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Published in:
P L o S One

DOI:
10.1371/journal.pone.0098870

Publication date:
2014

Document Version
Publisher's PDF, also known as Version of record

Citation for published version (APA):
Bielecki, J., Zaharoff, A. K., Leung, N. Y., Garm, A. L., & Oakley, T. H. (2014). Ocular and Extraocular Expression of Opsins in the Rhopalium of Tripedalia cystophora (Cnidaria: Cubozoa). DOI: 10.1371/journal.pone.0098870
Ocular and Extraocular Expression of Opsins in the Rhopalium of *Tripedalia cystophora* (Cnidaria: Cubozoa)

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**Abstract**

A growing body of work on the neuroethology of cubozoans is based largely on the capabilities of the photoreceptive tissues, and it is important to determine the molecular basis of their light sensitivity. The cubozoans rely on 24 special purpose eyes to extract specific information from a complex visual scene to guide their behavior in the habitat. The lens eyes are the most studied photoreceptive structures, and the phototransduction in the photoreceptor cells is based on light sensitive opsin molecules. Opsins are photosensitive transmembrane proteins associated with photoreceptors in eyes, and the amino acid sequence of the opsins determines the spectral properties of the photoreceptors. Here we show that two distinct opsins (*Tripedalia cystophora-lens eye expressed opsin* and *Tripedalia cystophora-neuropil expressed opsin*, or Tc-leo and Tc-neo) are expressed in the *Tripedalia cystophora* rhopalium. Quantitative PCR determined the level of expression of the two opsins, and we found Tc-leo to have a higher amount of expression than Tc-neo. *In situ* hybridization located Tc-leo expression in the retinal photoreceptors of the lens eyes where the opsin is involved in image formation. Tc-neo is expressed in a confined part of the neuropil and is probably involved in extraocular light sensation, presumably in relation to diurnal activity.

**Introduction**

Cubozoans are an emerging model system for understanding visual information processing through integrative studies of morphology, behavior and physiology. Morphologically, cubozoans accomplish image analysis with a limited, and therefore experimentally tractable, neural capacity of about one thousand neurons [1,2]. Coupled with this simple neural architecture is a complex visual system of 24 eyes: Cubozoans have six eyes on each of four sensory structures called rhopalia. Two eyes per rhopalium are lens eyes, comparable in morphology to vertebrate eyes (Figure 1). Behaviorally, cubozoans use vision to avoid obstacles [3–5], to navigate using terrestrial cues [6] and for phototaxis [7,8]. Multiple behaviors of cubozoan medusae are modulated by a swim pacemaker system that is influenced by light sensed by the lens eyes, pit eyes, and the neuropil [9,10]. Physiologically, the cubozoan *Tripedalia cystophora* possesses monochromatic vision with peak sensitivity in the blue-green part of the spectrum (504 and 512 nm for the upper and lower lens eyes respectively) [11,12]. Since the specific peak absorbance values were obtained by experimentation, the neuropil might express opsins because it influences the swim pacemaker in different light conditions [10]. In Cnidaria beside cubozoans, there is direct molecular evidence for extraocular opsin expression [14,19]. In the hydrozoans *Cladonema radiatum* and *Podocoryne carnea*, several opsins are expressed in tissues that lack obvious visual functions, including tentacles, gonads, and manu-
In Hydra magnipapillata, many different opsin-like sequences exist and some are expressed broadly in neurons dotting the ectoderm. Hydra magnipapillata has no eyes, and they use extraocular phototransduction in dermal photoreception and to modulate firing of nematocytes in different light levels.

Here, we report that the rhopalia of Tripedalia cystophora express at least two opsins; one identical to that found previously by Kozmik and one closely related to Cr-leo from C. rastonii. Based on quantitative PCR we found the two opsins to be expressed at significantly different levels in the rhopalium. Additionally, in situ hybridization localized the expression of the two opsins to different rhopaliaal structures, suggesting that one opsin serves an extraocular function. We find the opsin transcript found previously by Kozmik to be expressed in the neuropil of Tripedalia cystophora, but we see no evidence of expression of that transcript in the lens eyes. We refer to this gene as Tc-neo (Tripedalia cystophora neuropil expressed opsin).

