The crowns have eyes: Multiple opsins found in the eyes of the Crown-of-Thorns Starfish *Acanthaster planci* including the first r-opsin utilized by a deuterostome eye

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Abstract

Opsins are G protein-coupled receptors used for both visual and non-visual photoreception, and these proteins evolutionarily date back to the base of the bilaterians. In the current sequencing age, phylogenomic analysis has proven to be a powerful tool, facilitating the increase in knowledge about diversity within the opsin subclasses and, so far, nine paralogs have been identified. Within echinoderms, opsins have been studied in Echinoidea and Ophiuroidea, which do not possess proper image forming eyes, but rather widely dispersed dermal photoreceptors. However, most species of Asteroidea, the starfish, possess true eyes and studying them will shed light on the diversity of opsin usage within echinoderms and help resolve the evolutionary history of opsins. Using high-throughput RNA sequencing, we have sequenced and analyzed the transcriptomes of different *Acanthaster planci* tissue samples: eyes, radial nerve, tube feet and a mixture of tissues from other organs. At least ten opsins were identified, and eight of them were found significantly differentially expressed in both eyes and radial nerve, providing new important insight into the involvement of opsins in visual and nonvisual photoreception. Of relevance, we found the first evidence of an r-opsin photopigment expressed in a well developed visual eye in a deuterostome animal.

Keywords

Transcriptomics; Asteroidea, vision, photoreceptors, evolution, echinoderm

Introduction

Light carries an immense amount of information about the surroundings. Direct light from the sun, the moon, or the stars is used by various animals to set diurnal or annual clocks and to set direction during navigational tasks. Light reflected from the surroundings guides innumerous different behaviours as it provides information about objects with unprecedented details and speed. Light reception is thus widespread in the animal kingdom but interestingly the molecular machinery behind light reception shares many common features across all phyla. In most cases examined so far the first step in the phototransduction, the absorption of the photons in metazoan, is mediated by a specific protein family called opsins [1]. Opsins are seven transmembrane G protein-coupled receptors binding a chromophore, retinal, which undergoes a conformational change upon the absorption of light, thus triggering the rest of the transduction cascade. During the last couple of decades, several molecular studies have examined the diversity of the opsin family and found nine major clades [2]. What the work has also shown is that many animals have a surprisingly high number of opsin gene copies and that they can be expressed in almost any body region or organ [3]. In many of these cases, the functions remain unknown and may well be outside light reception [4,5].

Light reception is known from all major groups of echinoderms and is facilitated by different types of photoreceptors ranging from non-pigmented dermal photoreceptors to proper image forming eyes. A rather special case has been suggested in the brittlestar *Ophiocoma* with dispersed microlenses formed by skeletal elements [6]. Another dermal photoreceptor system is found in sea urchins which has also been suggested to support image forming vision [7,8]. The genome has been sequenced for the sea urchin *Strongylocentrotus purpuratus* and eight opsin genes were found belonging to the opsin clades c-opsins, r-opsins, Go-opsins, peropsin, neuropsin and echinopsins A and B [9,10]. The latter two groups were recently renamed as bathyopsins and chaopsins, respectively, [2]. The r-opsin Sp-opsin4 is expressed in cells at the base of the transparent tube feet and is putatively mediating the directional negative phototaxis described for a couple of species [8,11,12]. The brittle star *Amphiura filiformis* has even higher

opsin diversity with at least 13 gene copies [13], but here little is known about expression patterns and behavioural roles.

Dermal photoreception is also known from several species of starfish (Asteroidea) [14–16] but it is unknown if it is opsin based and if so whether it is the same opsin as found in the photoreceptors of the eyes. Remarkably, these echinoderms also have well defined eyes. Those eyes are found in most non-burrowing starfish species at the tip of each arm sitting at the base of the unpaired terminal tube foot as a direct extension of the radial nerve. They are compound eyes and structurally they resemble the eyes of arch clams and fan worms [17,18] with lensless ommatidia typically 20-40 µm in diameter. Depending on species, adult specimens have 50-300 ommatidia in each eye and recent studies have shown that this supports spatial resolution in the range of 8-17 degrees used for navigation [19–21]. These studies have also indicated that the ommatidia have a single population of photoreceptors which utilize an opsin with peak sensitivity in the deep blue part of the spectrum around 470 nm.

Recently, two draft genomes of the crown-of-thorns starfish (COTS) *Acanthaster planci*[1], relative to animals collected from Okinawa, Japan and Great Barrier Reef, Australia (GBR), were released. These two genomes shared 98.8% nucleotide identity and were determined to be the same species [22], which is in agreement with previous classification of the Pacific ocean COTS being one species [23]. Although *A. planci* is not the first Asteroidea with an assembled genome, it is the first species with well defined eyes to have an assembled genome. The GBR genome was released along with annotations for ~24,500 protein coding genes [22], and this presents an opportunity to study the mechanisms behind vision in a species with a well defined eye that is evolutionarily close to species with alternative methods of photoreception. Here we have used tissue specific transcriptomics to investigate the differential expression of opsin genes in *A. planci*. We found at least seven different paralogs and, by comparing expression levels in the eyes, in locomotory tube feet, in the radial nerve, and in a mixture of gonadal, stomach, and

[1] COTS is thought to be divided into four separate species distinguished by bodies of water [23]--Red Sea, the Pacific, the Northern and the Southern Indian Ocean, reclassifying the Pacific ocean species as *Acanthaster solaris [24]*. However, for consistency with the released genome we will refer to the GBR species as *A. planci*.

epidermal tissue, we are able to infer which opsins are most likely used in vision, in non-visual photoreception, and outside photoreception.

Material and methods

(a) Animals

The specimens of *A. planci* used in this study were hand collected on the Great Barrier Reef off the coast of Cairns, Australia. After collection the animals were kept in holding tanks with running seawater at 26 degrees for 2-3 days and then flown to Denmark. In Denmark they were kept under similar conditions at the Danish National Aquarium, The Blue Planet, where they were fed three times a week with a past of enriched squid and fish meat. Tissue samples were taken from four specimens with diameters of 15-23 cm. Three terminal tube feet including the eye, 3 locomotory tube feet, approx. 5 cm radial nerve, and pieces of the gonads, the stomas, and the epidermis were sampled from each of the four specimens and stored in RNAlater at 4ºC. Two additional animals were collected at the coastal reefs of Guam and a total of 12 eyes and 10 cm radial nerve were taken from them directly after collection and stored in RNAlater at 4ºC.

(b) RNA extraction and sequencing

The tissue samples were removed from the RNAlater, immediately frozen with liquid nitrogen and homogenized using a mortar and pestle. Powdered tissues were then dissolved in EuroGOLD RNAPure (EMR 506100) and processed using EUROzol RNA extraction protocol (EMR055100, euroclone), then subjected to LitCl (4M) purification. Library preps and sequencing were done at Università degli Studi di Salerno using SMART-Seq v4 Ultra Low Input RNA Kit.

Sequenced reads were examined using fastqc and then quality filtered and trimmed using trimmomatic (v0.33) [25]. Quality controlled reads were quasi-mapped and quantified to v1 great barrier reef *Acanthaster planci* transcriptome using salmon (v0.82) [26]. Transcripts per million (TPM), the normalized transcript counts [27]. Differentially expressed genes were identified using DESeq2 [28] (FDR $\leq$ 0.05 and -1.5 $\geq$ log2FC $\geq$ 1.5). All scripts can be found at https://github.com/elijahlowe/Acanthaster_opsins.git.

(c) Identification of opsin sequences

Opsin protein sequences were collected from echinoderms [10,13,29–34], hemichordates [35] (Freeman et al. 2008), molluscs [36–38], arthropods [39,40], vertebrates [41–47] and annelids [48] covering 40 species and 159 opsin sequences with an additional 7 melatonin receptor sequences to be used as an outgroup. These sequences were retrieved from various databases including Echinobase [49], and NCBI [50], as well as from publications themselves, as described in electronic supplementary material (SI 1). These sequences were used to perform Reciprocal Best Hits (RBH) BLAST against the *A. planci* (GBR) proteome. Additionally, pantherSCORE2.0 [51] was used to identify opsin sequences using hidden Markov models. The collected sequences were aligned with MAFFT (v7.215) [52,53] using L-INS-i algorithm which is better designed for divergence sequences and performed well when benchmarked against other multiple sequence aligners [54]. The aligned sequences were then trimmed using trimAl [55], removing gaps that occurred in 10% of the alignments while being sure to retain 60% of the total sequence length. Initial phylogenetic trees were generated using the aligned sequences with fasttree (v2.1.7) [56] and visualised with figtree (v1.4.3) [57]. Additional trees were then generated using iqtree [58] with 10,000 ultrafast bootstrap support [59] using the 'a Bayesian-like transformation of aLRT' (abayes) method [60], and LG+F+R6 amino acid substitution model selected using ModelFinder [61]. Modifications such as additional labels and visual effects were done using inkscape.

