



Transcriptomics reveal genes involved in bioluminescence and vision in marine deep-sea shrimp (Oplophoridae)



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Introduction

Oplophorid shrimp are deep-sea pelagic decapods that exhibit extraordinary mechanisms of bioluminescence (Figure 1). All secrete a luminescent spew, which is emitted as a bright cloud from the base of the antennae or legs, for defense against predators (Thomson et al., 1995) (Figure 2). In addition to the spew, three oplophorid genera possess luminescent photophores that can be found along the length of the body, which are used for counterillumination (Herring, 2007).

Eye pigment spectral sensitivities are often defined as long-, medium-, or short-wavelength sensitive (LWS, MWS, SWS). LWS detects wavelengths between 495-570 nm (red/green), MWS detects 470-540 nm (green), SWS2 detects 415-480 nm (blue), and SWS1 detects 355-450 nm (UV/violet) (Land and Nilsson, 2002). Previous studies have determined that oplophorid photophore luminescence emits at a slightly shorter wavelength (near-UV) than spew luminescence, which emits in the blue-green spectrum (Latz et al., 1988). Subsequent behavioral and physiological studies determined that species containing only the spew have a peak spectral sensitivity in the blue-green light spectrum (between 490-510 nm), whereas species containing both the spew and photophores possess an additional pigment which allows for a peak spectral sensitivity to near-UV light (about 410 nm) (Frank and Widder, 1994; Cronin and Frank, 1996). Here, we investigate representatives from two oplophorid genera that possess both bioluminescent spew and photophores. We also include a penaeid shrimp, *Benthesicymus bartletti*. Other dendrobranchiate shrimp, such as *Sergestes similis*, have been discovered to possess only one visual pigment with a peak sensitivity in the blue-green light spectrum (Lindsay et al., 1999).

Unfortunately, the study of bioluminescence and visual structures in the marine environment, particularly the deep sea, has been challenging due to the perils of collecting and observing live organisms. And although behavioral and physiological studies on the detection of the emitted light have been conducted, characterization of the genes involved in detection has not yet been examined. The advent of novel molecular techniques applied to transcriptomics allow us to study the evolution of bioluminescence at a genetic level and may provide insight into the genes involved in the detection of light. Proteins in the opsin family regulate the phototransduction pathway, the pathway in which a light signal from the environment is converted into an electrical signal in photoreceptor cells (Rivera et al., 2010). Two major opsin clades are the r- and c-opsins that, along with their associated pathway proteins, are typically separated into rhabdomeric or ciliary photoreceptors, which differ in morphology. Here, we take a transcriptomic approach to identify and/or verify genes expressed in the visual system that may play a role in detecting bioluminescence. In this current study, we focus on r- and c-opsin type genes. We hypothesize that two separate opsins may be responsible for blue-green and UV light detection, which we will investigate using transcriptomic analysis.



Figure 2. This photograph shows an oplophorid shrimp, *Acantheephyra purpurea*, utilizing a bioluminescent spew to distract or blind a viperfish, *Chauliodon danae*.