Methods

Animals

We collected medusae of Tripedalia cystophora, Conant 1897, near La Parguera, Puerto Rico (N17° 58' 22.49" W67° 04' 03.66"), in the mangrove where they feed on copepods aggregated in light shafts. Tripedalia cystophora is not an endangered or protected species and specific permissions were not required to collect the animals. We stored the medusae in RNAlater for transcriptome sequencing. In addition to the collected animals, we obtained medusae (7–9 mm in bell diameter) of Tripedalia cystophora from our cultures at the University of Copenhagen, Denmark. Cultures originated from gravid

| Primer name | Primer sequence 5’→3’ |
|-------------|-----------------------|
| 454 poly-T | AAG CAG TGG TAT CAA CGC AGA GTA CTCTTCT CTCTTCT |
| RT-PCR LEO-F | CTG GAA GGT GCG ATA GCA TT |
| RT-PCR LEO-R | AGG TTG CCG CCT TCT TTA TT |
| RT-PCR NEO-F | CGC TGG AAG CGC CTG TGG |
| RT-PCR NEO-R | TCA TTC CGG CTC AAC AGA ATT TCC |
| qPCR LEO-F | GCC CTG TCG TCA CCG CT |
| qPCR LEO-R | CGG CCA GGT GAT GGA GCA TCG C |
| qPCR NEO-F | CGC TGG AAG CGC CTG TGG |
| qPCR NEO-R | TGG TGT CCC GCT TCA AGG GAA GT |

Primer sequences used for the various molecular techniques: 454- pyrosequencing, reverse transcriptase PCR (RT-PCR) and quantitative PCR (qPCR). F and R denote forward and reverse primers respectively.

doi:10.1371/journal.pone.0098870.t001

Figure 1. Cubozoan visual system. The visual system of the cubozoan Tripedalia cystophora (A) comprises four sensory structures called rhopalia (B). Each rhopalium carries six eyes of four morphological types (lower lens eye LLE, upper lens eye ULE, pit eye PE and slit eye SE) and a light sensitive neuropil (NP, red broken line). The eyes are responsible for the image formation in the animal and the light sensitive neuropil is thought to be involved in diurnal activity.

doi:10.1371/journal.pone.0098870.g001
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Quantitative PCR (qPCR)

We used quantitative PCR to test the hypothesis that Tc-leo and Tc-neo are expressed at different levels in the rhopalium. We constructed cDNA as for reverse transcriptase PCR, except using only one rhopalium for each run. We performed qPCR using eight biological replicates (8 separate rhopalium from 8 different animals) with two to four technical replicates each to compare relative expression of Tc-leo and Tc-neo in the rhopalial transcriptome. In seven replicates we were able to include a sham (no RNA) negative control. We designed qPCR primers to each opsin. No introns are known from these genes, so we relied on DNase treatment to prevent DNA contamination. Primers used were qPCR Tc-leo-F & R qPCR Tc-neo-F & R (Table 1). Amplification conditions: RT step - 10 min at 50°C, Inactivation step - 5 min at 95°C, Cycle 35X - 10 sec at 95°C and 15 sec at 55°C. We deposited our sequence of Tc-leo in GenBank (accession no. KJ542646).

Table 2. Relative expression of the rhopalial opsins based on quantitative PCR.

| Biological Replicate | Tc-leo Average C_{T} ± SEM | Tc-neo Average C_{T} ± SEM |
|----------------------|-----------------------------|-----------------------------|
| 1                    | 15.86 ± 0.30                | 25.05 ± 1.91                |
| 2                    | 19.55 ± 2.01                | 28.30 ± 1.45                |
| 3                    | 20.55 ± 0.05                | 32.50 ± 0.00                |
| 4                    | 26.40 ± 4.53                | No C_T Value Given          |
| 5                    | 20.07 ± 0.38                | No C_T Value Given          |
| 6                    | 33.35 ± 0.15                | No C_T Value Given          |
| 7                    | 34.23 ± 1.01                | No C_T Value Given          |
| 8                    | 29.70 ± 1.85                | 31.5 ± 0.46                 |

Quantitative PCR ascertain the relative expression of Tc-leo and Tc-neo. In every run of the qPCR Tc-leo expressed relatively higher than Tc-neo. In every run the cycle threshold (C_{T}) was lower for Tc-leo. In four runs Tc-neo was not sufficiently expressed to return a C_{T} ascertaining the much higher relative expression of Tc-leo.

doi:10.1371/journal.pone.0098870.t002
40X - 10 sec at 95°C and 15 sec at 55°C, Melt Curve Analysis
80X - 30 sec every 0.5°C. We used the iScript One-Step RT-PCR Kit with SYBR Green (BioRad, Hercules, CA, USA) for all quantitative PCR runs (Figure 2 and Table 2).