Results

Diversity of opsins in *A. planci*

We identified thirteen putative opsin paralogs in *A. planci*'s proteome using Reciprocal Best Hits (RBH) BLAST and pantherSCORE2.0, which share high sequence similarity. Closer examination using the genome revealed that two of these sequences were actually one fragmented opsin (gbr.65.47.t1 and gbr.65.48.t1), so we manually edited the sequence, which can

be found in the opsin fasta file of electronic supplementary material (SI 2). These sequences were included in a phylogenetic analysis totaling 169 sequences spanning 40 different species. Our phylogenetic analysis from both FastTree and iqtree revealed ten *A. planci* opsins belonging to 7 groups and two sequences clustering with the melatonin receptors outgroup (figure 1): 1 rhabdomeric opsin (r-opsin), 4 ciliary opsins (c-opsins), 1 peropsin, 1 Go-opsin, 1 RGR opsin, 1 neuropsin, and 1 chaopsin. Chaopsins were first classified as echinoderm specific [9,10] but were later found to group with several cnidarian opsins [2]. *A. planci* possesses representatives of all so far known echinoderm opsin groups with the exception of bathyopsin (former echinopsin A), which thus has yet to be identified in any starfish. *A. planci* opsins grouped closest with those of *Patiria miniata*--an eyeless starfish--followed by *Asteria rubens* opsins.

In *A. planci* eyes, all opsins with the exception of ciliary opsin 1.1b and neuropsin were up-regulated in comparison to the mixed tissues (Figure 2). This was also the case when comparing opsin expression in the radial nerve to the mixed sample but to a lesser degree (Figure S1). Expression of opsins in the tube feet, however, was not observed to be up-regulated compared to the mixed tissue, in fact c-opsin 1.1b and neuropsin showed higher expression in the mixed tissue samples (figure 3).

In order to assess the putative functionality of the *A. planci* identified opsin sequences, an analysis of the key residues necessary for opsin function was performed (Table 1). In most opsins, the retinal binds to the K296 via a Schiff base bond, however the proton in the opsin protein is unstable and a counterion is needed and often supplied by the highly conserved Glutamic acid (E113) residue. There are cases, however, where this residue is replaced by a Tyrosine (Y), Phenylalanine (F), Methionine (M), or Histidine (H) and the other highly conserved Glutamic acid residue, E181, serves as the counterion [62]. This is the case with the majority of the opsins in *A. planci*, where E113 are replaced with a Tyrosine (Y), with the exception of Ap-Go-opsin which has a Isoleucine (I) in the position 113.

In addition to ten opsin sequences, we have also observed ten *A. planci* sequences that are potential G protein alpha subunits. Phylogenomic methods classified these sequences as 3 $G\alpha_s$, 1 $G\alpha_o$, 4 $G\alpha_i$, 1 $G\alpha_q$, and 1 $G\alpha_{12}$ (figure S2). All identified G protein alpha subunits with the

exception of 1 $G\alpha_s$ (gbr.231.19.t1), 1 $G\alpha_i$ (gbr.143.10.t1) and the $G\alpha_{12}$ are upregulated in the eyes of *A. planci* compared to the mixed tissue samples (figure S3).

(a) Ciliary and rhabdomeric opsins

There are four ciliary opsins identified in the *A. planci* genome, three of which are expressed in the eyes; c-opsin 1.1a, 1.2 and c-opsin 1.3. These three c-opsins were closely clustered on gbr_scaffold65 (~70 kb), and observed to be significantly differentially expressed in the animal, as neither are expressed in the mixed tissue (figure 4). Of the Ap-c-opsins, 1.1a is the highest expressed, followed by Ap-c-opsin 1.2 and 1.3. C-opsin 1.2 was observed to not have the K296 residue, which is required for the formation of the Schiff base, but instead an Arginine (R) residue is placed in this position. This is the only *A. planci* opsin missing this key residue. While the E113 counterion is replaced with tyrosine (Y) in all of the Ap-c-opsins, the E181 counterion is present in Ap-c-opsin1.1a, completely missing in Ap-c-opsin 1.1b and 1.2 and replaced with an Alanine (A) in Ap-c-opsin 1.3 The Ap-c-opsin 1.2 and Ap-c-opsin 1.3 are missing other important motifs, the C187 and C110 disulfide bond motifs [63], respectively (table 1).

A. planci's r-opsin, on the other hand, is the most highly differentially expressed of the opsins and of any other genes when comparing eye tissues to mixed tissue (figure 2). Further, the Ap-r-opsin was observed to be the most up-regulated in the starfish eye tissue and its sequence features the Lysine residue (K296), critical for the Schiff base formation, and a putative counterion (E181) (Table 1).

(b) Chaopsin

Chaopsin is a recently identified group of opsins. Ramirez et al. [2] found the formerly described groups of anthozoa I opsins [64] and the echinoderm echinopsin B [10] to cluster forming the chaopsin group. In agreement with D'Aniello et al. (2015) we found an *A. planci*'s chaopsin to cluster together with other ambulacrarian chaopsins between the r-opsin and c-opsin clades (figure 1). Ap-chaopsin is amongst the highest differentially expressed opsins in our *A. planci* transcriptomes, with ~9.7 $\log_2$ fold changes in the eye compared to the mixed tissue (figure 2). It

is also expressed in the radial nerve tissue, but to a far lesser degree, and appears to not be significantly expressed in the mixed tissues or the tube feet (figure 3 and S1).

(c) Peropsin RGR and Go opsins

Peropsin and RGR opsin are the highest expressed of the opsin in the tube feet, mixed, and radial nerve, although both still have higher expression in the eyes (figure 3 and S1). The disulfide bond linkage C110/C187, counterion sites and the Lysine for the Schiff base formation are all present. However, both *A. planci* RGR-opsin and peropsin contain variations of the NPxxY motif, NAALQ and NPLMF, respectively (Table 1). Additionaly, peropsin has a variation of the LxxxD motif, ASAGD. Ap-Go-opsin was expressed in both the eyes and radial nerve but was not found in the mixed sample or the tube feet (figure 2 and 3).

Discussion

Light sensing is an important aspect of life and much of it is mediated or initiated by the G-protein-coupled receptor proteins, opsins. The release of the annotated draft genome of *A. planci* has prompted us to investigate its opsin repertoire and expression in a tissue specific manner. This allowed us to classify the specific opsins and to infer possible function and further expand the knowledge of opsin evolution especially within deuterostomes. Ten opsins were identified spanning seven clades: r-opsin, c-opsin, Go-opsin, peropsin, neuropsin, RGR opsin and chaopsin. Opsins have also been sequenced from two other starfish species, *Asterias rubens* and *Patiria miniata*. Through a phylogenomic analysis, it was observed that *A. planci* opsins grouped closest to those in the eyeless *P. miniata*. This grouping is in accordance with the phylogenetic position of these starfish species [65], with *A. planci* as an Acanthasteridae more closely related to *P. miniata*, an Asterinidae, (both species belonging to Valvatida)- than to *A. rubens* belonging to Forcipulatacea. However, studies on tissue specific opsin expression is needed to reveal in which organs of the eyeless starfish *P. miniata* the respective opsin orthologs are expressed. To this point, it remains unclear if opsins potentially serving a visual function in the eye possessing *A. planci* might have switched functions in the eyeless representative, or if

their expression simply persists as a potential evolutionary remain, a finding known e.g. from blind cave salamanders which still express opsins inside their highly degenerated and pigmentless photoreceptors [66].

Rhabdomeric opsin

Electrophysiological recordings from the photoreceptors of *A. planci* have shown light absorption here to be utilizing a single opsin. Of the several opsins found to be expressed in the eyes of *A. planci*, r-opsin appeared as the highest differentially expressed gene in the eye transcriptome when compared to mixed samples, thus suggesting this to be the opsin utilized for vision. A similar function has been proposed for the sea urchin r-opsin in *S. purpuratus* tube feet [7]. Whereas rhabdomeric opsins have been described in many protostome species as the primary opsin for vision (reviewed in [67]), no deuterostome eye has previously been found to express an r-opsin. As *A. planci* eyes have been previously demonstrated to perform proper spatial vision [19–21], our findings thus provide first evidence for a deuterostome eye utilizing an r-opsin for spatial vision.

Ciliary opsin

Ciliary opsins are well known for their role in vertebrate vision and brain function in some invertebrates [68].[68]They are differentially expressed in *A. planci* eyes, with the exception of Ap-c-opsin 1.1b, which is not clustered on the same scaffold as the other 3 Ap-c-opsins. Interestingly, the three clustered opsins show a correspondence between their spatial ordering within the cluster (in the direction 3' to 5') and their expression level, with most 3’ Ap-c-opsin being the most highly expressed in both eye and radial nerve (figure 4). C-opsins appear clustered also in vertebrates, for example, human’s medium and long wavelength ciliary opsins (opsin1MW1, opsin1MW2, opsin1MW3 and opsin1LW) are clustered on chromosome X, while human’s short wavelength c-opsin is on chromosome 7 (Ensembl genome browser; [69]). Clustering of opsins has so far not been described outside deuterostomes, but it is also seen in the echinoderm, *S. purpuratus* where Go-opsins (opsin 3.1 and 3.2) clustered on scaffold26

(Echinobase; [49]). The significance of the occurrence of opsin clustering in echinoderms is not obvious and awaits more functional studies.