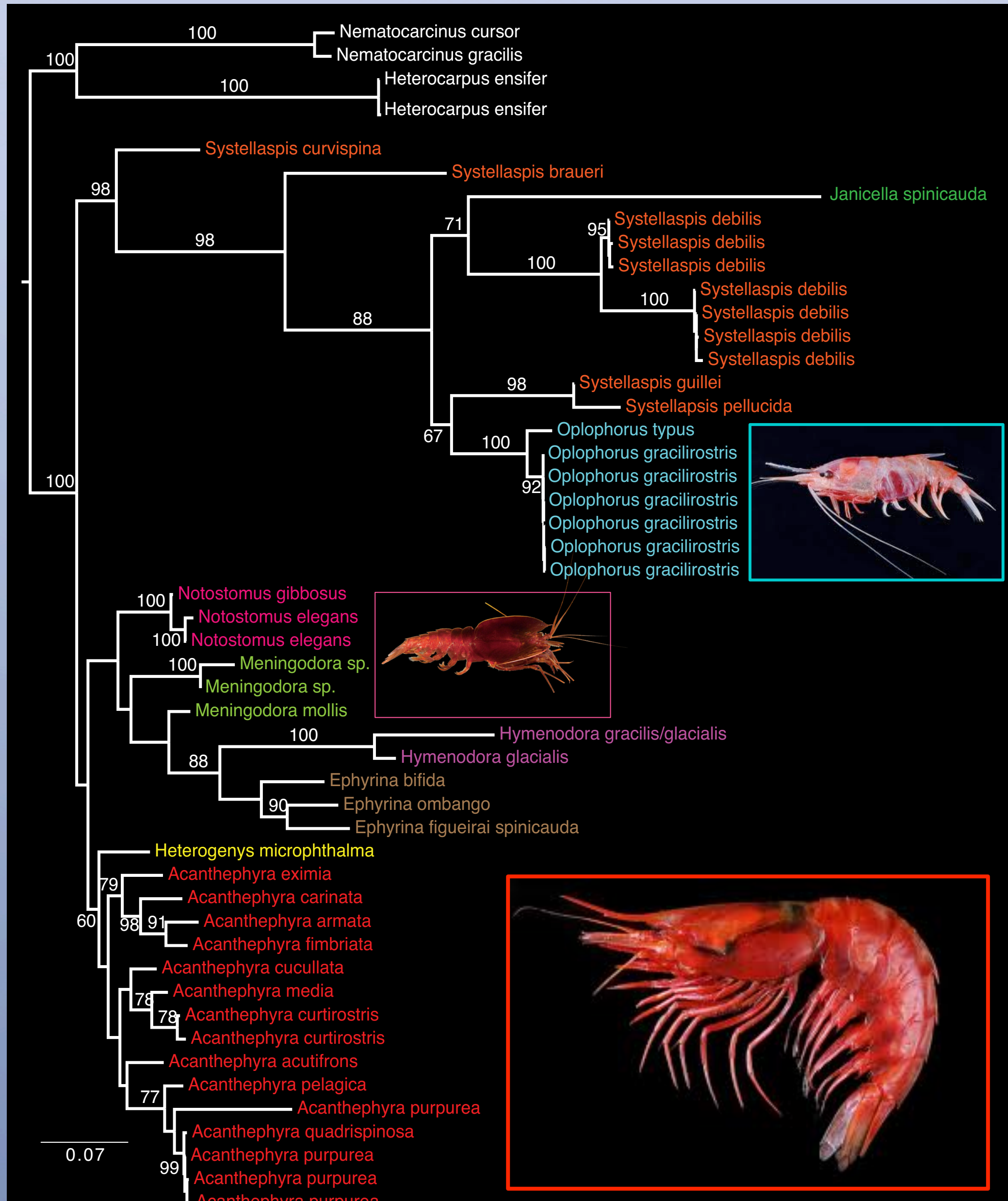


Figure 1. Phylogenetic tree of Oplophoridae, pelagic deep sea shrimps which excrete a bioluminescent spew. Some oplophorids additionally possess bioluminescent photophores. The spew has been shown to produce a slightly different emission spectra than the photophores (i.e. blue-green versus near-UV light). Photos by Tin-Yam Chan and Johnsen Lab, Duke University

Results

Table 1. A subset of the returned similarity results after running the sequences through BLAST. MWS, SWS2, and SWS1 type pigments are color-coded green, blue, and purple (representing UV). () = number of gene copies per opsin