**In situ Hybridization**

For the *in situ* hybridization experiments we fixed cultured medusa for 24 hrs in 4% PFA, rinsed 3×5 mins in PBS and dehydrated in a graded series of methanol (25, 50, 75, 90 and 100%). The experiments followed the colorimetric labeling protocol established by Grens et al. [26] with modifications by Plachetzk et al. [22]. To generate *in situ* hybridization probes, we used RT-PCR to amplify portions of each opsin, using the same primers described above (qPCR Tc-leo and qPCR Tc-neo). In addition to experimental anti-sense probes, we generated a sense control for both the opsins to verify the validity of the peroxidase staining. The rhopalia were whole mounted and the opsin expression was recorded by digital imaging (Figure 3). Tc-leo and Tc-neo expression was graphically illustrated (Figure 4).

**Phylogenetic Analyses**

We explored the phylogenetic relationship of the three known cubozoan opsins (Figure 5). We used the “O&O” opsin data set of Feuda et al. [27], which contains 104 sequences that are representative of all major opsin subfamilies, plus placopsin and melanin receptor outgroups. This data set already included Cr-leo, and we added both *Tripedalia* opsins, Tc-leo and Tc-neo, by using the ‘-add’ option of MAFFT 7.0 [28] to align those two protein sequences to the published alignment of Feuda et al. [27]. We searched for the Maximum Likelihood gene tree, assuming a GTR+gamma model of protein evolution, using RAxML [29]. We also gauged node support using 100 bootstrap pseudoreplicates, also implemented in RAxML. We performed all phylogenetic analyses in Osiris, within the Galaxy bioinformatics package [30,31]. All data and analyses are publicly available (http://galaxy-dev.cnsi.usc.edu/osiris/u/oestratodd/p/leo-neo).

**Light Microscopy and TEM**

To confirm the position of the opsin expression in the rhopalium, we made light microscopy and ultrastructural images from rhopalia prepared by standard EPON embedding and sectioning procedures (see [1]) (Figure 6).

**Results**

**454 Pyrosequencing**

Our search of resulting transcriptome data returned a previously unknown *Tripedalia cystophora* opsin sequence that is 94% identical to Cr-leo, the lens eye opsin described from * Carybdea rastoni* [17]. We did not find any other opsin sequences in the rhopalian 454 data, but this is probably due to the limited depth of coverage of our transcriptome, which may not detect sequences with low levels of expression.

**Reverse Transcriptase PCR (RT-PCR)**

We used reverse transcriptase PCR to verify expression in rhopalia of the previously published Tc-neo [18] and our newly found Tc-leo. We found both opsins to be expressed in cDNA prepared from rhopalia. However, a difference in band brightness on the gel suggested that Tc-leo was expressed at a higher level than Tc-neo (data not shown).

**Quantitative PCR (qPCR)**

Our qPCR results confirmed the differences in the level of expression of the two opsins in *T. cystophora* rhopalia. The level of expression of Tc-leo was higher than Tc-neo. In all 8 biological replicates Tc-leo returned a lower cycle threshold (C\textsubscript{T}) than Tc-neo (Table 2). This result is significant in a binomial test (p = 0.0078). In four replicates the Tc-neo expression was so low that it did not return a C\textsubscript{T} value, whereas Tc-leo was expressed in all replicates. These results clearly and conservatively show that Tc-leo has significantly higher expression than Tc-neo in the rhopalia of *T. cystophora*.