Chaopsin

Chaopsin (Ap-chaopsin) is the second most differentially expressed opsin in *A. planci* eyes and it has many of the motifs necessary for phototransduction, including the NPxxY binding motif in the 7 transmembrane domain involved in coupling with the G protein. Little is known about the functions of chaopsins (or opsin5) but they have been identified in Echinoidea, Asteroidea, and Ophiuroidea [10,13,30,34,49]. In the respective species, chaopsins have been found analyzing genomic data or transcriptomes of hypothesized photosensitive tissues, such as the tube feet in *Strongylocentrotus droebachiensis [30]* and *Strongylocentrotus intermedius [34]*. A chaopsin was identified in the genome of the eyeless *P. miniata* but was not found in transcriptome data of the eye possessing starfish *A. rubens*. The lack of chaopsin in *A. rubens* is probably a methodological artifact, since general arm tissue, including radial nerve and tube feet, but not eyes, has been used to generate these transcriptomic data [70]. In the Caribbean elkhorn coral, *Acropora palmata,* opsin3, which along with echinoderm opsin5 belongs to the chaopsin clade [2], has been demonstrated to couple with a Gq-protein in a light-dependent manner [71]. This leads us to hypothesize that the Ap-chaopsin may be important for phototransduction in *A. planci* and potentially in all echinoderms. The exact role of this opsin remains elusive, though.

Peropsin and RGR opsin

Peropsin and RGR opsin were expressed in the tube feet, the mixed tissue, and the radial nerve of *A. planci*, but both have the highest expression in the eyes. They are considered as photoisomerase enzymes and not as photopigments, since they bind to all-trans retinaldehyde to regenerate 11-cis-retinoids for pigment regeneration. This has been observed for peropsin in the vertebrate retinal pigment epithelium [72], in cephalopod photoreceptors [73] and in the jumping spider [74]. Knock-down mice [75], together with biochemical and spectroscopic studies in amphioxus [76], have demonstrated the same properties for RGR opsin in chordates. This family

of opsins is thus important for visual pigment regeneration [77]. While RGR opsins are known to not have the NPxxY binding motif in the 7 transmembrane domain involved in coupling with the G protein, this motif is often found in peropsins [74,76]. This was not the case in *A. planci*, where both RGR opsin and peropsin have varying NPxxY binding motifs. This could alter peropsin's ability to couple with G proteins, further supporting its function as photoisomerase. However, it is worth mentioning that, in chicken, the presence of both peropsin and RGR opsin is thought to serve in the visual cycle of the circadian clock [46].

Go-opsin

In *A. planci* eye and radial nerve transcriptomes, we found significant expression of a Go-opsin along with putative Go alpha subunit proteins. Evidence for Go-opsins in animals is rare. These opsins interact with a specific G-protein that differs from those in the c-opsin as well as the r-opsin transduction cascade [38,78]. In the retina of the bivalve *Patinopecten yessonensis*, Go-opsin is expressed in a layer of morphologically ciliary receptor cells, which do not express a ciliary opsin. However, in the marine annelid *Platynereis dumerilii*, Go-opsin is coexpressed with two r-opsins in the photoreceptor cells of the larval eye [79]. Knocking down Go-opsin did not lead to absence of phototaxis in *P. dumerilii* but did reduce the sensitivity to the blue-cyan part of the color spectrum (λ_{max}=488 nm). A similar absorbance spectrum (λ_{max}=483 nm) was observed in amphioxus, *Branchinostoma belcheri*, Go-opsin [76,80]. While the morphological and expression data on Go-opsin in *P. yessonensis* point towards a functioning in a visual context, no other studies have found this. Our data does not reveal if *A. planci* Go-opsin is co-expressed with any other opsins inside the same cells, nonetheless, the fact that this opsin is expressed in the starfish eye along with the results from annelids and amphioxus, opens up the possibility that it is involved in spectral tuning of vision in *A. planci*. Such a tuning would indeed be consisted with the spectral sensitivity curves obtained by electrophysiology in this starfish species [20].

Conclusion

In conclusion, our findings demonstrate that the eye of the starfish *A. planci* expresses an entire set of ten different opsin proteins. This starfish has recently been demonstrated to perform spatial vision in order to prey on its preferred coral food and its r-opsin, as the by far most highly expressed photopigment in its eyes, is likely to facilitate these sophisticated visually guided behaviours. *A. planci* is thus not only one more echinoderm possessing a much more complex photoreceptor system than previously assumed, but rather the first deuterostome animal that has been shown to navigate by the use of r-opsin expressing photoreceptors. The variety of opsins found differentially expressed in various starfish light sensitive tissues by our transcriptomic analyses also set the groundwork for comparative studies on evolutionary changes in photoreceptor function that occurred towards the vertebrate eye.

Data accessibility

Raw sequencing data will be submitted to NCBI SRA under the accession number SUB2926139. Additionally, fasta files containing all sequences can be found in the data section of the github repository https://github.com/elijahlowe/Acanthaster_opsins and in electronic supplementary material.

Authors' contributions

Collection of tissue samples were done by AG. RNA extraction was performed by (MIA) and EKL. Computational analysis was performed by EKL. Writing of the paper was performed by EKL, AG, IA and EUL. All authors contributed to the project design.

Competing interests

The authors declare that we have no competing interest.

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References

1. Land MF, Nilsson D-E. 2012 Light and vision. In *Animal Eyes*, pp. 23–45.(doi:10.1093/acprof:oso/9780199581139.003.0002)

2. Ramirez MD, Pairett AN, Pankey MS, Serb JM, Speiser DI, Swafford AJ, Oakley TH. 2016 The Last Common Ancestor of Most Bilaterian Animals Possessed at Least Nine Opsins. *Genome Biol. Evol.* **8**, 3640–3652. (doi:10.1093/gbe/evw248)

3. Suga H, Schmid V, Gehring WJ. 2008 Evolution and functional diversity of jellyfish opsins. *Curr. Biol.* **18**, 51–55. (doi:10.1016/j.cub.2007.11.059)

4. Shen WL, Kwon Y, Adegbola AA, Luo J, Chess A, Montell C. 2011 Function of rhodopsin in temperature discrimination in Drosophila. *Science* **331**, 1333–1336. (doi:10.1126/science.1198904)

5. Leung NY, Montell C. 2017 Unconventional Roles of Opsins. *Annu. Rev. Cell Dev. Biol.* (doi:10.1146/annurev-cellbio-100616-060432)

6. Aizenberg J, Tkachenko A, Weiner S, Addadi L, Hendler G. 2001 Calcitic microlenses as part of the photoreceptor system in brittlestars. *Nature* **412**, 819–822. (doi:10.1038/35090573)

7. Ullrich-Lüter EM, Dupont S, Arboleda E, Hausen H, Arnone MI. 2011 Unique system of photoreceptors in sea urchin tube feet. *Proc. Natl. Acad. Sci. U. S. A.* **108**, 8367–8372. (doi:10.1073/pnas.1018495108)

8. Ullrich-Lüter EM, D'Aniello S, Arnone MI. 2013 C-opsin expressing photoreceptors in echinoderms. *Integr. Comp. Biol.* **53**, 27–38. (doi:10.1093/icb/ict050)

9. Raible F, Tessmar-Raible K, Arboleda E, Kaller T, Bork P, Arendt D, Arnone MI. 2006 Opsins and clusters of sensory G-protein-coupled receptors in the sea urchin genome. *Dev. Biol.* **300**, 461–475. (doi:10.1016/j.ydbio.2006.08.070)

10. D'Aniello S *et al.* 2015 Opsin evolution in the Ambulacraria. *Mar. Genomics* **24 Pt 2**, 177–183. (doi:10.1016/j.margen.2015.10.001)

11. Blevins E, Johnsen S. 2004 Spatial vision in the echinoid genus Echinometra. *J. Exp. Biol.* **207**, 4249–4253. (doi:10.1242/jeb.01286)

12. Yerramilli D, Johnsen S. 2009 Spatial vision in the purple sea urchin Strongylocentrotus purpuratus (Echinoidea). *J. Exp. Biol.* **213**, 249–255. (doi:10.1242/jeb.033159)

13. Delroisse J, Ullrich-Lüter E, Ortega-Martinez O, Dupont S, Arnone M-I, Mallefet J, Flammang P. 2014 High opsin diversity in a non-visual infaunal brittle star. *BMC Genomics* **15**, 1035. (doi:10.1186/1471-2164-15-1035)

14. Yoshida M, Ohtsuki H. 1968 The phototactic behavior of the starfish, Asterias amurensis Lutken. *Biol. Bull.* **134**, 516–532.

