPT-like structures (dashed outline) among more irregularly shaped stereom elements. Dorsal view. **C**, **D**, Transverse sections through the arm plate show projections from the lateral nerve (arrowheads) to opsin-reactive cells and ciliary tufts at the surface, between the EPT-like structures. Chr, chromatophore; EPT, expanded peripheral trabecula; lat ner, lateral nerve; med ner, median nerve; ner, nerve bundles.