**In situ Hybridization**

The colorimetric *in situ* hybridization determined the location of Tc-leo and Tc-neo mRNA expression (Figures 3 and 4). Tc-leo mRNA was expressed in the cell bodies of the photoreceptors of the upper and lower lens eyes (Figures 3A, B and 6). The photoreceptors of cubozoans include an outer photoreceptive segment, a mid-section containing pigment granules and a basal cell body containing the nucleus (Figure 6). The lens eyes have everted retinas and the nuclei of the photoreceptors are located outside the pigment screen (Figures 6 and S1) [32]. The colorimetric labeling using our Tc-leo probe is located in the area of the photoreceptors corresponding to the nucleus and thereby the endoplasmatic reticulum. Tc-leo expression is limited to the retinal photoreceptors of the lens eyes; none of the surrounding tissue is stained and the sense control is devoid of colorimetric staining (Figure 3C). In contrast to Tc-leo, Tc-neo was expressed in the neuropil of the rhopalium (Figure 3D and E). The neuropil fills up most of the volume of the rhopalium between the epidermis and the gastrodermis, from the base of the stalk to the top of the lower lens eye [2]. Tc-neo was not expressed in the entire neuropil but in limited parts (Figure 3D and E). Curiously, we did not find evidence that either opsin is expressed in photoreceptors of the pit and slit eyes of the rhopalium (Figure 3).

**Phylogenetic Analysis**

We found (Figure 5) Tc-leo, Cr-leo and Tc-neo to fall into a clade with other cnidarian opsins, although with fairly low bootstrap support of 53%. This opsin clade contains genes from the hydrozoans *Hydra magnipapillata* and Cladoema radiatum and from the anthozoan *Nematostella vectensis*. This cnidarian clade of opsins may be called ‘cnidops’ [15]. In the analysis of Feuda et al. [27], cnidops is the sister-group of the bilaterian RGR/Go clade (or type IV opsins sensu [16]) of opsins. However, when we aligned Tc-leo and Tc-neo to the alignment of Feuda et al. [27], we did not recover this relationship. Instead, we find a group of opsins from *Nematostella* that Feuda et al. [27] found to be related to ciliary opsins to be the sister group of cnidops with very low support (17%). These results indicate that opsin phylogenetic results, especially for ancient nodes, are highly sensitive to which genes are included.

Within the cnidops clade, we found Tc-leo and Cr-leo to be well-supported (100% bootstrap) orthologs. These two orthologs form a sister group to multiple hydrozoan opsins from *H. magnipapillata* and *C. radiatum*. In contrast, Tc-neo is much more distantly related, and not placed with certainty in our analysis. Our results do not recover a close relationship between c-opsins and Tc-neo (for detailed phylogenetic analysis, please refer to Figure S2).

**Discussion**

With a relatively simple nervous system coupled with camera-type, image-forming eyes, cubozoans have great potential to...
become a model system to understand visual information processing. In addition to numerous publications about morphology, physiology and behavior, research into the molecular basis of cubozoan light sensitivity has also begun [17,18,33]. Here, we report expression of two different opsins in the cubozoan *Tripedalia cystophora*. We find *Tc-leo* to be expressed in the upper and lower lens eyes and *Tc-neo* to have extra-ocular expression in the neuropil. Even though previous techniques only found one opsin per species, our phylogenetic results indicate the duplication of *leo* and *neo* occurred before the origin of cubozoans. These results reconcile previous discrepancies between molecular and physio-

Figure 3. Opsin expression in the rhopalium of *Tripedalia cystophora*. *In situ* hybridization colorimetric staining places *Tc-leo* mRNA expression in the cell bodies of the retinal photoreceptors of the lens eyes (upper lens eye ULE and lower lens eye LLE) (**A,B**). The control with the sense probe (**C**) is devoid of colorimetric staining validating the positive results in **A** and **B**. *Tc-neo* mRNA is expressed in part of the neuropil (**D,E**), which is also known to have photosensitive properties [10]. *Tc-neo* sense control is seen in **F**. None of the opsins are expressed in the lesser eyes (pit eyes PE and slit eyes SE), suggesting that other opsins could be expressed in these eye types.

doi:10.1371/journal.pone.0098870.g003
logical data and provide the first direct molecular evidence of extraocular opsin expression in a cubozoan.