15. Yoshida M. 1979 Extraocular Photoreception. In *Comparative Physiology and Evolution of Vision in Invertebrates*, pp. 581–640. Springer, Berlin, Heidelberg. (doi:10.1007/978-3-642-66999-6_10)

16. Yoshida M, Takasu N, Tamotsu S. 1984 Photoreception in Echinoderms. In *Photoreception and Vision in Invertebrates*, pp. 743–771. Springer, Boston, MA. (doi:10.1007/978-1-4613-2743-1_22)

17. Nilsson D-E. 1994 Eyes as Optical Alarm Systems in Fan Worms and Ark Clams. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **346**, 195–212. (doi:10.1098/rstb.1994.0141)

18. Bok MJ, Capa M, Nilsson D-E. 2016 Here, There and Everywhere: The Radiolar Eyes of Fan Worms (Annelida, Sabellidae). *Integr. Comp. Biol.* **56**, 784–795. (doi:10.1093/icb/icw089)

19. Garm A, Nilsson D-E. 2014 Visual navigation in starfish: first evidence for the use of vision and eyes in starfish. *Proc. Biol. Sci.* **281**, 20133011. (doi:10.1098/rspb.2013.3011)

20. Petie R, Garm A, Hall MR. 2016 Crown-of-thorns starfish have true image forming vision. *Front. Zool.* **13**, 41. (doi:10.1186/s12983-016-0174-9)

21. Petie R, Hall MR, Hyldahl M, Garm A. 2016 Visual orientation by the crown-of-thorns starfish (Acanthaster planci). *Coral Reefs* **35**, 1139–1150. (doi:10.1007/s00338-016-1478-0)

22. Hall MR *et al.* 2017 The crown-of-thorns starfish genome as a guide for biocontrol of this coral reef pest. *Nature* **544**, 231–234. (doi:10.1038/nature22033)

23. Vogler C, Benzie J, Lessios H, Barber P, Wörheide G. 2008 A threat to coral reefs multiplied? Four species of crown-of-thorns starfish. *Biol. Lett.* **4**, 696–699. (doi:10.1098/rsbl.2008.0454)

24. Haszprunar G, Vogler C, Wörheide G. 2017 Persistent Gaps of Knowledge for Naming and Distinguishing Multiple Species of Crown-of-Thorns-Seastar in the Acanthaster planci Species Complex. *Diversity* **9**, 22. (doi:10.3390/d9020022)

25. Bolger AM, Lohse M, Usadel B. 2014 Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* **30**, 2114–2120. (doi:10.1093/bioinformatics/btu170)

26. Patro R, Duggal G, Love MI, Irizarry RA, Kingsford C. 2017 Salmon provides fast and bias-aware quantification of transcript expression. *Nat. Methods* **14**, 417–419. (doi:10.1038/nmeth.4197)

27. Li B, Ruotti V, Stewart RM, Thomson JA, Dewey CN. 2010 RNA-Seq gene expression estimation with read mapping uncertainty. *Bioinformatics* **26**, 493–500.

(doi:10.1093/bioinformatics/btp692)

28. Love MI, Huber W, Anders S. 2014 Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol.* **15**, 550. (doi:10.1186/s13059-014-0550-8)

29. Ooka S, Katow T, Yaguchi S, Yaguchi J, Katow H. 2010 Spatiotemporal expression pattern of an encephalopsin orthologue of the sea urchin Hemicentrotus pulcherrimus during early development, and its potential role in larval vertical migration. *Dev. Growth Differ.* **52**, 195–207. (doi:10.1111/j.1440-169X.2009.01154.x)

30. Lesser MP, Carleton KL, Böttger SA, Barry TM, Walker CW. 2011 Sea urchin tube feet are photosensory organs that express a rhabdomeric-like opsin and PAX6. *Proc. Biol. Sci.* **278**, 3371–3379. (doi:10.1098/rspb.2011.0336)

31. Cannon JT, Kocot KM, Waits DS, Weese DA, Swalla BJ, Santos SR, Halanych KM. 2014 Phylogenomic resolution of the hemichordate and echinoderm clade. *Curr. Biol.* **24**, 2827–2832. (doi:10.1016/j.cub.2014.10.016)

32. Mashanov VS, Zueva OR, García-Arrarás JE. 2014 Transcriptomic changes during regeneration of the central nervous system in an echinoderm. *BMC Genomics* **15**, 357. (doi:10.1186/1471-2164-15-357)

33. Elphick MR, Semmens DC, Blowes LM, Levine J, Lowe CJ, Arnone MI, Clark MS. 2015 Reconstructing SALMFamide Neuropeptide Precursor Evolution in the Phylum Echinodermata: Ophiuroid and Crinoid Sequence Data Provide New Insights. *Front. Endocrinol.* **6**, 2. (doi:10.3389/fendo.2015.00002)

34. Zhao C, Ji N, Tian X, Feng W, Sun P, Wei J, Chang Y. 2015 Opsin4,Opsin5, andPax6significantly increase their expression in recently settled juveniles of the sea urchinStrongylocentrotus intermedius(Echinodermata: Echinoidea). *Invertebr. Reprod. Dev.* **59**, 119–123. (doi:10.1080/07924259.2015.1041655)

35. Freeman RM Jr *et al.* 2008 cDNA sequences for transcription factors and signaling proteins of the hemichordate Saccoglossus kowalevskii: efficacy of the expressed sequence tag (EST) approach for evolutionary and developmental studies of a new organism. *Biol. Bull.* **214**, 284–302. (doi:10.2307/25470670)

36. Hall MD, Hoon MA, Ryba NJ, Pottinger JD, Keen JN, Saibil HR, Findlay JB. 1991 Molecular cloning and primary structure of squid (Loligo forbesi) rhodopsin, a phospholipase C-directed G-protein-linked receptor. *Biochem. J* **274 (Pt 1)**, 35–40.

37. Hara-Nishimura I, Kondo M, Nishimura M, Hara R, Hara T. 1993 Cloning and nucleotide sequence of cDNA for rhodopsin of the squid Todarodes pacificus. *FEBS Lett.* **317**, 5–11.

38. Kojima D, Terakita A, Ishikawa T, Tsukahara Y, Maeda A, Shichida Y. 1997 A Novel Go-mediated Phototransduction Cascade in Scallop Visual Cells. *J. Biol. Chem.* **272**,

22979–22982. (doi:10.1074/jbc.272.37.22979)

39. Carulli JP, Hartl DL. 1992 Variable rates of evolution among Drosophila opsin genes. *Genetics* **132**, 193–204.

40. Chou WH, Hall KJ, Wilson DB, Wideman CL, Townson SM, Chadwell LV, Britt SG. 1996 Identification of a novel Drosophila opsin reveals specific patterning of the R7 and R8 photoreceptor cells. *Neuron* **17**, 1101–1115.

41. Okano T, Kojima D, Fukada Y, Shichida Y, Yoshizawa T. 1992 Primary structures of chicken cone visual pigments: vertebrate rhodopsins have evolved out of cone visual pigments. *Proc. Natl. Acad. Sci. U. S. A.* **89**, 5932–5936.

42. Sun H, Gilbert DJ, Copeland NG, Jenkins NA, Nathans J. 1997 Peropsin, a novel visual pigment-like protein located in the apical microvilli of the retinal pigment epithelium. *Proc. Natl. Acad. Sci. U. S. A.* **94**, 9893–9898.

43. Tao L, Shen D, Pandey S, Hao W, Rich KA, Fong HK. 1998 Structure and developmental expression of the mouse RGR opsin gene. *Mol. Vis.* **4**, 25.

44. Provencio I, Rodriguez IR, Jiang G, Hayes WP, Moreira EF, Rollag MD. 2000 A novel human opsin in the inner retina. *J. Neurosci.* **20**, 600–605.

45. Kasper G *et al.* 2002 Different structural organization of the encephalopsin gene in man and mouse. *Gene* **295**, 27–32.

46. Bailey MJ, Cassone VM. 2004 Opsin photoisomerases in the chick retina and pineal gland: characterization, localization, and circadian regulation. *Invest. Ophthalmol. Vis. Sci.* **45**, 769–775.

47. Sakami S, Kolesnikov AV, Kefalov VJ, Palczewski K. 2014 P23H opsin knock-in mice reveal a novel step in retinal rod disc morphogenesis. *Hum. Mol. Genet.* **23**, 1723–1741. (doi:10.1093/hmg/ddt561)

48. Arendt D, Tessmar K, de Campos-Baptista M-IM, Dorresteijn A, Wittbrodt J. 2002 Development of pigment-cup eyes in the polychaete Platynereis dumerilii and evolutionary conservation of larval eyes in Bilateria. *Development* **129**, 1143–1154.

49. Cameron RA, Samanta M, Yuan A, He D, Davidson E. 2009 SpBase: the sea urchin genome database and web site. *Nucleic Acids Res.* **37**, D750–4. (doi:10.1093/nar/gkn887)

50. NCBI Resource Coordinators. 2016 Database resources of the National Center for Biotechnology Information. *Nucleic Acids Res.* **44**, D7–19. (doi:10.1093/nar/gkv1290)

51. Thomas PD, Campbell MJ, Kejariwal A, Mi H, Karlak B, Daverman R, Diemer K, Muruganujan A, Narechania A. 2003 PANTHER: a library of protein families and

subfamilies indexed by function. *Genome Res.* **13**, 2129–2141. (doi:10.1101/gr.772403)

52. Katoh K. 2002 MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Res.* **30**, 3059–3066. (doi:10.1093/nar/gkf436)

53. Katoh K, Standley DM. 2013 MAFFT: Iterative Refinement and Additional Methods. In *Methods in Molecular Biology*, pp. 131–146.(doi:10.1007/978-1-62703-646-7_8)

54. Thompson JD, Linard B, Lecompte O, Poch O. 2011 A comprehensive benchmark study of multiple sequence alignment methods: current challenges and future perspectives. *PLoS One* **6**, e18093. (doi:10.1371/journal.pone.0018093)

55. Capella-Gutiérrez S, Silla-Martínez JM, Gabaldón T. 2009 trimAl: a tool for automated alignment trimming in large-scale phylogenetic analyses. *Bioinformatics* **25**, 1972–1973. (doi:10.1093/bioinformatics/btp348)

56. Price MN, Dehal PS, Arkin AP. 2009 FastTree: computing large minimum evolution trees with profiles instead of a distance matrix. *Mol. Biol. Evol.* **26**, 1641–1650. (doi:10.1093/molbev/msp077)

57. Morariu VI, Srinivasan BV, Raykar VC, Duraiswami R, Davis LS. 2009 Automatic online tuning for fast Gaussian summation. In *Advances in Neural Information Processing Systems 21* (eds D Koller, D Schuurmans, Y Bengio, L Bottou), pp. 1113–1120. Curran Associates, Inc.