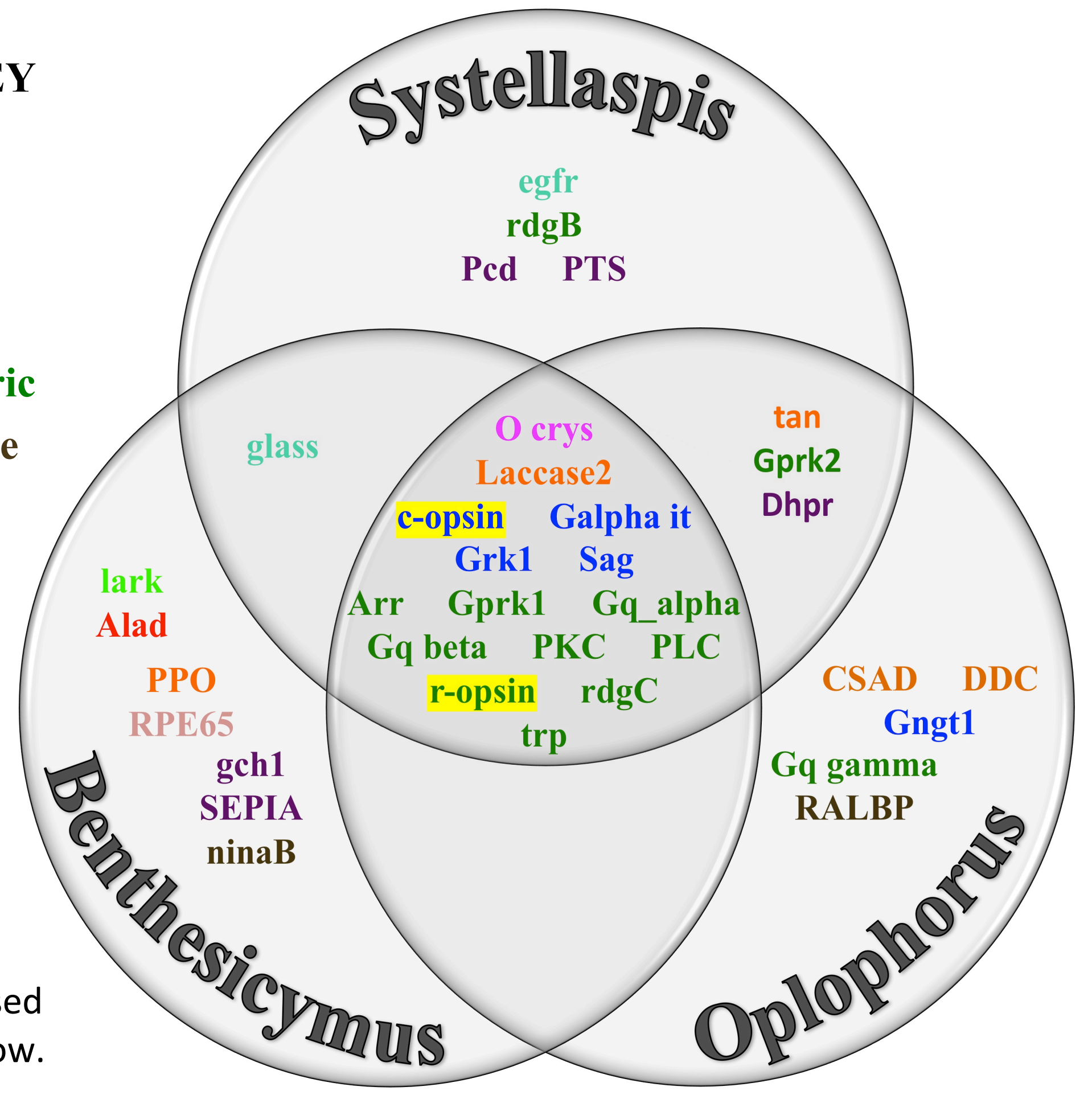
	<i>Benthesicymus</i> (1)	<i>Systellaspis</i> (2)	<i>Oplophorus</i> (2)
r-opsin		<i>Uca pugilator</i> (E=2e-64) SWS2 <i>Megoura viciae</i> (E=6e-58) UV-wavelength-like (SWS1) <i>Bombus impatiens</i> (E=2e-56) UV-sensitive (SWS1)	<i>Uca pugilator</i> (E=2e-124) SWS2 <i>Daphnia pulex</i> (E=2e-137) rhodopsin family, MWS
	<i>Neogonodactylus oerstedii</i> (E=4e-160) MWS/SWS2 <i>Neomysis americana</i> (E=4e-82) MWS	<i>Neognathophausia ingens</i> (E=1e-109) MWS	<i>Neogonodactylus oerstedii</i> (E=2e-175) MWS/SWS2
c-opsin		<i>Daphnia pulex</i> (E=3e-59) SWS2 <i>Limulus polyphemus</i> (E=4e-63) between MWS/SWS2 and SWS1 <i>Hemigrapsus sanguineus</i> (E=1e-59) MWS/SWS2 <i>Acyrtosiphon pisum</i> (E=7e-57) UV-sensitive-like (SWS1) <i>Bombus impatiens</i> (E=2e-56) UV-sensitive (SWS1)	<i>Limulus polyphemus</i> (E=3e-128), MWS/SWS2 and SWS1 <i>Hemigrapsus sanguineus</i> (E=2e-122) MWS/SWS2
	<i>Neomysis americana</i> (E=4e-82) MWS <i>Archaeomysis grebnitzkii</i> (4e-72) MWS	<i>Neogonodactylus oerstedii</i> (E=1e-175) MWS	<i>Neogonodactylus oerstedii</i> (E=2e-175) MWS/SWS2

The r- and c-opsin genes were found in all three shrimp species (Figure 4). Utilizing GenBank BLAST, similarity searches from other species revealed genes comparable to MWS, SWS2, and SWS1 eye pigments (Table 1).

FUNCTIONAL GENE SET KEY

- Pterin Synthesis
- Melanin Synthesis
- Photoreceptor Specification
- Phototransduction, Ciliary
- Phototransduction, Rhabdomeric
- Retinoid Pathway, Invertebrate
- Ommochrome Synthesis
- Heme Synthesis
- Diurnal Clock
- Crystallins

Figure 4. This Venn diagram shows all of the eye genes that were hit upon for the three species in question. Each gene has been categorized by functional gene set and is color-coded appropriately. The genes that we focused on (c- and r-opsin) are highlighted yellow.