Leo Expression in Lens Eyes

Our results indicate *leo* mRNA is expressed in the cell body of the lens eye photoreceptors, corresponding to the area of the cell nucleus (Figures 3, 6 and S1). Light microscopy and ultrastructural studies confirm the position of the nuclei in the cell bodies (Figure 6) [32,34]. Our *leo* expression results are consistent with Koyanagi et al. [17], but different from the results of Kozmik et al. [18] who reported a different gene (*Tc-neo*) to be expressed in lens eyes with a maximal sensitivity to blue light (470 nm) in *in vitro* expression analysis. In contrast to the 470 nm peak of *Tc-neo*, ERG experiments found a single sensitivity peak near 510 nm in lens eyes of the same species [11]. We find *Tc-leo* to be very similar and orthologous to an opsin (*Cr-leo*) of a related cubozoan and *Cr-leo* has an *in vitro* absorption maximum of 500 nm [17], much more closely matching the physiological results of *T. cystophora* lens eyes. These results can be reconciled if only *leo* (not *neo*) is expressed in lens eyes. One possible explanation for the discordant result is that Kozmik et al. [18] obtained non-specific antibody staining in the lens eyes, such that their antibody probed *Tc-leo* rather than *Tc-neo*. To generate their *Tc-neo* antibody, they used the c-terminal 55 amino acids of the opsin. In this region, we found that 30% of the amino acids are identical and 63% of amino acids have similar physicochemical properties between *Tc-leo* and *Tc-neo*. Because *in situ* hybridization used sequence-specific probes, and immunohistochemistry may be more prone to cross-hybridization, we suggest non-specific hybridization as the cause of the discordant expression results.

![Figure 4. Graphical representation of expression of *Tc-leo* and *Tc-neo*.](https://www.plosone.org/)

**Figure 4. Graphical representation of expression of *Tc-leo* and *Tc-neo*.** While the *Tc-leo* is expressed in the retinal photoreceptors of the lens eyes, *Tc-neo* is expressed in the neuropil. The green areas depict the rhopodial in situ hybridization colorimetric staining pattern of the *Tc-leo* and blue areas represent *Tc-neo* (A, side view and B, top view). Upper lens eye (ULE), lower lens eye (LLE), slit eye (SE) and pit eye (PE).
doi:10.1371/journal.pone.0098870.g004

![Figure 5. Cnidops phylogenetic tree.](https://www.plosone.org/)

**Figure 5. Cnidops phylogenetic tree.** Maximum likelihood phylogenetic analysis including representative animal opsins from the “O&O” data set of Feuda et al. [27] plus additional Cnidarian opsins indicates that *Tc-leo* and *Tc-neo* are distantly related opsins whereas *Tc-leo* and *Cr-leo* are closely related to each other. Illustrated here is a subset of all the genes analyzed, focusing on Cnidarian opsins; *Cladonema radiatum* (*Cr*), *Hydra magnipilata* (*Hm*) and *Nematostella vectensis* (*Nv*). The colors of the branches correspond to their phylogenetic placement in the analysis of Feuda et al. [27]. The full phylogeny, showing all genes analyzed is included in Figure S2. Numbers at nodes are bootstrap values based on 100 pseudoreplicated datasets, implemented in RAxML [29], assuming a GTR plus gamma model of protein evolution.
doi:10.1371/journal.pone.0098870.g005
Neo Expression in Neuropil

In addition to Tc-leo expression in lens eyes, we find Tc-neo to be expressed in the neuropil and to have a lower level of overall expression in rhopalia. These results and previous research suggest functional involvements of neo in the rhopalium, and we therefore hypothesize the neuropil to contain an aggregated/higher-order extraocular photoreceptor (sensu [19]). This is further supported by electrophysiological data where the neuropil was shown to modulate the pacemaker signal frequency when exposed to light [10]. Tc-neo has a peak absorbance of ~470 nm [18] consistent with other photosensitive pigments involved in diurnal activity pattern (entrainment), which often have absorption maxima in the blue spectrum of visible light [35,36]. T. cystophora display light mediated diurnal behavior [37] and since Tc-neo is expressed in the neuropil, which is transparent and exposed to ambient light, it is possible that this opsin is involved in the overall activity pattern of the animal based on the ambient light level [37]. The results on level of expression also fit well with receptor morphology where the membranes of cells with non-directional photoreception usually are considerably less folded than retinal photoreceptors used for spatial vision [38], and extraocular opsins should have lower expression than ocular opsins. From the qPCR experiments it is evident that Tc-neo is expressed around 200 times less than Tc-leo. This lower degree of expression of Tc-neo compared to Tc-leo is also evident when comparing the strength of colorimetric staining. Despite multiple lines of evidence suggesting Tc-neo involvement in neuropil-based light sensitivity functions, we caution that firm conclusions await direct experimental manipulation of Tc-neo, which await the advent of genetic manipulation techniques in cubozoans.