58. Nguyen L-T, Schmidt HA, von Haeseler A, Minh BQ. 2015 IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Mol. Biol. Evol.* **32**, 268–274. (doi:10.1093/molbev/msu300)

59. Minh BQ, Nguyen MAT, von Haeseler A. 2013 Ultrafast approximation for phylogenetic bootstrap. *Mol. Biol. Evol.* **30**, 1188–1195. (doi:10.1093/molbev/mst024)

60. Anisimova M, Gil M, Dufayard J-F, Dessimoz C, Gascuel O. 2011 Survey of branch support methods demonstrates accuracy, power, and robustness of fast likelihood-based approximation schemes. *Syst. Biol.* **60**, 685–699. (doi:10.1093/sysbio/syr041)

61. Kalyaanamoorthy S, Minh BQ, Wong TKF, von Haeseler A, Jermiin LS. 2017 ModelFinder: fast model selection for accurate phylogenetic estimates. *Nat. Methods* **14**, 587–589. (doi:10.1038/nmeth.4285)

62. Terakita A. 2005 The opsins. *Genome Biol.* **6**, 213. (doi:10.1186/gb-2005-6-3-213)

63. Karnik SS, Khorana HG. 1990 Assembly of functional rhodopsin requires a disulfide bond between cysteine residues 110 and 187. *J. Biol. Chem.* **265**, 17520–17524.

64. Hering L, Mayer G. 2014 Analysis of the opsin repertoire in the tardigrade Hypsibius dujardini provides insights into the evolution of opsin genes in panarthropoda. *Genome*

Biol. Evol. **6**, 2380–2391. (doi:10.1093/gbe/evu193)

65. Mah C, Foltz D. 2011 Molecular phylogeny of the Valvatacea (Asteroidea: Echinodermata). *Zool. J. Linn. Soc.* **161**, 769–788. (doi:10.1111/j.1096-3642.2010.00659.x)

66. Kos M, Bulog B, Szél A, Röhlich P. 2001 Immunocytochemical demonstration of visual pigments in the degenerate retinal and pineal photoreceptors of the blind cave salamander (Proteus anguinus). *Cell Tissue Res.* **303**, 15–25. (doi:10.1007/s004410000298)

67. Plachetzki DC, Serb JM, Oakley TH. 2005 New insights into the evolutionary history of photoreceptor cells. *Trends Ecol. Evol.* **20**, 465–467. (doi:10.1016/j.tree.2005.07.001)

68. Arendt D, Tessmar-Raible K, Snyman H, Dorresteijn AW, Wittbrodt J. 2004 Ciliary photoreceptors with a vertebrate-type opsin in an invertebrate brain. *Science* **306**, 869–871. (doi:10.1126/science.1099955)

69. Aken BL *et al.* 2017 Ensembl 2017. *Nucleic Acids Res.* **45**, D635–D642. (doi:10.1093/nar/gkw1104)

70. Delroisse J, Lanterbecq D, Eeckhaut I, Mallefet J, Flammang P. 2013 Opsin detection in the sea urchin Paracentrotus lividus and the sea star Asterias rubens. *Cah. Biol. Mar.* **54**, 721–727.

71. Mason B, Schmale M, Gibbs P, Miller MW, Wang Q, Levay K, Shestopalov V, Slepak VZ. 2012 Evidence for multiple phototransduction pathways in a reef-building coral. *PLoS One* **7**, e50371. (doi:10.1371/journal.pone.0050371)

72. Hao W, Fong HK. 1999 The endogenous chromophore of retinal G protein-coupled receptor opsin from the pigment epithelium. *J. Biol. Chem.* **274**, 6085–6090.

73. Hara T, Hara R. 1968 Regeneration of squid retinochrome. *Nature* **219**, 450–454.

74. Nagata T, Koyanagi M, Tsukamoto H, Terakita A. 2010 Identification and characterization of a protostome homologue of peropsin from a jumping spider. *J. Comp. Physiol. A Neuroethol. Sens. Neural Behav. Physiol.* **196**, 51–59. (doi:10.1007/s00359-009-0493-9)

75. Chen P *et al.* 2001 A photic visual cycle of rhodopsin regeneration is dependent on Rgr. *Nat. Genet.* **28**, 256–260. (doi:10.1038/90089)

76. Koyanagi M, Terakita A, Kubokawa K, Shichida Y. 2002 Amphioxus homologs of Go-coupled rhodopsin and peropsin having 11-cis- and all-trans-retinals as their chromophores. *FEBS Lett.* **531**, 525–528.

77. Dowling JE. 1960 Chemistry of visual adaptation in the rat. *Nature* **188**, 114–118.

78. Porter ML, Blasic JR, Bok MJ, Cameron EG, Pringle T, Cronin TW, Robinson PR. 2012 Shedding new light on opsin evolution. *Proc. Biol. Sci.* **279**, 3–14.

(doi:10.1098/rspb.2011.1819)

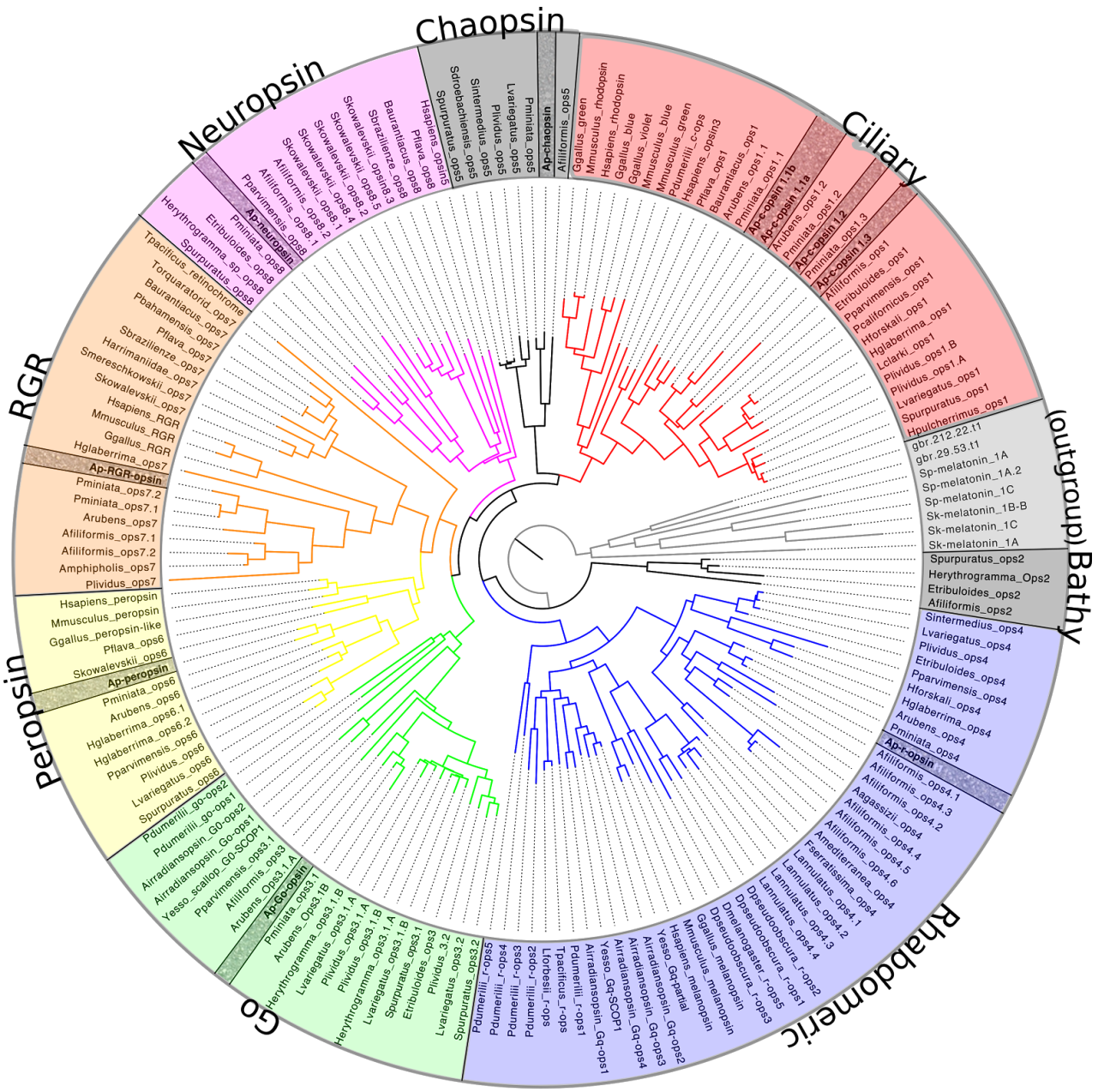
79. Gühmann M, Jia H, Randel N, Verasztó C, Bezares-Calderón LA, Michiels NK, Yokoyama S, Jékely G. 2015 Spectral Tuning of Phototaxis by a Go-Opsin in the Rhabdomeric Eyes of Platynereis. *Curr. Biol.* **25**, 2265–2271. (doi:10.1016/j.cub.2015.07.017)

80. Tsukamoto H, Terakita A, Shichida Y. 2005 A rhodopsin exhibiting binding ability to agonist all-trans-retinal. *Proc. Natl. Acad. Sci. U. S. A.* **102**, 6303–6308. (doi:10.1073/pnas.0500378102)

81. Cai K, Klein-Seetharaman J, Hwa J, Hubbell WL, Khorana HG. 1999 Structure and function in rhodopsin: effects of disulfide cross-links in the cytoplasmic face of rhodopsin on transducin activation and phosphorylation by rhodopsin kinase. *Biochemistry* **38**, 12893–12898.