Methods

- RNA was extracted from the eyes of 3 shrimp species (*Benthesicymus bartletti*, *Systellaspis debilis*, and *Oplophorus gracilirostris*) using a NucleoSpin RNA kit.
- Full-length mRNAs were made into cDNA using the Clontech SMARTer kit and normalized to trim high copy genes expressed in these tissues.
- Samples were submitted to the Brigham Young University next-generation sequencing center for purification, emPCR, and pyrosequencing on a 454 platform (Figure 3).
- Visual target genes were isolated from transcriptome data using the transcriptome annotation pipeline Phylogenetical Informed Annotation (PIA) of the Light Interaction Tool Kit (LIT) (developed by the Oakley Lab at UC Santa Barbara).
- Comparisons were made to genes reported in GenBank using BLAST ([http:// www.ncbi.nlm.nih.gov/BLAST/](http://www.ncbi.nlm.nih.gov/BLAST/)).

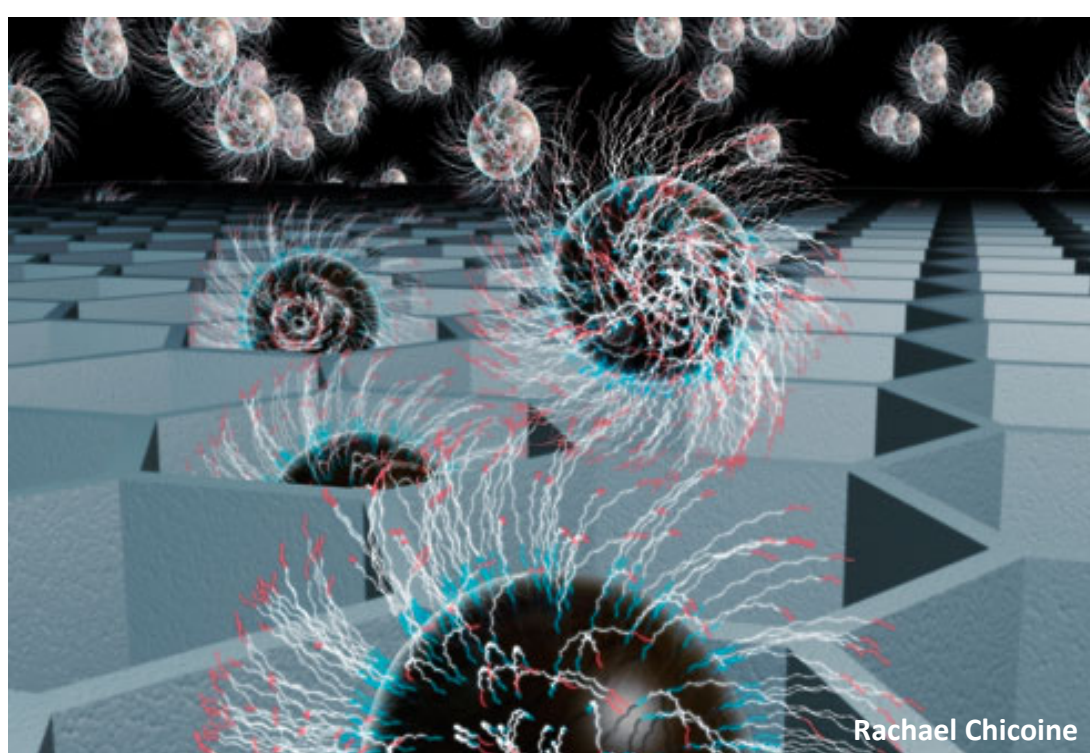


Figure 3. 454 pyrosequencing

Conclusions/Future Studies

- We found two copies each of the r- and c-opsins in *Oplophorus* and *Systellaspis*, and one copy of each in *Benthesicymus*.
- BLAST results suggest some sequences matched exclusively blue-green, while others match a mixture of blue-green and UV-like opsins. The sequences that match a mixture of blue-green and UV-like eye pigments may represent opsins that detect in the shorter wavelength end of the blue spectra, detecting near-UV light emitted from photophores.
- It is possible that species such as *S. debilis*, which possess both bioluminescent spew and photophores, have two types of eye pigments that are able to detect and distinguish between blue-green and near-UV light of their respective bioluminescent mechanisms.
- In the future, we would like to characterize other visual genes within Oplophoridae and link them to visual function.
- Future studies may include an expansion of this transcriptomic approach to include other caridean shrimp outside of the family Oplophoridae.

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