Leo and Neo are Distantly Related Opsins

The two opsins we found expressed in the rhopalium of T. cystophora are rather distantly related, yet both appear to be members of a cnidarian opsin clade that can be called cnidops [15]. The close relationship of one of these genes (Tc-leo) to another gene (Cr-leo) expressed in lens eyes, suggests that all cubozoan lens eyes express a member of this orthologous opsin clade. Our conclusion placing the second opsin (Tc-neo) within cnidops is different from a previous conclusion that Tc-neo is a c-opsin [18]. Despite different conclusions, the results are not drastically different. Kozmik et al. [18] found Tc-neo to be sister to

Figure 6. Microscopy of the eyes of Tripedalia cystophora. Light microscopy of the upper and lower lens eyes (A) and pit and slit eyes (B) show that the nuclei (+) are located in the cell bodies outside the zone of pigment granules (*). Transmission electron micrograph (C) shows the cell membrane (arrowhead) and numerous mitochondria (¤) that are located between the pigment granules and the nucleus suggesting the area of protein translation to be adjacent to the nucleus. The folded membranes of the cilium (○) are evident in the outer segments of the photoreceptor cells (C). Scale bar in (C) 2 µm.
doi:10.1371/journal.pone.0098870.g006
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Summary

From our combined qPCR and in situ hybridization results there is little doubt that the Tc-neo is expressed in the retinal photoreceptors of the lens eyes and is as such the first step in image formation. Tc-neo is expressed in the neuropil and highly likely involved in extraocular light sensation, presumably in relation to control the diurnal activity pattern [37].

Supporting Information

Figure S1 Graphical representation of cubozoan photoreceptor morphology. Sagittal section of the lower lens eye of Tripedalia cystophora (A) (modified from [34]). Light is absorbed in the ciliary layer by the photoreceptive outer segments (POS) (B) and the pigment layer prevents false light entering the eye. Pigment granules (pg) make up the pigment layer and are located in the pigmented region (PR) of the photoreceptors. The nuclei (n) are located in the nuclear region (NR) of the cell bodies. The photoreceptors are everted and the neural layer is thereby located outside the pigment layer. Gray et al. [40] found invaginated synapses (is) in the nuclear region but the significance of this discovery is largely unknown. It is thought that the photoreceptors articulate on second order neurons since the proximal end protrudes into a neural plexus that extends into the neuropil of the rhopalium. (TIF)

Figure S2 Detailed opsin phylogenetic tree. Maximum likelihood phylogenetic analysis including representative animal opsins from the “O&O” data set of Feuda et al. [27], plus the new Tc-leo gene. Feuda et al. [27] did not include Tc-neo, which we also added to their data set. We rooted animal opsins with melatonin receptor genes (black branches). The branch colors for animal opsins follow Feuda et al. [27]. Unlike Feuda et al. [27], we do not recover monophyletic ciliary opsins (red branches). Also differing from Feuda et al. [27], we do not find a sister-group relationship between ‘cnirops’ [41] genes and the clade called Type IV opsins by Porter et al. [16]. The difference between our topology and that of Feuda et al. [27] seems to be caused by the addition of Tc-neo. Numbers at nodes are bootstrap values based on 100 pseudoreplicated datasets, implemented in RAxML [29], assuming a GTR plus gamma model of protein evolution, the same model used by Feuda et al. [27]. (TIF)

Acknowledgments

The authors acknowledge the invaluable help collecting cubomedusae by the Row Boje Crew (Rebecca Lampe, Bryan Juarez, and Valerie Lovdahl), as well as Katia Jindrich, Sabrina Pankey and Des Ramirez for helping with procedures and their comments on the manuscript. JB acknowledges the financial support by the Journal of Experimental Biology (travel grant) and Danish Independent Research Council Grant DFF132500146. AG acknowledges Vilum-Kahn Rasmussen VKR022166. THO acknowledges National Science Foundation ISO-1045257.

Author Contributions

Conceived and designed the experiments: JB THO. Performed the experiments: JB AZ NL AG THO. Analyzed the data: JB AG THO. Contributed reagents/materials/analysis tools: JB AG THO. Wrote the paper: JB THO. Provided manuscript comments: AG.
References

1. Garm A, Ekstrom P, Boules M, Nilsson DE (2006) Rhopalial are integrated parts of the central nervous system in box jellyfish. Cell Tissue Res 325: 333–343.