82. Haskell-Luevano C, Cone RD, Monck EK, Wan YP. 2001 Structure activity studies of the melanocortin-4 receptor by in vitro mutagenesis: identification of agouti-related protein (AGRP), melanocortin agonist and synthetic peptide antagonist interaction determinants. *Biochemistry* **40**, 6164–6179.

83. Fritze O, Filipek S, Kuksa V, Palczewski K, Hofmann KP, Ernst OP. 2003 Role of the conserved NPxxY(x)5,6F motif in the rhodopsin ground state and during activation. *Proc. Natl. Acad. Sci. U. S. A.* **100**, 2290–2295. (doi:10.1073/pnas.0435715100)


Chaopsin
Ciliary
(outgroup)
Bathy
Rhabdomeric
Go
Peropsin
RGR
Neuropsin
Ap-chaopsin
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Ggallus_green
Mmusculus_rhodopsin
Hsapiens_rhodopsin
Ggallus_blue
Ggallus_violet
Mmusculus_blue
Mmusculus_green
Pdumerilii_c-ops
Hsapiens_opsin3
Pflava_ops1
Baurantiacus_ops1
Arubens_ops1.1
Pminiata_ops1.1
Ap-c-opsin 1.1b
Ap-c-opsin 1.1a
Arubens_ops1.2
Pminiata_ops1.2
Ap-c-opsin 1.2
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Ap-c-opsin 1.3
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Etribuloides_ops1
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Plividus_ops1.A
Lvariegatus_ops1
Spurpuratus_ops1
Hpulcherrimus_ops1
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gbr.29.53.t1
Sp-melatonin_1A
Sp-melatonin_1A.2
Sp-melatonin_1C
Sk-melatonin_1B-B
Sk-melatonin_1C
Sk-melatonin_1A
Spurpuratus_ops2
Herythrogramma_Ops2
Etribuloides_ops2
Afiliformis_ops2
Sintermedius_ops4
Lvariegatus_ops4
Plividus_ops4
Etribuloides_ops4
Pparvimensis_ops4
Hforskali_ops4
Hglaberrima_ops4
Arubens_ops4
Pminiata_ops4
Ap-r-opsin
Afiliformis_ops4.1
Afiliformis_ops4.3
Afiliformis_ops4.2
Aagassizii_ops4
Afiliformis_ops4.4
Afiliformis_ops4.5
Afiliformis_ops4.6
Amediterranea_ops4
Fserratissima_ops4
Lannulatus_ops4.1
Lannulatus_ops4.2
Lannulatus_ops4.3
Lannulatus_ops4.4
Dpseudoobscura_r-ops2
Dpseudoobscura_r-ops1
Dmelanogaster_r-ops5
Dpseudoobscura_r-ops3
Ggallus_melanopsin
Mmusculus_melanopsin
Hsapiens_melanopsin
Yesso_Gq-partial
Airradiansopsin_Gq-ops2
Airradiansopsin_Gq-ops3
Airradiansopsin_Gq-ops4
Yesso_Gq-SCOP1
Airradiansopsin_Gq-ops1
Pdumerilii_r-ops1
Pdumerilii_r-ops1
Tpacificus_r-ops
Lforbesii_r-ops
Pdumerilii_r-ops2
Pdumerilii_r-ops3
Pdumerilii_r-ops4
Pdumerilii_r-ops5
Pdumerilii_r-ops5
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Lvariegatus_ops3.2
Plividus_3.2
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Airradiansopsin_G0-ops2
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Pdumerilii_go-ops2
Spurpuratus_ops6
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Pminiata_ops6
Ap-peropsin
Skowalevskii_ops6
Pflava_ops6
Ggallus_peropsin-like
Mmusculus_peropsin
Hsapiens_peropsin
Plividus_ops7
Amphipholis_ops7
Afiliformis_ops7.2
Afiliformis_ops7.1
Arubens_ops7
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Pminiata_ops7.2
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Hglaberrima_ops7
Ggallus_RGR
Mmusculus_RGR
Hsapiens_RGR
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Smereschkowskii_ops7
Harrimaniidae_ops7
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Pflava_ops7
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Torquaratorid_ops7
Tpacificus_retinochrome
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Herythrogramma_sp_ops8
Etribuloides_ops8
Pminiata_ops8
Ap-neuropsin
Pparvimensis_ops8
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Afiliformis_ops8.2
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Skowalevskii_ops8.2
Skowalevskii_opsin8.3
Skowalevskii_ops8.5
Skowalevskii_ops8.4
Sbrazilienze_ops8
Baurantiacus_ops8
Pflava_ops8
Hsapiens_opsin5
Spurpuratus_ops5
Sdroebachiensis_ops5
Sintermedius_ops5
Plividus_ops5
Lvariegatus_ops5
Pminiata_ops5

Figure 1.

Eyes vs mixed
log fold change
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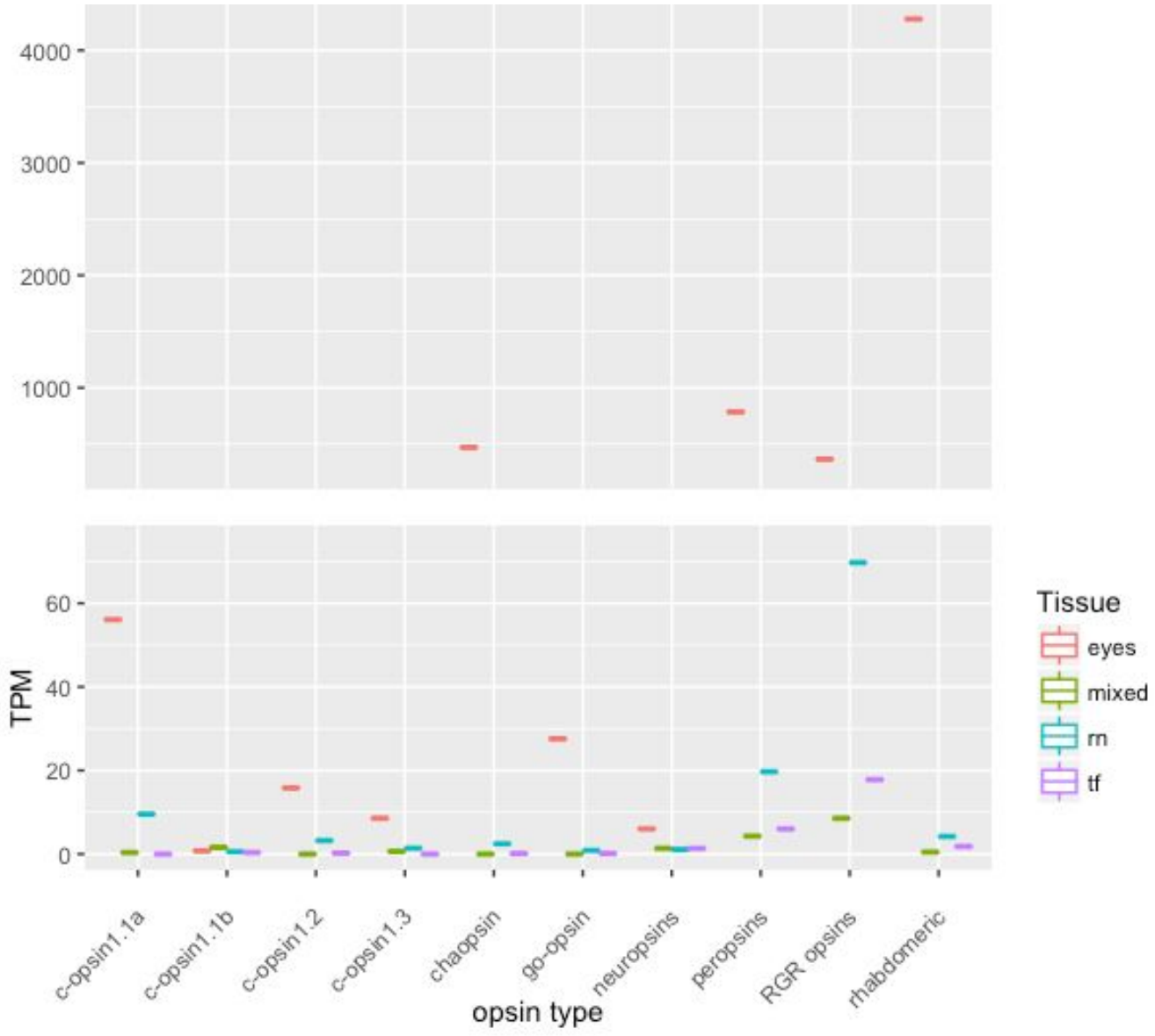
5
0
-5
-10
1e-01
1e+01
1e+03
1e+05
mean of normalized counts