2. Singh C, Garm A, Nilsson DE, Ekstrom P (2006) Bilaterally symmetrical rhopalial nervous system of the box jellyfish *Tripedalia cystophora*. J Morphol 267: 1391–1405.

3. Hamner WM, Jones MS, Hamner PP (1995) Swimming, feeding, circulation and vision in the Australian box jellyfish, *Chironex fleckeri* (Cnidaria: Cubozoa). Marine and Freshwater Res 46: 985–990.

4. Matsumoto GI (1995) Observations on the anatomy and behaviour of the box-jellyfish *Carybdea rastonii* Haacke. Mar and Fresh Behav and Physiol 26: 139–140.

5. Garm A, O’Connor M, Parkefelt L, Nilsson DE (2007) Visually guided obstacle avoidance in the box jellyfish *Tripedalia cystophora* and Chiropelidae bronzie. J Exp Biol 210: 3616–3623.

6. Garm A, Oskarsson M, Nilsson DE (2011) Box jellyfish use terrestrial visual cues for navigation. Curr Biol 21: 798–803.

7. Petrie R, Garm A, Nilsson DE (2011) Visual control of steering in the box jellyfish *Tripedalia cystophora*. J Exp Biol 214: 2009–2017.

8. Buskey E (2003) Behavioral adaptations of the cubozoan medusa *Tripedalia cystophora* for feeding on copepod (*Dioithona oualata*) swarms. Mar Biol 142: 225–232.

9. Garm A, Bielecki J (2008) Swim pacemakers in box jellyfish are modulated by the visual input. J Comp Physiol A 194: 641–651.

10. Garm A, Mori S (2009) Multiple photoreceptor systems control the swim pacemaker activity in box jellyfish. J Exp Biol 212: 3951–3960.

11. Garm A, Coates MM, Gad R, Seymour J, Nilsson DE (2007) The lens eyes of the box jellyfish *Tripedalia cystophora* and *Chirolophus boops* are true and color-blind. J Comp Physiol A 193: 547–557.

12. Coates MM, Garm A, Theobald JC, Thompson SH, Nilsson DE (2007) The dermal light sense in the context of integrative photoreceptor cell biology. Vis Neurosci 18: 535–549.

13. Terakita A (2005) The opsins. Genome Biol 6: 213.

14. Koyanagi M, Takada E, Nagata T, Tsukamoto H, Terakita A (2013) Homologs of vertebrate OPN3 potentially serve as a light sensor in nonphotoreceptive tissue. PNAS 110: 4998–5003.

15. Plachetzki DC, Fong CR, Oakley TH (2010) The evolution of phototransduction from an ancestral cyclic nucleotide gated pathway. Proc Biol Sci 277: 1963–1969.

16. Plachetzki DC, Fong CR, Oakley TH (2012) Cnidocyte discharge is regulated by light and opsin-mediated phototransduction. BMC Biol 10: 19.

17. Desrosier SF, Madden TL, Schijff AA, Zhang J, Zhang Z, et al. (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 25: 3389–3402.

18. Greis A, Mason E, Marsh JL, Bode HR (1995) Evolutionary conservation of a cell fate specification gene: the Hydra achaete-scute homolog has neuronal activity in Drosophila. Development 121: 4027–4035.

19. Feuda R, Hamilton SC, McKinney JO, Pisani D (2012) Metazoan opsin evolution reveals a simple route to animal vision. PNAS 109: 18086–18092.

20. Karoh K, Stanselley DM (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol Biol Evol 30: 772–780.

21. Stamatakis A (2006) RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22: 2680–2681.

22. Oakley TH, Alexandrou MA, Ngo R, Pankey MS, Looijker KB (In Press) Oisir: Accessible and reproducible phylogenetic and phylogenomic analyses within the Galaxy workflow management system. BMC Bioinformatics.

23. Goecks J, Nekrutenko A, Taylor J, The Galaxy Team (2010) Galaxy: a comprehensive approach for supporting accessible, reproducible, and transparent computational research in the life sciences. Genome Biol. 27 11(8): R86.

24. Yatsn N (1917) Notes on the physiology of *Carybdea rantonii*. Journal of the College of Science, Tokyo Imperial University 40: 1–14.

25. Piasek L, Kozmik Z, Copula sivickisi. J Comp Physiol A 195: 557–569.

26. Levy O, Appelbaum L, Leggat W, Gothlif Y, Hayward DC, et al. (2007) Light-responsive cryptochrome from a simple multicellular animal, the coral *Acropora millepora*. Science 318: 467–470.

27. Garm A, Bielecki J, Petie R, Nilsson DE (2012) Opposite patterns of diurnal activity in the box jellyfish *Tripedalia cystophora* and *Cephea cypri*id. Biol Bull 222: 35–45.

28. Nilsson DE (2013) Eye evolution and its functional basis. Vis Neurosci 30: 5–20.

29. Rivera AS, Ozturk N, Fahey B, Plachetzki DC, Degnan BM, et al. (2012) Light-responsive cryptochrome from a simple multicellular animal, the coral *Acropora millepora*. Science 318: 467–470.

30. Garm A, Bielecki J, Petie R, Nilsson DE (2012) Opposite patterns of diurnal activity in the box jellyfish *Tripedalia cystophora* and *Cephea cypri*id. Biol Bull 222: 35–45.

31. Goecks J, Nekrutenko A, Taylor J, The Galaxy Team (2010) Galaxy: a comprehensive approach for supporting accessible, reproducible, and transparent computational research in the life sciences. Genome Biol. 27 11(8): R86.

32. Stamatakis A (2006) RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22: 2680–2681.

33. Oakley TH, Alexandrou MA, Ngo R, Pankey MS, Looijker KB (In Press) Oisir: Accessible and reproducible phylogenetic and phylogenomic analyses within the Galaxy workflow management system. BMC Bioinformatics.

34. Goecks J, Nekrutenko A, Taylor J, The Galaxy Team (2010) Galaxy: a comprehensive approach for supporting accessible, reproducible, and transparent computational research in the life sciences. Genome Biol. 27 11(8): R86.

35. Yatsn N (1917) Notes on the physiology of *Carybdea rantonii*. Journal of the College of Science, Tokyo Imperial University 40: 1–14.

36. Piasek L, Kozmik Z, Copula sivickisi. J Comp Physiol A 195: 557–569.

37. Levy O, Appelbaum L, Leggat W, Gothlif Y, Hayward DC, et al. (2007) Light-responsive cryptochrome from a simple multicellular animal, the coral *Acropora millepora*. Science 318: 467–470.

38. Garm A, Bielecki J, Petie R, Nilsson DE (2012) Opposite patterns of diurnal activity in the box jellyfish *Tripedalia cystophora* and *Cephea cypri*id. Biol Bull 222: 35–45.

39. Nilsson DE (2013) Eye evolution and its functional basis. Vis Neurosci 30: 5–20.

40. Rivera AS, Ozturk N, Fahey B, Plachetzki DC, Degnan BM, et al. (2012) Light-responsive cryptochrome from a simple multicellular animal, the coral *Acropora millepora*. Science 318: 467–470.

41. Garm A, Bielecki J, Petie R, Nilsson DE (2012) Opposite patterns of diurnal activity in the box jellyfish *Tripedalia cystophora* and *Cephea cypri*id. Biol Bull 222: 35–45.

42. Plachetzki DC, Oakley TH (2010) The evolution of phototransduction from an ancestral cyclic nucleotide gated pathway. Proc Biol Sci 277: 1963–1969.

43. Plachetzki DC, Fong CR, Oakley TH (2012) Cnidocyte discharge is regulated by light and opsin-mediated phototransduction. BMC Biol 10: 19.

44. Chou IH, Holmes MH (2001) DNA sequence quality trimming and vector removal. Bioinformatics 17: 1093–1104.

45. Altschul SF, Madden TL, Schijff AA, Zhang J, Zhang Z, et al. (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 25: 3389–3402.

46. Greis A, Mason E, Marsh JL, Bode HR (1995) Evolutionary conservation of a cell fate specification gene: the Hydra achaete-scute homolog has neuronal activity in Drosophila. Development 121: 4027–4035.

47. Feuda R, Hamilton SC, McKinney JO, Pisani D (2012) Metazoan opsin evolution reveals a simple route to animal vision. PNAS 109: 18086–18092.

48. Karoh K, Stanselley DM (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol Biol Evol 30: 772–780.