Figure 2.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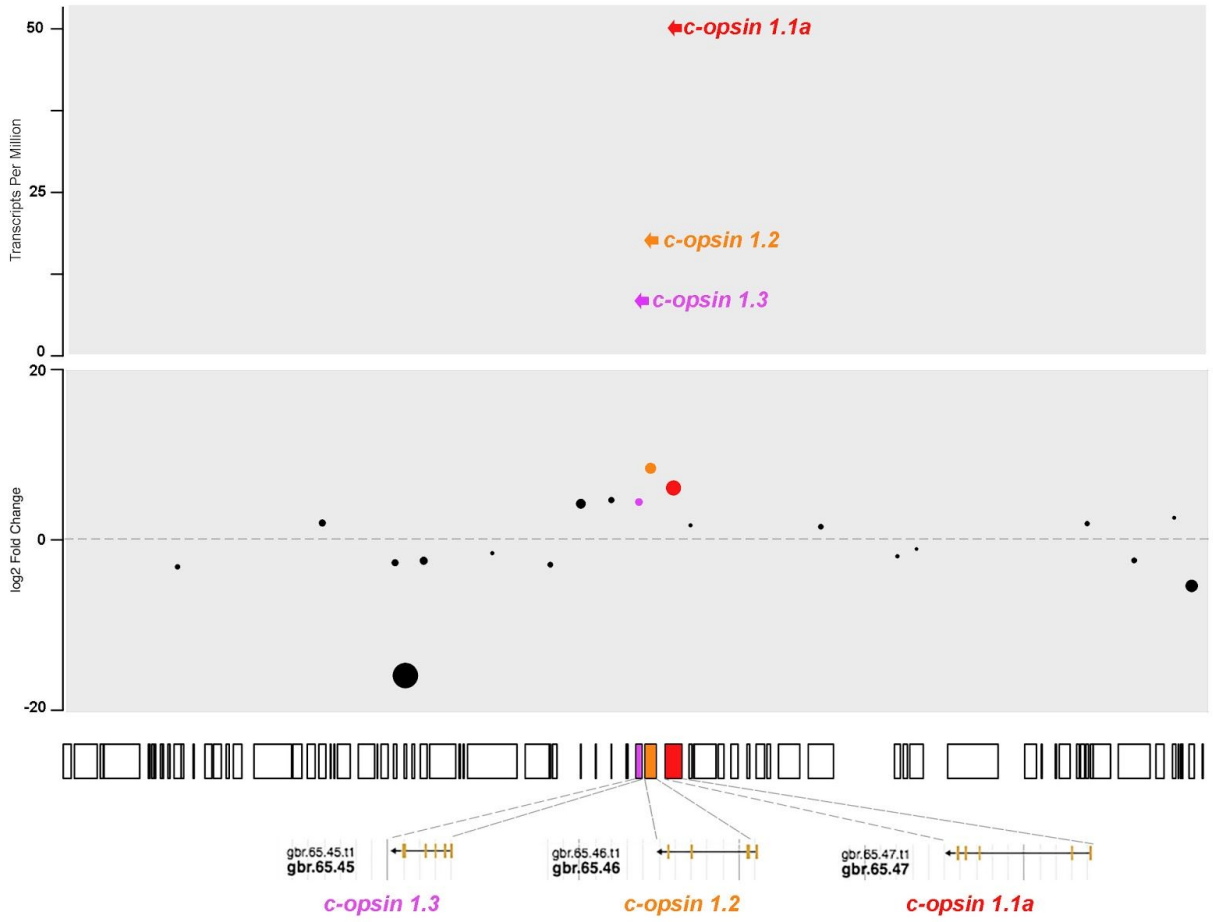
c-opsin1.1b
c-opsin1.2
c-opsin1.3
chaopsin
go-opsin
neuropsins
peropsins
RGR opsins
rhabdomeric
opsin type
Tissue
eyes
mixed
m
tf

Figure 3.


c-opsin 1.1a
c-opsin 1.2
c-opsin 1.3
Transcripts Per Million
50
25
0
log2 Fold Change
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0
-20
gbr.65.45.t1
gbr.65.45
gbr.65.46.t1
gbr.65.46
gbr.65.47.t1
gbr.65.47
c-opsin 1.3
c-opsin 1.2
c-opsin 1.1a

Figure 4.

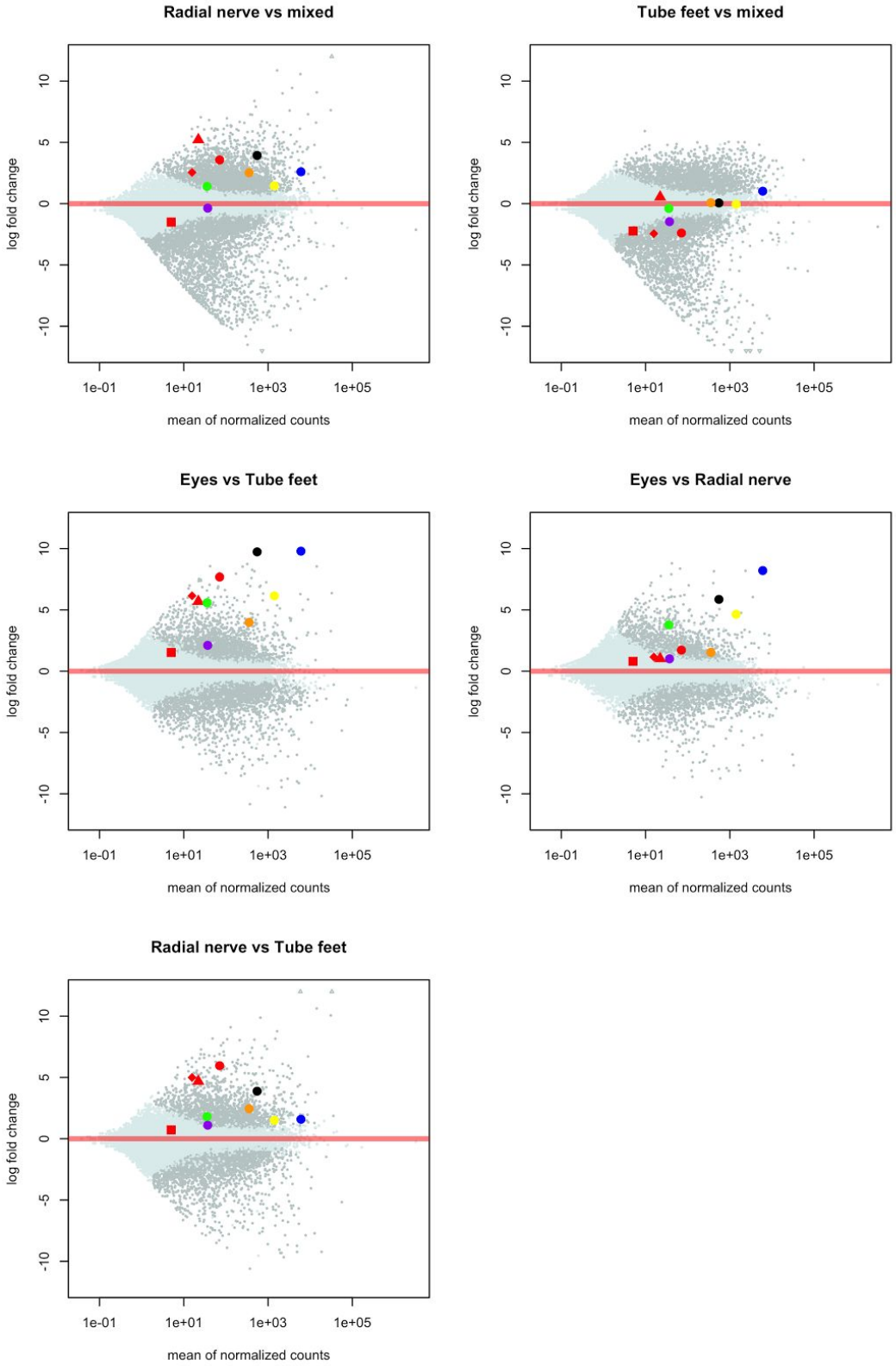
Radial nerve vs mixed
Tube feet vs mixed
Eyes vs Tube feet
Eyes vs Radial nerve
Radial nerve vs Tube feet
log fold change
mean of normalized counts
10
5
0
-5
-10
1e-01
1e+01
1e+03
1e+05

Figure S1.

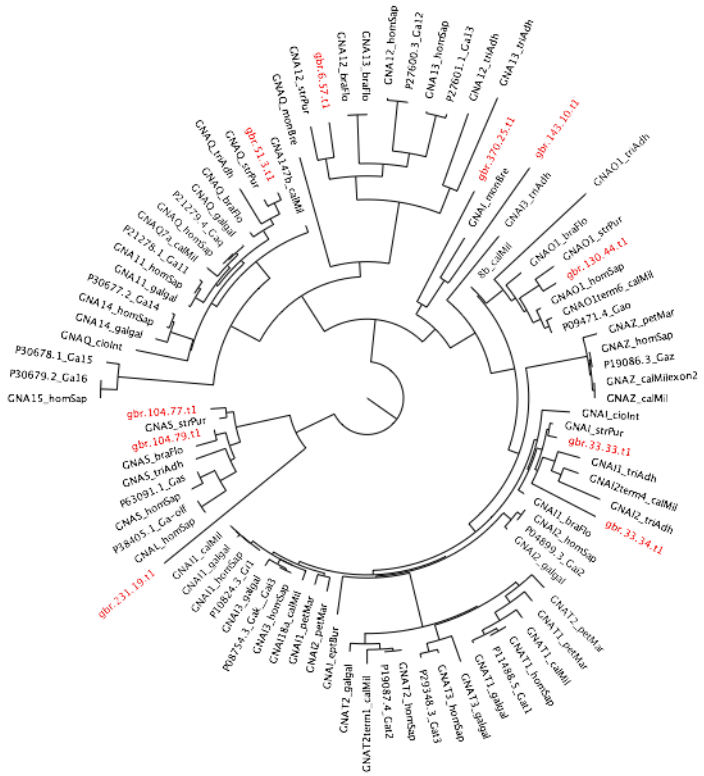

Figure S2.

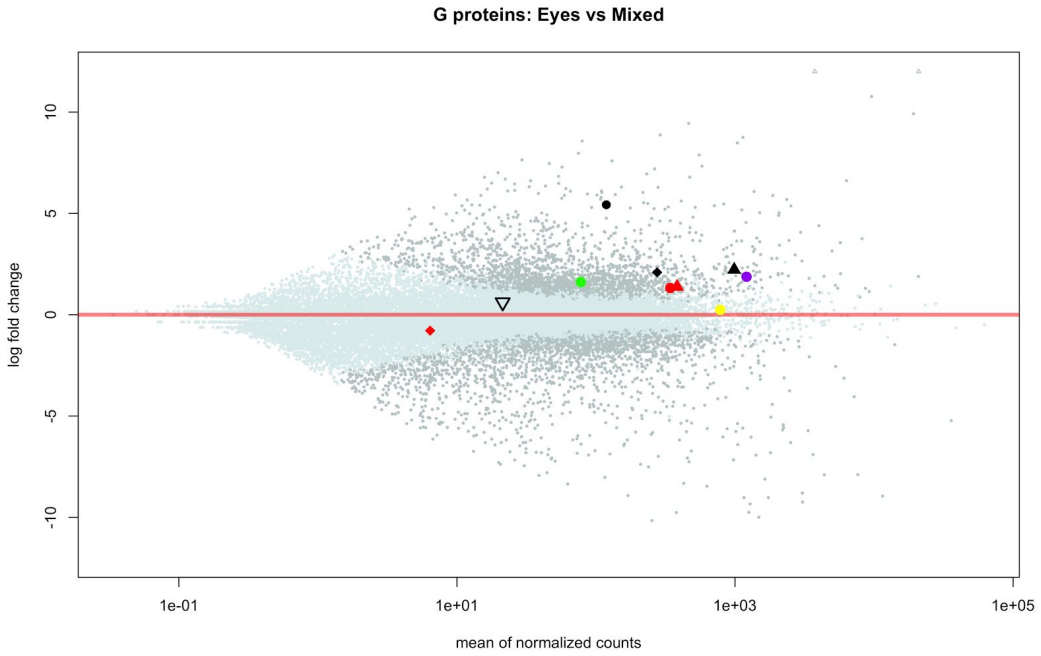

Figure S3.

Table 1. Analysis of known typically highly conserved key residues and amino acid motifs

*A. planc*i Opsin	Disulfide bond[a] (C110/C187)	LxxxD[b] (79)	Counterion[c] (E113/E181)	Schiff base[d] (K296)	NPxxY[e] (302)
C-opsin 1.1a (gbr.65.47.t1)	C/C	ICVAD	Y/E	K	NPVIY
C-opsin 1.1b (gbr.508.2.t1)	C/C	VCVAD	Y/-	K	NPVIY
C-opsin 1.2 (gbr.65.46.t1)	C/F	ISVGD	Y/-	R	N----
C-opsin 1.3 (gbr.65.45.t1)	L/C	VCEA-	Y/A	K	NPIIY
Go-opsin (gbr.470.6.t1)	C/C	MAVSD	I/E	K	NPLIY
R-opsin (gbr.176.5.t1)	C/C	LAFSD	Y/E	K	NPLVY
Chaopsin (gbr.176.10.t1)	C/C	LSGSD	Y/E	K	NPIIY
Peropsin (gbr.31.87.t1)	C/C	ASAGD	Y/E	K	NPLMF
RGR opsin (gbr.37.113.t1)	C/C	LCAGD	Y/E	K	NAALQ
Neuropsin (gbr.37.57.t1)	C/C	LAVSD	Y/E	K	NPIIY

a. Motif required for recognition of rhodopsin by G-protein [81]
b. Motif interacting with NPxxY motif upon receptor activation for structural constrains [82]
c. Glutamic acid residues stabilizing the Schiff base bond
d. Lysine residue forming Shiff base bond with retinal Motif providing structural constraints in response to photoisomerization during formation of the G protein-activating Meta II [83]

Figure Legends

Figure 1. Phylogenomic tree of 169 opsin sequences with melatonin receptors as the outgroup. There are 10 *A. planci* opsins (bold and checkered background) which classify into 7 different groups: 4 c-opsins, 1 chaopsin, 1 r-opsin, 1 peropsin, 1 RGR opsin, 1 go-opsin, and 1 neuropsin. No bathyopsin was found in *A. planci*, and has yet to be identified in any starfish species. The tree was generated by iqtree [58] with 10,000 ultrafast bootstrap support [59] using the 'a Bayesian-like transformation of aLRT' (abayes) method [60], and LG+F+R6 amino acid substitution model.

Figure 2. Summary of differential expression data in various *A. planci* tissue samples focusing on opsin expression. (A) Transcripts per million (TPM) counts of each opsin for each tissue type. For eyes (green) the highest expressed opsins are r-opsin, peropsin, chaopsin, RGR opsin, and go-opsin, with c-opsin 1.2, c-opsin 1.3, and neuropsin being expressed at low amounts, while no expression is observed in c-opsin 1.1b. RGR opsin and peropsin were the highest expressed amongst the other tissues. The mixed tissue and tube feet (tf) have little to no expression (TPM < 0.5) of c-opsin 1.2, c-opsin 1.3, go-opsin, and chaopsin.

Figure 3. Differential expression of the eye tissue samples versus the mixed tissue samples. R-opsin is the most differentially expressed, followed by chaopsin. With the exception of c-opsin 1.1b and neuropsin, all opsins are differentially expressed in the eyes of *A. planci* compared to the mixed tissue samples.

Figure 4. The boxes below the two panels represent "gbr_scaffold65" from the Great barrier reef assembly of the *A. planci* genome. Each box represents a gene and the size of the box correlates the to size of the gene. Three of the four Ap-c-opsins are located on the same scaffold all being transcripted 3' to 5'; Ap-c-opsin 1.1a (red), Ap-c-opsin 1.2 (orange) and Ap-c-opsin 1.3 (purple). Bottom panel: The log2 fold change of significantly differentially expressed genes (FDR < 0.05) comparing *A. planci* eyes the mixed tissue samples, with the three Ap-c-opsins highlighted. Top panel: Expression of the three Ap-c-opsins clustered on "gbr_scaffold65", the expression level correlates with the spatial ordering within the cluster (in the direction 3' to 5'), the most 3' having the highest expression and the most 5' having the lowest expression.

Figure S1. Differential gene expression in all *A. planci* tissue samples with the opsins highlighted: c-opsins in red, go-opsins in green, chaopin in black, neuropsin in purple, peropsin in yellow, r-opsin in blue and RGR opsin in orange. The y-axis in the $\log_2$ fold-change, as the distance from the y-axis increases the more differentially expressed a gene is in one tissue versus the other. The x-axis represents counts per million (CPM), an increase on the this axis shows genes with more reads counts.

Figure S2. Phylogenomic tree of 92 G-protein alpha subunit sequences. *A. planci* sequences ID's are highlighted in red. Of the 10 *A. planci* sequences 3 classified as $G\alpha_s$, 1 as $G\alpha_o$, 4 as $G\alpha_i$, 1 as $G\alpha_q$, and 1 as $G\alpha_{12}$.

Figure S3. Differential gene expression in the *A. planci* eye samples compared to mixed tissues, with the G protein alpha subunits highlighted: 3 $G\alpha_s$ (red), 1 $G\alpha_o$ (green), 4 $G\alpha_i$ (black), 1 $G\alpha_q$ (purple), and 1 $G\alpha_{12}$ (yellow). All identified g protein alpha subunits with the exception of 1 $G\alpha_s$ (gbr.231.19.t1), 1 $G\alpha_i$ (gbr.143.10.t1) and the $G\alpha_{12}$ are upregulated in the eyes of *A. planci* compared to the mixed tissue samples.